Exxon Valdez Oil Spill
Restoration Project Final Report

Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

Restoration Project 99025
Final Report

Volume 1

L. E. Holland-Bartels, Editor

U.S. Geological Survey
Alaska Biological Science Center
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Study History: This project began with the acceptance of the 5-year study plan by the Trustee Council in March 1995. The FY 95 funds were provided to develop sampling protocols, test methodologies, and to initiate those portions of the overall study that could begin in late summer 1995. The first full field season for this study was initiated in FY 96, followed by a similar field effort in FY 97, and focused reduced effort in FY 98. Program reviews by the Chief Scientist and Trustees of work reported in this document occurred in February 1996 and 1997 and January 1998. The final report has undergone external scientific peer review conducted through the Chief Scientist's office as well as journal review as noted in the individual chapters.

Abstract: The 1989 spill of some 42 million L of crude oil into Prince William Sound, Alaska, represents not only the largest tanker spill in United States history, but the world's largest spill in northern waters. Acute effects have been studied extensively. However, efforts to quantify the spill's long-term chronic effects and develop defensible restoration measures have been plagued by varying levels of scientific uncertainty. That such uncertainty exists is not unexpected. The spill occurred in Prince William Sound's highly variable physical setting typified by its complex oceanography and fjord-like geomorphology. Additionally, uncertainty was driven by the scarcity of precise pre-spill population estimates and spotty life-history information for most species. The research reported herein in, structured in eight primary papers and 27 supporting papers (appendices), documents the state of recovery and assessments of continuing constraints to population recovery for four vertebrate predators (sea otter *Enhydra lutris*, harlequin duck *Histrionicus histrionicus*, river otter *Lontra canadensis*, and pigeon guillemot *Cepphus columba*) whose recovery status remained uncertain some 5 years after the *Exxon Valdez* oil spill. These species are used in a collective weight of evidence approach to better understand the process of coastal community recovery. Each species is examined for the strength of information it brings in health, population, and trophic metrics to support or reject the hypothesis of continuing oil effects in the nearshore system versus the alternatives that food constraints or demographic bottlenecks limit these focal species. While data for individual species contain various levels of uncertainty, scientific confidence is developed in the following picture when examined across species, metric, and hypothesis: Within the nearshore coastal environment, sporadic releases of residual oil are occurring, and benthic species, primarily invertebrates, are being exposed in a temporally and spatially patchy manner sufficient to transport oil up through the food chain. Thus, for the two invertebrate-feeders, sea otter and harlequin duck, evidence exists over several lines of investigation to suggest that local-scale populations continue to be constrained not by food availability or natural demographic processes, but by increased levels of mortality coincident with continued exposure to residual oil. Conversely, weight of evidence suggests that only limited direct oil-related effects are being transferred through the fish trophic pathway. Sufficient evidence suggests recovery is occurring in river otter populations, while the lack of

recovery in pigeon guillemot may be attributed to food limitations (both natural and indirectly related to the spill) and/or slow demographic response to initial acute mortalities. Individual lines of investigation often contained uncertainty, but the collective weight of evidence presented in this multipaper volume indicates lack of full recovery of the nearshore ecosystem from the *Exxon Valdez* oil spill nearly a decade following the event. Integrated, multispecies approaches can allow sufficient weight of evidence to develop despite inherent system variability or data limitations and, thus, facilitate both better societal understanding of such pollution events and development of appropriate restoration responses.

Key Words: Alaska, Barrow's goldeneye, biomarkers, body mass, *Cepphus columba*, clams, condition indices, cytochrome P450, demography, diet, ecosystem, emigration, *Enhydra lutris*, *Exxon Valdez* oil spill, food limitation, habitat selection, harlequin ducks, health, hematology, *Histrionicus histrionicus*, home range, hydrocarbons, immigration, intertidal, *Lontra canadensis*, masked greenlings, mortality, mussels, nearshore, pigeon guillemots, plasma biochemistry, pollution, population recovery, predator-prey interaction, prey, prey availability, prey consumption rate, prey demography, Prince William Sound, reproduction, river otters, sea otters, sea urchins, serum chemistry, sex-ratio, subtidal, surveys, survival, trophic.

Project Data: Final Restoration Report 99025, a collaborative and multiagency effort, used an integrated approach to assess recovery status of the nearshore ecosystem of Prince William Sound following the *Exxon Valdez* oil spill of 1989. As a result of this design, scientists from some 15 research organizations located in over 10 states participated and were required to openly share research results to all participants in near real-time. This distributed-organization and research-sharing requirement necessitated the development of a detailed data management plan and a process by which data could be shared and remotely accessed. Such a design was developed and documented in Holland-Bartels (1996)[1]. For the period of active study, all study data were served for project scientists by the U.S. Geological Survey's Alaska Biological Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503 (Table 1). At project completion, all study data were returned to principal investigators to be managed and archived per policy of their respective agencies. Access to these data is made by arrangement with senior authors (or agency) of the report chapters.

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[1]Holland-Bartels, L. 1996. Mechanisms of impact and potential recovery of nearshore vertebrate predators: Restoration Project 95025 Annual Report. Report to the *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska, USA.

Table 1. Data management summary for Nearshore Vertebrate Predator Study at study completion, 1998.

				Files present?			Compliance?	
NVP component	Files (#)	Total size (mb)	Data on file	History	Metadata	SOP	History	Metadata
Focal Species								
Sea otters	501	165.0	all:1995–98	some	some	yes	some	yes
Harlequin ducks	108	3.18	all:1995–98	yes	yes	yes	yes	yes
River otter	15	3.41	all:1996–98	yes	yes	yes	yes	yes
Pigeon guillemot	24	4.07	1996–97 (no 98)	yes	yes	yes	yes	yes
Prey Data								
Duck food	23	0.55	all:1995, 1997	yes	yes	yes	yes	yes
Intertidal clams	71	3.28	all:1995–97	yes	yes	yes	yes	yes
Mussels	137	6.99	1996 (no 97, 98)	yes	yes	yes	yes	yes
Subtidal clams	21	1.53	all:1995–97	yes	no	yes	yes	-
Subtidal fishes	51	1.25	all:1995–97	yes	yes	yes	yes	yes
Sea urchins	94	2.48	all:1996–97	yes	yes	yes	yes	yes
Other Files								
Invertebrate predators	22	1.88	all:1995–96	yes	one	yes	yes	yes
Side-scan sonar	72	4.40	all:1995	yes	yes	yes	yes	yes

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EXECUTIVE SUMMARY

This report is structured in a series of six main-body chapters covering perspectives on the four sentinel species, a multispecies assessment of health, and an overview of results.

Chapter 1 Synthesis: The 1989 spill of some 42 million L of crude oil into Prince William Sound, Alaska, represents not only the largest tanker spill in United States history, but the world's largest spill in northern waters. Acute effects have been studied extensively. However, efforts to quantify the spill's long-term chronic effects and develop defensible restoration measures have been plagued by varying levels of scientific uncertainty. That such uncertainty exists is expected. The spill occurred in Prince William Sound's highly variable physical setting typified by its complex oceanography and fjord-like geomorphology. Additionally, uncertainty was driven by the scarcity of precise pre-spill population estimates and spotty life-history information for most species. In this paper, I use information from four vertebrate predators whose recovery status was uncertain (sea otter *Enhydra lutris*, harlequin duck *Histrionicus histrionicus*, river otter *Lontra canadensis*, and pigeon guillemot *Cepphus columba*) in a collective weight of evidence approach to better understand the process of recovery. In this approach, each species is examined for the strength of information it brings in health, population, and trophic metrics to support or reject the hypothesis of continuing oil effects in the nearshore system versus the alternatives that food constraints or demographic bottlenecks are limiting the recovery of these focal species. For example, we have excellent sampling and methodological approaches for the invertebrate-feeding sea otter and fish-feeding pigeon guillemot that allows us to confidently address food limitation hypotheses through study of these species. On the other hand, the invertebrate-feeding harlequin duck and fish-feeding river otter are less useful in this assessment because precise methodologies are lacking or sample variability is high for various prey or feeding metrics. Three of four species provide strong independent models to address the question of demographic constraints. Finally, while there is only an intermediate level of confidence in individual studies of health status and oil biomarkers for any given species, their collective story is compelling. Collectively, scientific confidence is developed in the following picture: Within the nearshore coastal environment, sporadic releases of residual oil are occurring, and benthic species, primarily invertebrates, are being exposed in a temporally and spatially patchy manner sufficient to transport oil up through the food chain. Thus, evidence exists over several lines of investigation for sea otter and harlequin duck to suggest that local-scale populations continue to be constrained by increased levels of mortality coincident with continued exposure to residual oil rather than by food availability or natural demographic processes. Conversely, there is little evidence to suggest that direct oil-related effects are being transferred through the fish trophic pathway. Populations of the piscivorous river otter appear to be recovering. The continued lack of recovery in the piscivorous pigeon guillemot may be attributed to food limitations (both natural and indirectly related to the spill) and/or slow demographic response to initial acute mortalities. In summary, individual lines of investigation often contained uncertainty, but the collective weight of evidence from this project indicates lack of full recovery of the nearshore ecosystem from the *Exxon Valdez* oil spill nearly a decade following the event. Integrated, multispecies approaches can allow sufficient weight of evidence to develop despite

inherent system variability or data limitations. Thus, a better societal understanding of such pollution events can evolve and appropriate restoration efforts developed.

Chapter 2 Biomarkers: A major component of the Nearshore Vertebrate Predator ecosystem study addressed the potential of continuing exposure to residual oil from the 1989 *Exxon Valdez* oil spill as a factor limiting recovery of top predators. To evaluate exposure, we measured induction of cytochrome P450 1A (CYP1A), a protein involved in metabolism of aromatic hydrocarbons, in harlequin ducks *Histrionicus histrionicus*, pigeon guillemots *Cepphus columba*, river otters *Lontra canadensis*, and sea otters *Enhydra lutris* from oiled and unoiled areas of western Prince William Sound during 1995–99. We also assessed health of individual members of these species using hematologies, serum chemistries, and body condition as indicators. Two additional species were sampled for CYP1A: masked greenlings *Hexagrammos octogrammus* in 1996 and Barrow's goldeneyes *Bucephala islandica* in 1997. For all species, we found evidence of greater CYP1A induction in oiled areas compared to unoiled areas. Residual oil from the spill, rather than other petroleum or organochlorine contaminants in the environment, is the apparent source of the contaminant exposure. Species that prey on benthic invertebrates, including sea otters and seaducks, showed marked differences between areas, whereas species that consume primarily fishes showed either relatively small differences (river otters, pigeon guillemot adults) or no differences (pigeon guillemot chicks). These findings suggest that exposure resulted through consumption of contaminated invertebrate prey or contact with sediments during foraging. Body condition generally was equivalent between animals in oiled and unoiled areas, and few differences in blood values were noted. Red blood cell counts were lower in both adult harlequin ducks and pigeon guillemots in oiled areas compared to unoiled areas, possibly indicating a mild anemia. Sea otters in oiled areas had higher concentrations of serum gamma glutamyl transferase, an enzyme indicating liver dysfunction, but differences were not large and were less than in previous post-spill studies, perhaps due to declining oil toxicity and loss of the most severely affected individuals from the population. A negative relation was found between body mass and CYP1A induction in harlequin ducks, but not for the other species. Continuing exposure to residual *Exxon Valdez* oil spill may be limiting recovery of harlequin ducks and possibly sea otters, but does not appear to be a factor in recovery of river otters or pigeon guillemots in western Prince William Sound.

Chapter 3 Sea Otter Part A. Sea Otter Population Status and the Process of Recovery from the 1989 *ExxonValdez* Oil Spill: Sea otter (*Enhydra lutris*) populations were severely affected by the 1989 *Exxon Valdez* oil spill in western Prince William Sound, AK, and had not fully recovered by 2000. Here we present results of population surveys and incorporate findings from related studies to identify current population status and factors affecting recovery. Between 1993 and 2000, the number of sea otters in the spill-area of Prince William Sound increased by about 600 to nearly 2700. However, at Knight Island, where oil exposure and sea otter mortality in 1989 approached 0.90, no increase has been observed. Sea otter reproduction was not impaired and the age and sex structure of animals captured are consistent with both intrinsic reproduction and immigration contributing to recovery. However, low resighting rates of marked animals at Knight Island compared to an unoiled reference area, and a high proportion of young animals in beach cast carcasses through 1998, suggest that the lack of recovery was caused by relatively

poor survival or emigration of potential recruits. Significantly higher levels of cytochrome P4501A (CYP1A), a biomarker of hydrocarbons, were found in sea otters at Knight Island in 1996-98 compared to unoiled Montague Island, implicating oil effects in the lack of recovery at Knight Island. Delayed recovery does not appear to be directly related to food limitation. Although food availability was relatively low at both oiled and unoiled areas, we detected significant increases in sea otter abundance only at Montague Island, a finding inconsistent with food as a principal limiting factor. Persistent oil in habitats and prey provides a source of continued oil exposure and, combined with relatively low prey densities, suggests a potential interaction between oil and food. However, sea otters foraged more successfully at Knight Island and young females were in better condition than those at Montague Island. We conclude that progress toward recovery of sea otters in Prince William Sound is evident, but that in areas where initial oil effects were greatest, recovery may be constrained by residual spill effects, resulting in elevated mortality and emigration. It is evident that internal reproduction and immigration of juveniles has been the primary means of population recovery, as opposed to broad scale redistribution of adults from outside affected areas. The result is a recovery period protracted by long-term spill effects on survival and emigration and intrinsic limits to population growth.

Chapter 3 Sea Otter Part B. Food Limitation and the Recovery of Sea Otters Following the *Exxon Valdez* Oil Spill: We examined the potential role of food limitation in constraining recovery of sea otters in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. The spill resulted in the removal of a large number of sea otters in 1989, and as of 1998, the portion of the population in the heavily oiled northern Knight Island region had not fully recovered. Between 1996 and 1998, prey consumption rate was higher and the condition of sea otters was better at northern Knight Island than in an unoiled area of the sound (Montague Island). Estimates of prey energy available per unit mass of sea otter were about 4 times higher at Knight than Montague Island, albeit not significantly different between the two areas. Over this same period, the number of sea otters remained constant at northern Knight Island but increased at Montague Island. These data suggest that food was at least as abundant at Knight than at Montague Island, and that recovery of sea otters via intrinsic population growth was limited by factors other than food. However, the availability of food, the prey consumption rate, and the condition of sea otters were all much lower at both Knight and Montague Islands than in areas newly occupied by sea otters where the population growth rate was near the theoretical maximum. It is possible that the relative short supply of food (compared to areas where sea otter population growth rate was high) may have inhibited immigration or interacted with other factors (e.g., oil-induced mortality or predation) to restrict sea otter population growth. Nonetheless, these data suggest that impacts of anthropogenic disturbances on large, often food-limited vertebrate predators can persist in spite of the availability of food resources that are sufficient for intrinsic population growth.

Chapter 3 Sea Otter Part C. Trophic Linkages among Sea Otters and Bivalve Prey in Prince William Sound, Alaska, in the Aftermath of the *Exxon Valdez* Oil Spill: Implications for Community Models in Sedimentary Habitats: We exploited the *Exxon Valdez* oil spill in Prince William Sound (PWS), Alaska, to evaluate effects of reduced sea otter densities on prey

populations in sedimentary habitats. We considered the need for and characteristics of new models for trophic effects of sea otters on coastal marine benthic communities. We viewed evidence for nonlinear or uncertain patterns of prey response to varying sea otter density as particularly significant for new model structure.

We specifically examined responses of densities and size distributions of populations of mussels and clams (several taxonomic and habitat categories), all important sea otter prey in PWS, to reduction in sea otter density caused by the oil spill. We utilized two primary criteria for determining the consistency of prey demographic responses to reduced sea otter densities as predicted by null hypotheses consistent with existing published models. First, prey populations subject to reduced influence by sea otters should be denser and contain proportionately more large individuals than prey populations strongly influenced by sea otter predation. Second, response times of prey demography to reduced otter densities should be similar to response times of prey to increased otter densities, the latter as indicated in existing published models.

Results were disparate with regard to expectation for the six categories of prey evaluated. With few exceptions, density data indicated nonconformance with demographic expectations. In contrast, size data for prey indicated conformance with expectation in about half the categories evaluated. We suggest that lingering effects of the oil spill, nonlinear relationships of sea otters and prey that involve thresholds in otter density, uncertainties in prey recruitment patterns, spatial differences in natural disturbance rate, and differences between areas in effects of competing predators are the main factors possibly accounting for patterns in our data. Recruitment and disturbance effects in particular may include significant stochastic components, especially in a temporal context. We suggest that recovered sea otter populations and their prey do not necessarily exist in long-term stable equilibria, and that development of new models incorporating both trophic thresholds and trophic stochasticity will be important in understanding community-level responses to variable sea otter numbers.

Chapter 4 Harlequin Duck: Following the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, we studied the status of recovery of harlequin duck (*Histrionicus histrionicus*) populations during 1995-1998. We evaluated potential constraints to full recovery, including (1) exposure to residual oil, (2) food limitation, and (3) intrinsic demographic limitations on population growth rates. In this paper, we synthesize the findings from our work and incorporate information from other harlequin duck research and monitoring programs to provide a comprehensive evaluation of the response of this species to the *Exxon Valdez* spill. We conclude that harlequin duck populations had not fully recovered by 1998. Furthermore, adverse effects continued as many as 9 years after the oil spill, in contrast to the conventional paradigm that oil spill effects on bird populations are short-lived. These conclusions are based on the findings that (1) elevated cytochrome P450 induction on oiled areas indicated continued exposure to oil in 1998, (2) adult female winter survival was lower on oiled than unoiled areas during 1995-1998, (3) fall population surveys by the Alaska Department of Fish and Game indicated numerical declines in oiled areas during 1995-1997, and (4) densities on oiled areas in 1996 and 1997 were lower than expected using models that accounted for effects of habitat attributes. Based on hypothesized links between oil contamination and demography, we suggest that harlequin duck population recovery was constrained primarily by continued oil exposure. Full population recovery also will be delayed by the time necessary for intrinsic population growth to allow

return to pre-spill numbers following cessation of residual oil spill effects. Although not all wildlife species were affected by the *Exxon Valdez* oil spill, and some others may have recovered quickly from any effects, harlequin duck life history characteristics and benthic, near-shore feeding habits make them susceptible to both initial and long-term oil spill effects.

Chapter 5 River Otter: Integration of individual-based and population-level studies is essential to understanding effects of pollution on populations and ecosystems. Here we provide an example of such integration from our exploration of effects of the *Exxon Valdez* oil spill (*EVOS*) on river otters (*Lontra canadensis*) inhabiting the terrestrial-marine interface in Prince William Sound, Alaska, USA. Our research was divided into 2 phases: an early phase (1989-92) immediately following the oil spill; and a late phase (1996-99), which focused on potential chronic effects of oil contamination in the Sound. We used a variety of measurements that considered the physiological status and health of individual river otters, as well as aspects of their ecology, behavior, and demography. We then conducted meta-analysis to explore interactions between individual-based and population-level data in demonstrating injury and subsequent recovery of otters from ill effects of *EVOS*. During both phases of our studies, we first conducted intensive research at 2 study sites (oiled and "nonoiled"), and then expanded our investigations throughout similar areas of Prince William Sound. Nonetheless, our data are best interpreted as differences between heavily oiled areas and lightly oiled sites because later information indicated that our reference sites were lightly oiled. In the later phase, we were part of a broader ecosystem-based project (Nearshore Vertebrate Predators) designed to assess the long term effects of *EVOS* on a suite of key organisms, and to determine whether those species had recovered from that catastrophic accident.

We used radiotelemetry to locate carcasses of animals that died from natural causes, and documented that searching beaches immediately following the spill was not a reliable method for locating dead river otters. Our early research (1989-92) demonstrated that river otters living in oiled areas had lower body mass ($P < 0.04$) and elevated biomarkers ($P < 0.05$) in their blood (e.g., haptoglobin [Hp], interleukin-6 immunoreactive [IL-6 *ir*], aspartate aminotransferase [AST]) than otters inhabiting "nonoiled" areas. Likewise, otters from oiled areas had higher levels of fecal porphyrins ($P < 0.001$), ate a less-diverse diet ($P <0.001$), had larger home ranges ($P < 0.05$), and selected habitats differently ($P < 0.01$) than otters living in areas that were not heavily oiled. A mark-recapture analysis based on radiotracers in otter feces during 1990 indicated no difference ($P > 0.10$) between density of otters in Herring Bay (oiled) or Esther Passage ("nonoiled"), but no prespill data were available. Likewise, by 1992, biomarkers (Hp, IL-6 *ir*, AST) did not differ ($P > 0.05$) between oiled and "nonoiled" areas.

During the later phase of research, hydrocarbons on the pelage of river otters and the elevation of endothelial *P*450-1A, a biomarker sensitive to hydrocarbon exposure, indicated that river otters were exposed to oil still present in Prince William Sound. Nonetheless, body mass of otters continued to increase on oiled areas over time ($P < 0.05$), and eventually did not differ from otters living in "nonoiled" sites ($P > 0.05$). All blood biomarkers (Hp, IL-6 *ir*, AST) were markedly reduced from the early phase of our research, and no longer differed ($P > 0.10$) between oiled and "nonoiled" sites. We used Principal Component Analysis (PCA) to determine that few differences existed in an array of blood characteristics for otters inhabiting oiled and "nonoiled" sites, and those differences that did exist likely were related to diet. Corpoporphyrin

III, a key biomarker in heme synthesis, was reduced ($P = 0.008$) from post-spill collections made in 1990 in the oiled area, and no longer differed ($P > 0.05$) between oiled and "nonoiled" areas in 1996. We used stable isotope analysis to investigate differences in diet of river otters inhabiting oiled and "nonoiled" areas in 1996-97. When we controlled for otters inhabiting extensive freshwater habitats (which did not occur in our early studies), no differences in diet or the trophic level of otters were identified ($P > 0.20$) for otters living in oiled versus "nonoiled" sites. Similarly, density of marine fishes ($\geq$8 cm in total length) on underwater transects did not differ ($P = 0.97$) between oiled and "nonoiled" areas, although an area by year interaction occurred ($P = 0.01$). Habitat selection by otters also was altered from the early phase; river otters on both study areas selected vegetated slopes that were not steep, and selected sites with more understory (brush) and greater exposure; selection for those characteristics was more pronounced in the oiled area. Otters on both sites avoided (use < availability) gravel and small rocks. Although selected variables differed between oiled and "nonoiled" sites ($P < 0.001$), the direction of selection did not differ between areas. Moreover, tidal slope did not enter any of the models, in contrast to our early studies, indicating that differences in selection were not related to avoidance of oiled shores. Home-range size declined ($P < 0.05$) for otters living in oiled areas, and no longer differed ($P > 0.7$) from animals inhabiting "nonoiled" sites. We enumerated populations from oiled and "nonoiled" areas using a combination of live-captured individuals and DNA fingerprinting using microsatellite from otter feces at latrines. We also performed a conventional reconstruction based on age structure to calculate population size in 1997. Those methods indicated that most animals in the population were recruited following the oil spill and both methods characterized slowly ($\lambda = 1.03$-1.06) growing or stable population in the oiled area. Age structure of river otters in the Sound differed neither between oiled and "nonoiled" areas ($P > 0.36$), nor from a harvested population of river otters in Maine ($P > 0.49$). Finally, survivorship of river otters did not differ ($P > 0.2$) between oiled and "nonoiled" areas of Prince William Sound and was high compared with data on other otter populations in North America. Our data indicate that although river otters continued to be exposed to low levels of crude oil, effects of that exposure were no longer sufficient to cause obvious injury. We cautiously conclude that river otters have recovered from the more pernicious effects of *EVOS*.

Based on our experiences in this research, we provide theoretical considerations for use of biomarkers in wildlife studies and describe statistical approaches, including principal component analysis blood variables, which may assist researchers with interpreting complicated results of multiple variables and data sets. Likewise, we describe how dose-response curves should be used in understanding population-level responses to pollutants. We hope that this monograph will provide valuable insights for other wildlife biologists on the process of integration of toxicological data with that of ecological data useful for studying effects of pollution on wildlife populations and their habitats.

Chapter 6 Pigeon Guillemot: We conducted a study to determine mechanisms constraining population recovery of Pigeon Guillemots following the 1989 T/V *Exxon Valdez* oil spill. We asked whether recovery was limited by continuing exposure to residual oil, limitations imposed by prey availability, or other causes. Our approach was to compare demographic, physiological, and behavioral parameters between an oiled site pre- and post-spill, and between the oiled site and an unoiled site post-spill. Adult mass, body condition, and nestling survival were

significantly lower at the oiled site post-spill compared to pre-spill. After the spill guillemots increased in number at the unoiled site and chicks fledged at significantly heavier weights than at the oiled site, where populations remained depressed. Elevated CYP1A, LDH, and AST enzyme activities detected in adult guillemots a decade after the spill at the oiled site suggest that continued exposure to residual oil may have limited population recovery, although reduced availability of sand lance, a preferred forage fish, may have also played a role. Previous studies conducted at the oiled site demonstrated that guillemot chick growth and reproductive success were positively related to the percentage of high-lipid forage fish, such as sand lance, in the chick diet. Aspects of sand lance life history and the pattern of *Exxon Valdez* oil deposition strongly suggest that sand lance were impacted by the spill, although we lack direct evidence of this, and reductions in this species' abundance may have also resulted from natural causes. Our study suggests that the recovery of a top-level generalist predator may be constrained by both direct effects (continued exposure to residual oil) and indirect effects (reduced availability of a key prey species) following a large-scale perturbation. Furthermore, it demonstrates that recovery following oil spills may take considerably longer for certain species than the few years that have been proposed as typical for marine birds.

Introduction to Final Report

Introduction to Final Report: Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

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Resource managers often face great uncertainty when required to respond to, assess, and develop strategies to mitigate environmental catastrophes. Federal, state, and local resource agency interests often differ in focus and responsibilities. In addition, agency resource interests are strongly influenced by perspectives of the greater public. What results is a complex response matrix that reflects society's desire to understand impacts from the individual-level for some species (e.g., charismatic megafauna) to broader population-level (e.g., for commercial or agency trust species) or community-level impacts for others. Developing confidence in estimates of impacts at these different levels has proven difficult.

The need for scientific confidence is heightened when the catastrophe is human-made and when legal or societal controversies ensue. The 1989 *Exxon Valdez* oil spill (EVOS) and resulting efforts to assess impacts and develop restoration strategies represents such an event. It is not disputed that the T/V *Exxon Valdez* grounded on Bligh Reef, Prince William Sound, Alaska, on March 24, 1989. Nor is it disputed that some 42 million L of Alaskan crude oil spilled into the surrounding coastal waters of the Sound. However, the full scope of natural resource consequences of this event has been less clear. The early history and scientific assessments of the impacts of the oil spill on animals and society are documented in an extensive suite of books, symposia, and scientific journal papers. However, in 1994, some 5 years after the oil spill, populations of many species either still appeared unrecovered or the status of their recovery (unrecovered, recovering, or recovered) remained scientifically uncertain.

This report represents a collective presentation of a collaborative research program conducted 1995–99 entitled "Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators following the 1989 *Exxon Valdez* Oil Spill," hereafter referred to as the NVP Study (this report). Four top vertebrate predators, all listed as injured by the *Exxon Valdez* Oil Spill Trustee Council, were studied under a common research design. Each of these species depends on the nearshore coastal environment of Prince William Sound for all or critical parts of its life cycle. However, each species also offers scientists a different ecological window or perspective that we propose can be used to develop a suite of independent models upon which to build a weight of evidence on recovery status and any factors that may be affecting recovery. We propose that such a weight of evidence is sufficient to mitigate data uncertainties that have limited confident assessments in the past. These four species are (1) sea otter (*Enhydra lutris*), an invertebrate-feeding mammal present in the Sound year-round and dependent on intertidal to

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deep (< 200 m) subtidal areas; (2) harlequin duck (*Histrionicus histrionicus*), an invertebrate-feeding bird that overwinters in the Sound and depends on intertidal and shallow subtidal areas; (3) river otter (*Lontra canadensis*), primarily a piscivorous mammal that inhabits intertidal and coastal terrestrial zones and is present in the area year-round; and (4) pigeon guillemot (*Cepphus columba*), a piscivorous bird that nests and reproduces in colonies located on various islands in the Sound. The papers in this report address a variety of lessons learned from each of these species, as well as new methodologies and technical insights obtained from the NVP Study.

Holland-Bartels (Chapter 1) discusses the experimental premise for the NVP Study and lays the groundwork for the individual species assessments presented later in the report. A discussion outlines how the multispecies approach of NVP strengthens confidence in status and recovery assessments despite many data limitations. An integration of the various species models is presented in a weight of evidence assessment to bring greater confidence to evaluations of overall system recovery and potential restoration approaches.

Ballachey et al. (Chapter 2) outline the NVP efforts to evaluate continued oil exposure in our four test species through measurement of induction of cytochrome P450 1A, a protein involved in metabolism of aromatic hydrocarbons. Health of individuals was also assessed using hematologies, serum chemistries, and body condition.

Three integrated studies examine aspects of the status and recovery of the sea otter, one of two invertebrate feeders in the NVP Study. Bodkin et al. (Chapter 3 Part A) present a synthesis and overview of the status of recovery for this species by combining pre-spill abundance data with recovery models. Their work is followed by two papers that examine sea otter recovery status through information gleaned from studies of this species invertebrate prey. Briefly put, the sea otter-prey paradigm developed in the literature over the last decade suggests that in the presence of a sea otter population at equilibrium with its food supply, the prey populations should be less dense, contain fewer large individuals, and have more individuals in cryptic microhabitats than one would find in locations lacking sea otters. Dean et al. (Chapter 3 Part B) build on this theoretical model to determine the degree to which food limitations may be acting to restrict sea otter population growth in the study areas. VanBlaricom et al. (Chapter 3 Part C) take a closer look at this theoretical relation by focusing on the response of four critical prey taxa to the significant local reduction in sea otter density following the spill.

Esler et al. (Chapter 4) synthesize information regarding the status of recovery of populations of harlequin ducks following EVOS and present new data on health, food limitation, and demography. Population status is evaluated based on a synthesis of a variety of federal and state agency bird surveys. Adult female overwinter survival rates, obtained from extensive NVP telemetry efforts, are incorporated into population models to assess potential recovery rates. Blood chemistries and cytochrome P450 data are compared between oiled and nonoiled sites to examine health. Finally, the potential of habitat or food to explain population limitations is modeled.

Bowyer et al. (Chapter 5) evaluate their NVP-generated data in light of their significant early post-spill research to determine if the abnormal health and population characteristics viewed early post-spill continue to constrain recovery of river otters. The authors present significant advances in the state-of-knowledge and technical approaches to understanding this species.

Golet et al. (Chapter 6) focus on recruitment factors of pigeon guillemots nesting in oiled and nonoiled areas of Prince William Sound. Because of this species' nesting and chick-feeding characteristics, various critical pieces of data can be obtained with great confidence, including egg production and chick provisioning, growth, and survival information. Thus, data from this species can provide us with excellent insight into the fish-based food web of Prince William Sound and the potential for oil exposure through that trophic pathway.

Finally, some 30 appendices[2] are included to outline additional or more detailed insights gained during the NVP Study from subjects such as hematology (e.g., Appendix BIO-01; Appendix HD-07) to valuable information on prey species (e.g., Appendix SO-05).

Collectively, these papers provide a significant advancement in the state-of-knowledge for four apex vertebrate species, their prey, and the nearshore coastal waters of Alaska.

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[2]Holland-Bartels, L.E., editor. 2002. Mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill, volume 2 - appendices. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska.

Chapter 1. Synthesis

Long-term Consequences of the *Exxon Valdez* Oil Spill: Developing a Weight of Evidence Through an Integrated Multispecies Approach

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ABSTRACT

The 1989 spill of some 42 million L of crude oil into Prince William Sound, Alaska, represents not only the largest tanker spill in United States history, but the world's largest spill in northern waters. Acute effects have been studied extensively. However, efforts to quantify the spill's long-term chronic effects and develop defensible restoration measures have been plagued by varying levels of scientific uncertainty. That such uncertainty exists is expected. The spill occurred in Prince William Sound's highly variable physical setting typified by its complex oceanography and fjord-like geomorphology. Additionally, uncertainty was driven by the scarcity of precise pre-spill population estimates and spotty life-history information for most species. In this paper, I use information from four vertebrate predators whose recovery status was uncertain (sea otter *Enhydra lutris*, harlequin duck *Histrionicus histrionicus*, river otter *Lontra canadensis*, and pigeon guillemot *Cepphus columba)* in a collective weight of evidence approach to better understand the process of recovery. In this approach, each species is examined for the strength of information it brings in health, population, and trophic metrics to support or reject the hypothesis of continuing oil effects in the nearshore system versus the alternatives that food constraints or demographic bottlenecks are limiting the recovery of these focal species. For example, we have excellent sampling and methodological approaches for the invertebrate-feeding sea otter and fish-feeding pigeon guillemot that allows us to confidently address food limitation hypotheses through studies of these species. On the other hand, the invertebrate-feeding harlequin duck and fish-feeding river otter are less useful in this assessment because precise methodologies are lacking or sample variability is high for various prey or feeding metrics. Three of four species provide strong independent models to address the question of demographic constraints. Finally, while there is only an intermediate level of confidence in individual studies of health status and oil biomarkers for any given species, their collective story is compelling. Collectively, scientific confidence is developed in the following picture: Within the nearshore coastal environment, sporadic releases of residual oil are occurring, and benthic species, primarily invertebrates, are being exposed in a temporally and spatially patchy manner sufficient to transport oil up through the food chain. Thus, evidence exists over several lines of investigation for sea otter and harlequin duck to suggest that local-scale populations continue to be constrained by increased levels of mortality coincident with continued exposure to residual oil

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rather than by food availability or natural demographic processes. Conversely, there is little evidence to suggest that direct oil-related effects are being evidenced up through the fish trophic pathway. Populations of the piscivorous river otter appear to be recovering. The continued lack of recovery in the piscivorous pigeon guillemot may be attributed to food limitations (both natural and indirectly related to the spill) and/or slow demographic response to initial acute mortalities. In summary, individual lines of investigation often contained uncertainty, but the collective weight of evidence from this project indicates lack of full recovery of the nearshore ecosystem from the *Exxon Valdez* oil spill nearly a decade following the event. Integrated, multispecies approaches can allow sufficient weight of evidence to develop despite inherent system variability or data limitations. Thus, a better societal understanding of such pollution events can evolve and appropriate restoration efforts developed.

Key words: Alaska, *Cepphus columba*, cytochrome P450 (CYP1A), demography, ecosystem, *Enhydra lutris*, *Exxon Valdez* oil spill, harlequin ducks, health, *Histrionicus histrionicus*, intertidal, *Lontra canadensis*, nearshore, pigeon guillemots, river otters, sea otters, subtidal, trophic.

INTRODUCTION

The 1989 spill of some 42 million L of crude oil into Prince William Sound, Alaska, represents the largest tanker spill in United States history as well as the world's largest spill in northern waters. Crude oil that spilled from the grounded T/V *Exxon Valdez* moved with tides and currents, and ultimately spread over some 3,500 km of coastline. This oiling caused acute mortalities across a wide suite of marine-dependent species (Spies et al. 1996) from an estimated 2,650 sea otters (Garrott et al. 1993) to about 250,000 marine birds (Piatt and Ford 1996). However, most efforts to define the spill's long-term chronic effects and to develop defensible restoration measures have been plagued by varying levels of scientific uncertainty. That such uncertainty exists is expected. The spill occurred in Prince William Sound's highly variable physical setting typified by its complex oceanography and fjord-like geomorphology. Pre-spill population estimates were imprecise, life-history information spotty, and quantitative analytical criteria for judging individual or population health often nonexistent. Thus, it has been difficult to reach a consensus on some of the most basic issues critical to informed restoration; for example, what proportion of a given population died, whether recovery to pre-spill levels has occurred, or what the continuing oil-related effects might be at the population level?

Many studies of the physical and biological impacts of the spill can be found in three scientific volumes—Loughlin (1994), Wells et al. (1995), and Rice et al. (1996). Collectively, these >100 papers attest to successful biological investigation in the remote and complex environment of Prince William Sound and adjacent waters. However, they highlight the often limited capacity of research efforts to create certainty in assessments of spill impacts, population status, and ecosystem recovery. In 1994, based on the strengths and weaknesses of these and other early post-spill investigations, the *Exxon Valdez* Oil Spill Restoration Plan (EVOSTC 1994a) encouraged future restoration science activities to take a multidisciplinary ecosystem-based approach. This paper and those following in this volume represent the collaborative results of one such effort entitled "Mechanisms of Impact and Potential Recovery of Nearshore

Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill," hereafter referred to as the NVP Study. Our focus was the recovery of the nearshore ecosystem as assessed through a multispecies top-predator approach. Our design was driven by several factors:

(1) The nearshore ecosystem of Prince William Sound served as a repository for much of the oil spilled by the T/V *Exxon Valdez*. Wolfe et al. (1994) estimate that in the weeks immediately after the spill over 40% of the oil became beached within Prince William Sound and that some 10% persisted either beached or buried in shallow subtidal sediments through the end of their assessment in 1992. As of 1995, microbial analyses indicated that oil was still several order of magnitude more common in sediments of contaminated versus nonoiled areas of the Sound (Braddock et al. 1995), but that a continuous decline in oil concentration was occurring (Short et al. 1996). Even recently, however, some level of oil persistence has been documented (Broderson 1999; Hayes and Michel 1999) on beaches of Prince William Sound, buried in sediments and under cobble-boulder armor habitats. These persistent oil fractions are expected to dissipate slowly (Hayes and Michel 1999);

(2) As of 1994, continued biological exposure was evident for many species (e.g., Duffy et al. 1993; Patten et al. 1998). While buried oil is not subject to normal biodegradation, it can become biologically available and toxic when resuspended during storms and by tidal action (Braddock et al. 1995; Short et al. 1999). Evidence suggests that remaining oil and biological exposure are highly patchy (e.g., Harris et al. 1996). Thus, effects from the *Exxon Valdez* oil spill may be long term and chronic, but also difficult to detect; and

(3) Many of the taxa classified as still injured in 1994 by EVOSTC (1994a) were linked closely to the nearshore environment. Of the nearshore species, some intertidal and subtidal organisms were considered as recovering; clam and river otter *Lontra canadensis* status was unknown; while harlequin ducks *Histrionicus histrionicus*, pigeon guillemots *Cepphus columba*, sea otters *Enhydra lutris*, and some intertidal and subtidal organisms were considered not recovering.

The NVP Study provides a broadly based assessment of the recovery of the nearshore ecosystem based on detailed studies of four top-level predators (and their prey) whose status was unknown or classified as not recovering (EVOSTC 1994a) and for which there was evidence of continued oiling effects at the individual or population-level in 1994—sea otter, harlequin duck, river otter, and pigeon guillemot (Table 1). The central question posed for each of these species was—"Has population recovery occurred?" If recovery of each species was not evident, was recovery constrained by food limitations, continued exposure to *Exxon Valdez* oil, or insufficient time for intrinsic demographic processes to result in discernable recovery? The premise behind the study design was that an integrated, multispecies approach could mitigate many of the biological data limitations responsible for the scientific uncertainty that heretofore constrained effective recovery and restoration assessments. The effects of limited species-specific pre-spill data could be ameliorated. Project goals could be reached without each study objective being thoroughly addressed for each species. Also, the collective information (across species, methods, and hypotheses) could create a sufficient weight of evidence to judge ecosystem recovery more confidently.

In this paper, I discuss the experimental premise for the NVP Study and lay the groundwork for various perspectives gained from the studies of the four test species that are presented in subsequent papers in this volume. Each species is examined for the strength of

information it brings in health, population, and trophic metrics to support or dismiss the hypothesis of continued oiling effects in the nearshore system versus the alternatives that food constraints or demographic bottlenecks are limiting attainment of population recovery goals (Table 1 status/recovery strategy). Finally, the collective weight of evidence is presented to better understand the process of recovery in the nearshore ecosystem.

EXPERIMENTAL DESIGN

During the earliest phase of this study, investigators were tasked with creating a study design that ideally would (1) be sensitive to subtle differences in species' responses to their environment, (2) allow unambiguous inference to oil effects, and (3) not be prohibitively costly. No such design exists because of a number of obstacles. As stated above, pre-spill, baseline data were sparse to nonexistent for some study species and for many parameters of interest. This precluded application of more standard statistical approaches, such as before-after-control-impact designs (Stewart-Oaten et al. 1986). Second, the oil spill was unreplicated resulting in a single oiled area with its own unique attributes affecting parameters of interest even in the absence of oil-spill effects (Paine et al. 1996). Finally, to sample broadly at randomly selected sites within oiled and nonoiled areas at an intensity to achieve reasonable statistical power to detect differences—as would have been necessary to achieve our goal of reducing scientific uncertainty—proved to be cost-prohibitive. Therefore, the resulting NVP Study design represents a series of informed decisions some of which mitigate the above issues while others limit the study's geographic scale and scope of scientific inference.

In overview, the study employed a three-dimensional matrix design in which (1) the four apex predators at (2) selected oiled and nonoiled sites were examined for (3) a suite of parameters descriptive of their health, trophic status, and demographic characteristics to collectively provide insight into study hypotheses. The working approach was to assess the status of recovery of injured populations through estimates of abundance, demographic characteristics, measures of health, and abundance and distribution of prey; while concurrently determining factors that might constrain recovery. These hypothesized constraints were (1) oil toxicity examined through bioindicators of exposure to oil and measures of health, (2) food-based limitations through measurements of such things as prey availability and consumption rates, and (3) demographic processes that in the absence of continued oiling effects or food limitations result in a low rate of population increase assessed generally through simple population models or oiled versus nonoiled population comparisons.

Logic for and limitations in the selection of species and study sites are outlined below.

Species Selection

One key premise of the NVP Study was that top-level predators would be good indicators of full systemic recovery as their populations assimilate and integrate lower-level perturbations and their demographic characteristics tend to result in longer recovery times. Each of these species depends on the nearshore coastal habitats of Prince William Sound for all or critical parts of its life cycle. However, each species also has a unique combination of use patterns that provides investigators with different perspectives into any continued oil effects and what

mechanism might be at play (Fig. 1, Table 2). Despite the unique position occupied by each species within the Prince William Sound ecosystem, some critical characteristics are replicated within the study suite to increase the robustness of the weight of evidence model—taxonomic group (i.e., birds and mammals), potential for oil exposure through trophic pathways (i.e., low or high), and physical contact (i.e., low or high; Table 2). Establishing such dichotomy can be useful. For example, mammals and birds vary significantly in hematological characteristics (Duncan and Prasse 1989) that might result in different expressions of health through bioindicators. The study species represent the two principal trophic pathways in the nearshore ecosystem. One pathway leads through fish to the apex predator, the other through benthic invertebrates. Important to the NVP Study, bivalve mollusks and other subtidal and intertidal prey of harlequin ducks and sea otters appear unable or only marginally able to metabolize oil (e.g., Albers 1995). They bioaccumulate persistent organic pollutants to concentrations much higher than found in their surrounding environment. Thus, they can magnify, store, and function to move remaining hydrocarbons in the benthic environment up through the food chain. On the other hand, fish metabolize and do not bioaccumulate hydrocarbons. Thus, we would expect fish to be a less significant trophic pathway of hydrocarbon exposure. Finally, we selected species based on their susceptibility to oil exposure through physical contact based on preening and haul-out characteristics. Perspectives that were expected *a priori* from assessment of each study species follow:

Sea Otter.—The sea otter, an invertebrate-feeding mammal, is present in the intertidal and subtidal nearshore environments of the Sound year-round. They rely on their pelage rather than fat to maintain body temperature (Costa and Kooyman 1982), which requires a high metabolic rate and, thus, high caloric intake. They exhibit home ranges of few to >40 km of coastline (Lensink 1962; Kenyon 1969; Garshelis and Garshelis 1984; Riedman and Estes 1990) out to 40-m water depths in Prince William Sound (J. L. Bodkin, U.S. Geological Survey, Anchorage, Alaska, USA, unpublished data). Thus, they integrate environmental effects over large areas. Foraging for its preferred prey in Prince William Sound—clams—is done by excavating benthic sediments; this and its haul-out behavior make sea otters highly susceptible to contaminants through contact and food routes. Sea otters provide the highest potential to address the alternate hypothesis of food constraints within the invertebrate-feeder pathway. The species brings food to the surface to consume; prey composition, size structure, success rate, and other trophic metrics can be directly observed. Thus, high-quality prey data can be obtained. Population information for various critical prey species can be estimated, although some with high variability (Chapter 3 Part B). Additionally, the role of sea otters as a keystone species that exerts strong top-down control on community structure is well documented; prey density and size frequency are reduced as sea otter populations near carrying capacity (Estes and Palmisano 1974; Estes et al. 1978; Kvitek et al. 1992). While direct methods to assess many demographic metrics are well established for this species (e.g., Bodkin et al. 1997; Appendix SO-01), the stereotypical effect that sea otters have on their prey could be used as an ancillary indirect indicator of population status. Thus, this species can provide significant insight into weight of evidence discussions requiring demographic information.

Harlequin Duck.—The other invertebrate-feeder, the harlequin duck, molts and overwinters in Prince William Sound arriving in late summer from widely dispersed breeding areas. Like other seaducks, they are long-lived with relatively low annual reproductive output (Goudie et al. 1994). Within the Sound, wintering site fidelity is high (Chapter 4). Harlequin ducks are inextricably linked to intertidal and shallow subtidal zones and feed on an array of small benthic invertebrates (Dzinbal and Jarvis 1982; Goudie and Ankney 1986; Goudie and Ryan 1991; Patten et al. 1998). In general, seaducks rely on stable and predictable habitats to accommodate harsh conditions encountered in northern wintering areas; this may be especially true for harlequin ducks because of their small body size (Goudie and Ankney 1986). Life-history traits of harlequin ducks, coupled with the concentration of oiling in nearshore habitats, suggest that these birds will be particularly sensitive indicators of ecosystem recovery. In addition to the species' high potential for a food-based route of oil exposure as an invertebrate feeder, a contact route is also probable. Harlequin ducks reside in nearshore waters during winter, a habitat and season when storms could resuspend buried oil. Unlike the sea otter, this species provides less insight into the alternative hypothesis of food limitation since consumed prey type and size cannot be observed without sacrificing animals. Additionally, prey are logistically difficult to sample resulting in abundance estimates of high variability (Appendix HD-01). However, the species does contribute significantly to demographic discussions. Pre-spill survey data exist. The high phylopatry of individuals facilitates confident repeated mark-recapture assessments. Finally, telemetry-based methods are available to assess survival (Appendix HD-02).

River Otter.—Unlike the previous two species, the river otter is more terrestrially based, but concentrates its feeding activities in intertidal and subtidal zones of Prince William Sound (Larsen 1984; Woolington 1984; Bowyer et al. 1994). It, too, has extremely large home ranges (20–40 km of shoreline; Bowyer et al. 1995) and integrates effects of pollution over wide areas. They are long-lived ($\geq$12 years; Docktor et al. 1987) and consume a diet dominated by intertidal and subtidal fishes while also consuming a wide variety of marine invertebrates (Larsen 1984; Stenson et al. 1984; Bowyer et al. 1994). Unlike the sea otter, however, these animals are secretive and their feeding is not easily observed. Thus, both demographic and trophic assessments are difficult. Their prey base (fish) can be quantified but sample variability is high (Chapter 5). Like sea otters, river otters are susceptible to exposure to oil through contact because of their intertidal foraging behavior, but they have a lower potential for trophic exposure because of their reliance on fish.

Pigeon Guillemot.—Guillemots are one of the most neritic members of the marine bird family Alcidae. In winter, guillemots are widely dispersed among the protected coves and bays of Alaska's coastline. From May to late August, guillemots congregate in small nesting aggregations along the rocky shorelines and islands of Prince William Sound. Guillemots generally start to breed at 3 to 4 years age and produce small clutches (2 eggs). Their annual adult survivor rate (85%; Nelson 1991) is slightly lower than reported for other seabirds (Frederiksen and Petersen 1999). The semi-colonial nesting habits and high nesting fidelity of established breeders (Drent 1965; Ewins 1993) allow long-term comparisons in trends of breeding success between oiled and nonoiled habitats. However, adults are difficult to capture

and come from various wintering areas with unknown contaminant histories. Therefore, much of the NVP Study focused on metrics obtained on chicks produced in the study areas. Chicks remain in their burrows until they reach adult body size. Adults provide their young with demersal or surface-schooling fish. Thus, the species as represented by the chick is the only one of the test species with a low probability of oil exposure through contact or food. Unlike the other species, the combination of being able to observe and quantify chick feeding and monitor chick response (Chapter 6) gives a good picture of the linkage between food and production (Table 2).

Study Site Selection and Inference

General Design.—Various studies conducted shortly after the *Exxon Valdez* oil spill (1989–91) and designed to quantify injury applied random sampling for inference to the whole spill area. Such straight stratified random sampling often resulted in insufficient power to detect important effects at this level of inference as discussed by Sundberg et al. (1996). In some cases, within-site pseudo-replication was used to artificially increase statistical power (Page et al. 1995). This often resulted in an increase in the probability of falsely accepting that no differences existed between oiled and nonoiled sites (increased Type II error). Even application of an *a posteriori* method such as an After, Control-Impacted Paired design (Osenberg and Schmitt 1996) resulted in excessive observational error where patterns could not be generalized (e.g., Stekoll et al. 1996).

The NVP Study, however, is an integrated series of observational efforts designed to address specific issues of cause and effect. As such, the study was constrained to a selected sampling approach rather than random sampling of all potential oiled and nonoiled areas for several reasons. In addition to the sampling limitations seen in early post-spill studies, we knew that persistence of oil through time varies by habitat (Wolfe et al. 1994); species and individual variation exist in biological response to contamination (Mulcahy and Ballachey 1994; Dean et al. 1996; Highsmith et al. 1996; Loughlin et al. 1996; Spies et al. 1996); and that our nearshore vertebrate predators were highly mobile, thus, making definition of subpopulation oiling history difficult under a random sampling design. Finally, we desired that all four focal species be located at each study site to benefit from a collective weight of evidence and to limit intrinsic area differences as well as extrinsic factors (e.g., weather, oceanographic patterns). Thus, our nominal design was based on the dichotomy of a single heavily oiled area contrasted against a single minimally or nonoiled area within western Prince William Sound. These areas were to be sufficiently close to minimize extrinsic environmental differences yet ensure minimal exchange of study animals.

Within-area sampling was designed and stratified as appropriate for specific taxa to allow inference to study area. For example, sea otters were surveyed for abundance and distribution in two strata (high and low density) distinguished by distance from shore and depth, with a strip transect and intensive search unit approach (Bodkin and Udevitz 1999). Intertidal mussels, an important sea otter prey, were sampled at randomly selected quadrats along transects set vertically across the intertidal zone and post-stratified by two shoreline types (rocky and mixed substrate; Appendix SO-05). Other components were similarly sampled with appropriately customized designs.

These informed design choices bring with them an understanding that statistical inference of population measures are to area only (Hurlbert 1984). Nonetheless, the primary selected-site sampling scheme coupled with appropriate species-specific sampling and stratification protocols results in the level of confidence in both prey and predator estimates needed to meet the cause and effect assessment requirements of the study. Thus, our statistical inferences are more relational than place-based in nature. Clearly, final assessment of oil-spill effects results from attempts to account for intrinsic area differences (Wiens and Parker 1995) and from professional judgment based on consideration of the weight of evidence generated from data for competing hypotheses.

Specific Study Sites.—Not all criteria of the nominal design could be realized in western Prince William Sound for the selected test species for a variety of reasons. Selection of a single oiled and single nonoiled site was not possible; thus, generalized oiled and nonoiled areas were selected and studies co-located when possible (Fig. 2). The focal oiled area, the Naked Island-northern Knight Island complex, was among the most extensively studied oiled sites within the Sound (see Spies et al. 1996 for examples). Shoreline reaches here were generally classified as heavy-moderate oil in the Oil Spill Impact (OSI) database maintained by the Alaska Department of Environmental Conservation (Figs. 1–2 in Sundberg et al. 1996). All four species existed in this area, generally in sufficient numbers to effectively test NVP Study hypotheses. Here, sea otters and their prey were studied mainly along two, noncontiguous bays, Herring Bay and Bay of Isles, where many dead sea otters had been removed post-spill, and surveys suggested a remaining remnant population (J. L. Bodkin, U.S. Geological Survey, Anchorage, Alaska, USA, unpublished data). River otters were studied along the shoreline in Herring Bay, an area with reasonable river otter habitat and where historical data were available (Bowyer et al. 1994; Testa et al. 1994). Harlequin ducks also were studied here, but the oiled study zone was expanded out concentrically from the island complex to allow for sufficient sample size of birds and included the western shores of Green Island to the east and oiled areas along the islands and mainland to the west (Appendix HD-02). Pigeon guillemots were studied at their Naked Island nesting area.

While our nominal design also called for a single contrasting nonoiled study area, no such site existed in the general investigation area where all four species were resident. Therefore, the nonoiled assessment was conducted at two sites, split by trophic group to facilitate cost-effectiveness in prey estimates, with sea otters and harlequin ducks studied along the western shore of Montague Island (OSI database sites classified as mainly no oil with some very light oil sites) and river otters and pigeon guillemots around Jackpot Island (OSI database: no oil). Our study was faced with the same limitations experienced by many attempting to select sites to assess the biological impacts of the *Exxon Valdez* oil spill. While both of our reference study sites were outside the direct path of the oil, over time some patchy contamination did occur (e.g., see Clair et al. 1986). In addition, some heavily oiled sites could have been at the movement extremes of the study populations. Therefore, our study is based on comparisons between heavily oiled and no to lightly oiled sites and yields a conservative assessment of study objectives.

RESULTS

What do we know now at the end of the NVP Study relative to our central question of nearshore recovery as viewed through four sentinel top predators of that system? Not surprisingly, the NVP Study results paint a somewhat different picture for each species relative to this question. Such differences in outcome reflect each species' unique ecological position in the Sound (Table 2), the methods applied to assess demography, health, and trophic questions (Table 3, Figs. 3–4), the level of statistical confidence in measurement of various metrics for each species, and the strength of the relation between the metric and a given hypothesis.

Species-specific Assessments

Sea Otter.—The 1989 spill-related mortality of sea otters in Herring Bay, an area within the NVP Study's oiled site, was high (0.88; Bodkin and Udevitz 1994). Some 10 years post-spill, no evidence of population recovery exists (annual growth rate [AGR] = 0.00; 1993–98), while population estimates for both the NVP Study's nonoiled site (AGR = 0.16; 1995–98) and the greater western Prince William Sound (AGR = 0.16; 1996–98) have increased noticeably (Chapter 3 Part A). Because of extensive post-spill survey development efforts (Bodkin and Udevitz 1999), a high level of confidence is associated with these estimates. Thus, the recovery metric—population abundance returning to pre-spill levels (Table 1)—has not been attained for the population residing in the heavily oiled area assessed by the NVP Study (Table 4).

The metrics for the three potential limiting mechanisms—food, oil, demography—in general, could be confidently assessed for the sea otter. However, while we began with a reasonable scientific basis for interpretation of cause and effect, we find our results equivocal in some instances. Dean et al. (Chapter 3 Part B) outline many lines of evidence (e.g., prey abundance, consumption, sea otter condition) that suggest trophic limitations are unlikely to be constraining sea otter recovery. Much of the prey size frequency evidence presented in VanBlaricom et al. (Chapter 3 Part C) also support that sea otters in the heavily oiled Knight Island complex are below carrying capacity and, thus, not food limited. However, the authors present some evidence that cannot be fully reconciled with this conclusion. Various trophic metrics at both study sites were less than in other regions where sea otter populations are actively expanding and not food limited. The comparatively low level of prey at both sites may have inhibited immigration or interacted with other factors (Chapter 3 Part B). Also, not all metrics for all prey species pointed to the same outcome relative to the food structuring paradigm proposed by Estes and Palmisano (1974) and others that sea otters at capacity cause a reduction in density and a shift in size frequency toward smaller prey individuals (Chapter 3 Part C). Nonetheless, the weight of evidence suggests the Knight Island complex could support a substantially higher sea otter population as it did pre-spill. Many demographic characteristics could be confidently quantified, such as abundance, reproduction, and population sex and age composition (Chapter 3 Part A). Most of these values are similar between study areas, yet population growth has not occurred at the oiled Knight Island complex. For example, the higher proportion of young animals of both sexes at Knight Island, compared to Montague Island, is consistent with recovery either through reproductive or immigration recruitment to the area (Chapter 3 Part A). However, when this information is coupled with the high dependent to

independent ratios and lack of population growth in the Knight Island population, it is suggestive of a population constrained by either mortality or emigration. Monson et al. (Appendix BIO-03) provide indirect evidence for why population growth has not resulted despite availability of adequate food and reproduction. They found that sea otters had decreased survival rates in the years following the spill, spill effects on annual survival increased rather than dissipated for adult animals, and that even sea otters born after the 1989 spill showed some continuing negative effects though 1998. What mechanism might be behind this increased mortality and concomitant stagnation in population growth? Sea otters averaged an expression of the hydrocarbon biomarker cytochrome P450 (CYP1A) some >18 times higher in the oiled versus nonoiled population (method: reverse transcriptase polymerase chain reaction [RT-PCR], Chapter 2). The individual or population-level consequences of these levels are unknown. However, when this information is coupled with the observed statistically significant elevation in a key biomarker of liver damage (gamma glutamyl transferase [GGT], Appendix BIO-01), it suggests continued oil exposure as a factor in the lack of recovery (Table 4). As pointed out by Bodkin et al. (Chapter 3 Part A), a relatively small loss of animals annually at Knight Island (e.g., seven) could easily offset the estimated annual growth increment seen in the greater Prince William Sound. Furthermore, other sources of mortality, including harvest and predation, could have a disproportionate impact on this depressed population in contrast to the Montague Island population.

Harlequin Duck.—In 1989, some 212 harlequin duck carcasses were recovered throughout the spill zone, with a resulting estimate of 1,298 mortalities (J. Piatt, personal communication). The winter Sound-wide population estimate is about 14,000 birds (Lance et al. 1999). Recovery status for this species has been equivocal since early post-spill, in part, because most assessments have been based on surveys or studies of seabirds in general; efforts were not designed to be sensitive to critical life-history metrics of harlequin ducks (e.g., Wiens et al. 1996; Murphy et al. 1997; Lance et al. 1999). However, various comparisons of oiled versus nonoiled areas in Prince William Sound are consistent with a hypothesis of continued oil injury (Chapter 4). It also appears that assessment of recovery status of harlequin ducks is particularly sensitive to geographic scale because of this species' high level of molt and winter site fidelity (Cooke et al. 2000), more so than for the other NVP Study focal species. During the NVP Study, ~90% of birds returned to the same shoreline segment of their original capture, while an additional ~6% were recaptured in an adjacent shoreline segment. Adult females had highest site fidelity (same segment, 96%; adjacent, 4%). Such high-site fidelity suggests that any limitations to population recovery are manifested primarily by the habitat quality, oiling/cleanup histories, and persistence of oil expressed on a small (200-m or so) geographic scale. Thus, over time different groups of harlequin ducks likely develop unique exposure histories and recovery probabilities making the spatial scale of assessment critical. Esler et al. (Appendix HD-01) effectively document an oiling history effect on duck distribution after accounting for intrinsic habitat differences between NVP Study areas. Thus, the recovery metric (Table 1; no differences between oiled and nonoiled areas) has not been attained for the population residing in the heavily oiled area assessed by the NVP Study (Table 4).

Assessments of the metrics for the three potential limiting mechanisms—food, oil, demography—for harlequin ducks were made with varying degrees of confidence (Table 4).

Confidence is lowest for trophic assessments because estimates of abundance of small invertebrate prey were highly variable and direct observation of consumption was not possible. However, sufficient evidence exists to suggest that food availability is unlikely to limit recovery. Food models by Esler et al. (Appendix HD-01) document that variation in food data explained little of the variation in duck densities beyond that attributed to habitat attributes. Also, prey density and abundance per duck were similar between study areas. A high level of confidence could be placed in demographic measures particularly survival estimates. Cumulative female winter survival estimates were about 78% (SE ~3%) in oiled areas and 84% (SE~3%) in nonoiled areas, resulting in estimated annual population declines of about 5% in oiled study segments versus approximately stable populations in nonoiled study segments (Chapter 4). Thus, overwinter survival of females seems to be a key factor in the lack of recovery of this species. Ballachey et al. (Chapter 2) suggest that exposure to oil persists based on their findings of significantly higher CYP1A expression in samples taken from oiled- than nonoiled-site birds (ethoxyresorufin O-deethylase [EROD]: 205 versus 71 pmol/min/mg protein). Although direct evidentiary links between hydrocarbon exposure and survival are speculative, some laboratory efforts (Holmes et al. 1978, 1979) support this hypothesis for wild birds (Chapter 4).

River Otter.—The weight of evidence for the river otter suggests that this species is recovering (Table 4) based on its recovery criteria (Table 1; habitat use, food habitats, and physiological indices returning to pre-spill conditions). However, because the river otter is the most secretive of the four NVP Study focal animals and has the fewest pre-spill data, no single piece of recovery evidence carries high confidence. Instead, the recovery assessment is based on the preponderance of evidence that exists across trophic, health/oil, and demographic assessments (Table 3). For example, Bowyer et al. (Chapter 5) compared 1997 population point estimates at the oiled Herring Bay with early post-spill estimates to calculate an annual growth rate between 1990 (Testa et al. 1994) and 1997 of 1.3–6.4%. While suggestive of population growth, the 1997 point estimate of 46 otters falls within the 95% confidence interval for the 1990 population estimate (32–55 otters; Teska et al. 1994). Another indirect demographic measure used by Duffy et al. (1994a) to suggest injury—abandonment of latrines (1991: oiled area abandonment = ~15%; nonoiled abandonment <4%)—was inconsistent in later surveys where high rates of abandonment (>60%) occurred regardless of the area's oiling history. Both age structure (1997) and survivorship (1997–99) demonstrated no relation with oiling history. While variation and uncertainty are quite high for all the demographic measures, each of these measures—many once reflective of oil impacts—no longer demonstrate a significant relation to oiling history. In 1990, significant differences in habitat selection existed that suggested otters were avoiding oiled beaches (Bowyer et al. 1995). Some habitat selection was seen during the NVP Study, but was not suggestive of this earlier avoidance of oiled beaches. Concomitantly, the once larger home ranges on oiled areas that resulted from such habitat avoidance declined between 1990 and 1997–99. Similarly, trophic parameters are highly variable, but point toward a rejection of food limitation as a constraint to river otter population recovery. The once substantial differences in diets of otters between oiled and nonoiled areas (Bowyer et al. 1994) no longer exist. Also, 1996–97 assessments of prey abundance showed no consistent differences between study areas. Thus, while trophic variations exist, none appear sufficiently consistent to suggest that food limitations or oil-food related constraints continue. Finally, during the early post-spill

period, the strongest direct evidence of health impacts could be found in a wide variety of biomarkers. While continued exposure to hydrocarbons was evidenced through pelage swab (Chapter 5) and CYP1A assay (Chapter 2) data, exposure is less than in earlier post-spill studies (pelage swabs) or variable (CYP1A). No definitive explanation exists for the annual variations observed in CYP1A expression (Fig. 5). However, immunohistochemical staining (IHC) scores were elevated in males of both oiled and control population, perhaps reflective of the unexpected movement of males between study areas (Chapter 5). Females who did not demonstrate movement between study areas had near zero IHC scores for 2 of 3 study years, but elevated scores at both sites in 1997. Nonetheless, exposure levels seem insufficient to challenge health of the area's river otters. Elevations in blood-serum parameters seen immediately after the spill and diminished by 1992 (Duffy et al. 1993, 1994a,b) continued to decline. One such parameter—haptoglobin—remained elevated in 1996 samples from the oiled area (Chapter 5) yet was substantially lower than in previous years. Similarly, the earlier-observed elevation in fecal porphyrins was not seen in 1996. Thus, based on the consistency of these data, Bowyer et al. (Chapter 5) support that this species is recovering.

Pigeon Guillemot.—In 1989, some 1,500 to 3,000 pigeon guillemots died as a result of the *Exxon Valdez* oil spill (Piatt et al. 1990). Because only one survey was conducted during the two decades before the 1989 spill, documentation of the relative impact of this injury based on population or census data is problematic. The population data that exist—a survey in 1972 (about 15,000 birds) and then following the spill (3,500 in mid-1990s)—cover a period when marked changes have occurred in the ocean climate and fish community of the North Pacific, commonly known as the regime shift (Pitt and Anderson 1996; Agler et al. 1999; Pearson et al. 1999). Thus, for the pigeon guillemot, a seasonal piscivore, the question of "Is it food?" or "Is it oil?" is confounded. Nonetheless, recent censuses between oiled and nonoiled areas suggest that colonies have not compensated for the oil-related mortalities over the last 10 years (Hayes and Kuletz 1997; Kuletz et al. 1997), while colonies in nonoiled areas have expanded (Chapter 6). Thus, the recovery metric—stable or increasing populations (Table 1)—has not been attained (Table 4).

The metrics used to assess the three potential limiting mechanisms—food, oil, demography—in general, could be confidently measured during the NVP Study for pigeon guillemot chicks. Because of the potential confounding effects of the regime shift, we focused on how the nonoiled Jackpot Island colony functions in this changed environment. Survey methodology is now well defined and colony censuses can be made with some certainty. These data indicate that population growth is occurring in nonoiled areas of the Sound, some 36% between 1993 and 1998 at Jackpot Island (Chapter 6). Nesting effort was high and nesting sites were not limiting. In addition, while the mean productivity here was lower than compared to either the 1970s or post-spill data for the oiled Naked Island colony, it was sufficient to support significant population growth. Collectively, these data support the conclusion that reproductive parameters are unlikely to be limiting recovery of pigeon guillemots. Nor did any of the wide variety of blood parameters examined in chicks as indicators of liver, kidney, or immune function, the haematopoietic system, or electrolyte balance by Seiser (Appendix PG-01) at Jackpot and Naked Island colonies support physiological injury. Additionally, CYP1A expression in chicks was low between oiled- and nonoiled-site birds (Chapter 2). However, Golet et al. (2000) suggest that oil toxicity constraints to recovery cannot be fully discounted. While

CYP1A expression was extremely low in chicks and not correlated to oiling history or metrics of health, samples of adults (1999) showed low but significant differences in expression between oiled and nonoiled areas (Chapter 2) that correlated to elevations of serum enzymes indicative of liver damage (Golet et al. 2000). Nonetheless, while some level of continued oil exposure may exist, there is strongest evidence of a trophic mechanism driving lack of recovery of this species.

Providing the foundation for this hypothesis, Golet et al. (2000) found that guillemot chicks fed demersal fish had lower growth rates, peak fledge weights, and survival rates than chicks fed surface-schooling fish (e.g., herring and sand lance), fish with higher energy density than demersal fish (Van Pelt et al. 1997). Herring stocks of Prince William Sound crashed in 1993 because of poor nutritional status, disease, and other factors (Pearson et al. 1999). Less is known about the factors leading to decline of sand lance in the diet of birds at Naked Island documented by Hayes and Kuletz (1997). However, oil and the ensuing cleaning of beaches did affect abundance and biomass of many intertidal fish (Barber et al. 1995). Golet et al. (Chapter 6) compared historical and recent prey composition information for both Jackpot and Naked Islands and found strong evidence that compositional differences in diet might be constraining recovery of pigeon guillemots in our oiled study area. Chick diet at the expanding Jackpot Island colony was composed of roughly 40% surface-schooling fish (primarily herring) in 4 of 5 study years. These values are less than 1979–81 data from Naked Island (48%) but are significantly higher than presently seen for that colony (23%; Oakley and Kuletz 1996; Hayes and Kuletz 1997; Golet et al. 2000). Such patterns continue in mean fledge weight; a parameter that has been well documented to affect survival. Some physical differences between the nearshore areas of Jackpot and Naked Islands confound comparison and may be partly responsible for the higher abundance of herring at Jackpot Island (Stokesbury et al. 1997) and, thus, colony productivity. Nonetheless, overall evidence strongly suggests that the inability of the pigeon guillemot colony at Naked Island to compensate for 1989 mortalities is linked to factors regulating food quality.

Multispecies Perspective

Compelling evidence has been amassed to reduce many of the uncertainties in assessments of the mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill. The NVP Study has shown that each species gives us a different perspective on oiling injury and recovery and that such differences can be used to mitigate known weaknesses in individual lines of investigation through a collective weight of evidence approach. The collective evidence supports the hypothesis that patchy, persistent oil in the Sound is still being sufficiently mobilized some 10 years post-spill to constrain recovery within the nearshore ecosystem. Such mobilization was seen across the full spectrum of the nearshore community some 3-5 years after the spill (Table 1, reviewed by Spies et al. 1996). However, mobilization now is seemingly restricted to the invertebrate trophic pathway and primarily reflected at the population level in obligate top consumers in that pathway. The NVP Study species sea otter and harlequin duck represent that component of the nearshore ecosystem. We originally hypothesized that our four study species were at various levels of risk through oil exposure routes from very low (- -) to very high (+ +) as follows: pigeon guillemot (focus chicks)–minimal through contact or food (- -,- -), river otter mainly through contamination of pelage and minimally through food (+ +, -), sea otter through pelage

(but less than river otter) and food (+, + +), and harlequin duck through both preening and food (+ +, + +). In fact, CYP1A data support this ranking (Chapter 2). The river otter is deemed recovering. Our pigeon guillemot chick-based assessment suggests that species' recovery is constrained largely by food quality, although Golet et al. (2000) propose some oil-based impact to adults has potential to exacerbate this food mechanism. Per our prediction, both the sea otter and harlequin duck demonstrate a lack of recovery that seems to be tied to survival issues. This is directly evidenced by disparate survival rates in harlequin ducks and for sea otters by a lack of population growth despite adequate reproduction. In both instances, oiled-site populations exhibit negative or zero growth rates, while nonoiled-site populations are in growth modes. No consistent pattern exists across the many lines of investigation to support that natural demographic or food constraints exist. Data for both species do suggest, however, that exposure to hydrocarbons is occurring over multiple years of assessment (1996–98) as indicated by elevated expression of the biomarker CYP1A with oiling history. Direct evidentiary links between this biomarker and health or survival data are individually weak, but when viewed cross-species weight of evidence strengthens. Relations between CYP1A, some health variables (e.g., GGT: Chapter 2; sodium and glucose: Chapter 4), and relative survival are reflected at the study area level, but correlations among variables often do not hold true at the individual level (e.g., CYP1A and GGT in sea otters). Ballachey et al. (Chapter 2) indicate the lack of CYP1A and GGT correlation in individuals is not unexpected given that the former reflects recent exposure, while the latter reflects a response developed over long term. One notable exception to this lack of direct correlation of measures was in the significant negative relation between CYP1A and body mass in harlequin ducks.

It seems we no longer have populations under acute stress, but rather that components of the invertebrate-based nearshore community are still under chronic, but decreasing levels of stress. This stress is observed not at a regional level where both sea otters and harlequin ducks are stable or expanding (Lance et al. 1999; Chapter 3 Part A), but in those areas of the Sound most heavily oiled by the 1989 *Exxon Valdez* oil spill. Even in these most heavily oiled areas, effects do not have to be expressed in a large part of the population to cause the observed stagnant or declining patterns of abundance. For example, overwinter mortality in female harlequin ducks of 22% in the oiled areas versus some 16% in the base population translates to an annual 5% population loss—not recovery—in the oiled-site group as modeled by Esler et al. (Chapter 4). Unlike the harlequin duck study, we could not obtain direct mortality for sea otters because of the high cost of such studies. Nonetheless, similar small but consequential expressions of stress were observed; GGT elevation—indicative of liver damage—was high in about 15% of animals in oiled areas and similarly about 15% of animals had high expression of CYP1A (Chapter 2). In a depressed population, small changes in stress and survival can easily regulate population growth and, thus, recovery. Therefore, weight of evidence supports the hypothesis that sporadic release of hydrocarbons from subtidal sediments may still occur; intertidal and subtidal prey are exposed in a temporally and spatially patchy manner sufficient to transport oil up through the food chain.

DISCUSSION

As suggested by Carpenter et al. (1998), testing of alternative hypotheses in ecosystem experiments may be more informative than simply testing the null hypothesis. To accomplish this, however, multiple experimental perspectives or units are most valuable. While these authors were speaking of intentional manipulations in multiple systems, this philosophy is valuable in a broader context. The accidental manipulation in our study—the *Exxon Valdez* oil spill—does not lend itself to replication (Wiens and Parker 1995). Nonetheless, we were able to test alternatives to the direct oil hypothesis through multiple perspectives—the four sentinel nearshore species of our study—to similarly great benefit. The NVP Study applied far more of its effort in examining alternative hypotheses of demographic or food constraints (natural or indirectly related to oil) than towards the null oil hypothesis. This is a departure from the philosophy of much of the early post-spill science that often focused on documenting damage from direct exposure to oil (but see Peterson 2001). But given the level of system variability and uncertainty in cause and effect relations, our approach seems appropriate and productive.

This assessment of recovery of the nearshore ecosystem following the *Exxon Valdez* oil spill was based on the premise that lack of recovery of any of the study species would indicate lack of full system recovery. While we recognize that population recovery can be defined in different ways (Paine et al. 1996), we argue that evidence for continuing effects of the oil spill (e.g., lower survival of harlequin ducks corresponding to oil exposure) is clear justification for concluding lack of full recovery. We submit that evidence of population numbers not returning to pre-spill estimates (e.g., sea otters at Knight Island), despite increasing abundance in nonoiled areas, also suggests a lack of recovery. We disagree that occurrence of a full complement of species in the absence of other data is evidence of community or systemwide recovery (Wiens et al. 1996) as individuals of a species will be present even as populations are rapidly declining. We recognize that not all species were injured and that some recovered quickly (Bowman et al. 1995, 1997). Admittedly, our study species were those that showed evidence of injury and for which there were concerns about population recovery. However, we argue that these vulnerable species are particularly useful to track system recovery under the assumption that a recovered, functioning ecosystem is one in which direct and indirect effects of the spill no longer exist for any component population.

We chose a top-down approach, focusing on apex predators. These were the species that had better data available describing population injury following the spill and, thus, were most useful for assessing system recovery. Further, we reasoned that upper trophic levels would assimilate effects of the oil spill throughout the system. However, while we argue that apex-predator approaches are effective in focusing assessments of perturbations in complex environments, we also caution that it is shortsighted to follow this approach in too narrow a manner. Although our focus for lower trophic studies was the estimation of prey availability for our study species, our design encouraged and facilitated efforts to gain important insights into the recovery status and ecology of many of these lower trophic species (e.g., Appendix SO-03; Appendix SO-04; Appendix SO-05; Appendix SO-06; Appendix SO-07). Such approaches provide a more holistic examination of the environment of interest, while maintaining a study focus.

From a broad perspective, it is worth considering attributes that make species useful for monitoring or assessment of injury. Sentinel species, those species that are sensitive or vulnerable to perturbations, are clearly good and early indicators of systemic change. Keystone species, those species that have large direct and indirect effects on community structure, can be important to address given the cascading effects that can result from changes in their populations. Sea otters are a well-documented example of such a keystone species in the nearshore marine ecosystem as discussed earlier in this paper. However, keystone species are not necessarily good sentinel species as they may be resistant (insensitive) to environmental variability. Along the specialist-to-generalist continuum, generalists may be less sensitive to perturbations as they are able to adjust through prey or habitat switching and, thus, not show effects of environmental change. However, generalists assimilate a broad range of effects and may be useful to assess indistinct or indiscriminate perturbations. Specialists would be particularly sensitive to change in the food or habitat upon which they specialize; however, they may not respond to alterations outside of their area of specialization. We suggest that the choice of species for conducting ecosystem-level studies is an important one and recommend that investigators think about life-history traits, life-cycle events, sensitivity to environmental perturbations, and ecological role of species when considering study species. Our combination of species, taken together, provides a much better picture of nearshore recovery than any single species considered separately.

Geographic scale has a great deal to do with the assessment of status for the three study species that continue to express recovery constraints—pigeon guillemot, sea otter, and harlequin duck. Pigeon guillemot populations seem challenged less by direct oil exposure at a local scale, than by factors represented at a large regional scale descriptive of a theorized marine regime shift where food quality may now be insufficient to support the population growth needed to replace 1989 mortalities. Conversely, sea otters and harlequin ducks evidence recovery at the broader scale of the western Prince William Sound, while showing a lack of recovery at the scale represented by our heavily oiled study sites. At this smaller scale, however, compelling evidence exists over many lines of investigation to suggest that both species continue to be constrained by increased levels of mortality relative to oiling history. A weight of evidence suggests that neither demographic factors nor food availability control this process. Patchy, irregular exposure of these invertebrate-feeding species to persistent hydrocarbons is likely. It also seems that only minor increases in mortality are needed to continue to constrain these depressed populations. Thus, given the small spatial scale and likely irregular temporal nature of this exposure, it seems ill advised to focus on direct mitigation of future oil exposure; conversely, unaided recovery in the heavily oiled study areas seems doubtful in the near future. Since relatively slight elevations in mortality seem to be driving population levels, any enhancement of survival might be beneficial. For example, voluntary redirection of legal harvest of sea otters and harlequin ducks out of the study area should be discussed. This is particularly true for those younger sea otters with evidence of lower natural mortality.

Temporal scale is also critical to further understand the mechanisms still outstanding as constraints to population growth. The NVP Study effort was designed under an adaptive management philosophy with a defined window of study (three field seasons) to assess probabilities of each hypothesis and to prioritize likely constraints. That has been accomplished. However, the next step to understanding this system perturbation requires investigation at a

temporal scale appropriate for the constraint under study. This scale will vary and must be defined for each species. One such scale is that required to understand control and change in the marine food web (regime shift) critical to production issues constraining pigeon guillemot recovery—likely a decadal or greater scale. For those species where population growth seems constrained by survival issues (sea otter, harlequin duck), the scale of assessment should focus on the appropriate season where stress is elevated—winter—if a goal of understanding population control is to be furthered. Timescale issues are also central to adequately understanding species' response to stress events.

Beyond some of these general insights, three specific lessons learned stand out from the NVP Study effort related to blood chemistries as metrics of health, methods for hydrocarbon exposure assessment, and statistical methods in integrated studies. Firstly, although each component element of the NVP Study found no dramatic indication of health problems, much of our assessment rests on the use and interpretation of a panel of blood chemistries collected in snapshots, on an irregular timescale, and primarily during late spring and summer. We have learned a great deal about what these panels can and cannot tell us. While the blood panels used were clearly appropriate to assess acute health impacts immediately after the spill, they may not adequately reflect the chronic, low-level health issues occurring now, or even acute responses that may occur in a narrow timescale. In fact, we are not tracking individuals through time, but randomly sampling a population for which normal often includes a wide range of values. As in clinical veterinary cases, many of the blood chemistry values likely depart only from normal just before an animal's death. Both our sampling intensity and temporal distribution of samples make it unlikely that we would encounter an animal in this narrow window of opportunity. This seems particularly true when only slight elevations in mortality could be constraining population-level growth. The NVP Study continued with the various panels originally used during early post-spill efforts to demonstrate the point at which they and our standard methodologies could no longer track the original damage seen after the spill. These panels also tell us what is not a problem anymore. For example, in our most recent samples, we see little or declining evidence of the liver damage that was documented for several years after the spill in river and sea otters. More real-time assessments associated with periods of environmental (e.g., winter or storm periods) or behavioral (e.g., reproductive periods) stress should be considered in future efforts. For example, in late summer 1997, we used a portable blood analyzer to measure a suite of parameters in harlequin ducks and obtained interesting insight into the narrow timescale under which some blood variables deflect from and return to normal at a population level of sampling. During the first 17 days of sampling, the serum chemistries obtained were normal (Fig. 6). On the day following a 3-day storm, all of the sampled ducks had low potassium levels, most at a level of clinical concern. Some of these birds also had low blood glucose levels. The low values were again seen in a smaller percentage of sampled birds the following day and only normal values were detected thereafter. Such low serum potassium and glucose levels would be expected in birds suffering a combination of increased physical exertion and decreased feeding. It was during this period that most of the limited handling mortalities in this study occurred (Chapter 4). Since we see the survival curve of our oiled harlequin duck population depart from that of the reference population in midwinter during the height of the storm season, mortalities may reflect disparate responses to the storm challenge in the two populations.

Secondly, since vertebrates metabolize hydrocarbon, this characteristic makes them not only susceptible to toxic effects as the body processes the contaminant, but also makes it difficult to measure analytically the many resulting metabolites (e.g., >60 in sea otters; Mulcahy and Ballachey 1994). Thus, at the start of the NVP Study, we did not expect to be able to measure hydrocarbon concentrations directly in the body, nor did we wish to kill animals to collect the necessary tissues for such analyses. Therefore, we turned to cytochrome P450 (CPY1A), previously explored in the literature, to assess potential exposure of vertebrates to various hydrocarbons. This biomarker is expressed during exposure and dissipates in a period of perhaps days to a few weeks after exposure (Stegeman et al. 1992). We began with noninvasive efforts (skin punches and IHC method) and found this technique was highly insensitive to the present day levels of exposure (see comparative tissue and method analyses for Barrow's goldeneye *Bucephala islandica*; Chapter 2). The liver EROD method was clearly more sensitive but again required invasive methods. We were able to develop appropriate veterinary techniques to collect the required liver tissue in the field with minimal risk to sampled animals, but only for the birds species. Finally, we turned to RT-PCR methods that required only the collection of blood samples and were successful in developing methods for sea otters (Appendix BIO-02) and later for river otters. However, because of the nature of bird blood, we were unable to develop and apply this approach to harlequin ducks or pigeon guillemots. Thus, we made significant advances in methodology for future monitoring, but remain without a full matrix of data under a single technique for the NVP Study. More important, we observed inter- and intra-species' variation in expression of CYP1A. The factors controlling these variations and the actual consequences of such expression to health and survival are far from known.

Finally, ecosystem studies are difficult to interpret because of the complexity and number of pathways that may affect a phenomenon of interest. Also, there is a high likelihood that not all components can be fully investigated because of funding, logistical, or technical reasons. This holds true for the NVP Study. Many lines of investigation exist, not all lines suggest the same outcome, nor do they hold similar confidence or weight of evidence (e.g., Table 4). Thus, two major constraints to interpretation develop (1) how to build individual analyses into a informative whole and (2) how to weigh what has been observed in the context of uncertainty. Additionally, an interdisciplinary effort, such as the NVP Study (>15 principal investigators), is complicated by the real constraints of developing unified paradigms (Pickett et al. 1999) acceptable to the group. The NVP Study experimented with two statistical approaches to address these issues—meta analysis (Hedges and Olkin 1985) and Bayesian methodology (Kass and Raftery 1995). However, not surprisingly, meta analysis proved a casualty of the NVP Study design. Each of our species was selected because of the unique perspective it could give researchers; thus, the possibility of different outcomes was expected. While we nominally balanced key elements of those perspectives (physiological group, oil exposure through trophic pathways, and exposure through physical contact), each species demonstrated a variety of ecological nuances and methodological challenges that effectively negated the meaningful application of our balanced design when meta analysis was applied. Thus, with an actual sample size of $n = 1$ for each perspective, meta analysis was uninformative. Another approach was more useful. Adkison et al. (Appendix SYN-01) used Bayesian methodology to document, quantify, and incorporate subjective elements of weight of evidence into an ecosystem study focusing on the multiple lines of evidence within the sea otter window of the study. The end product of this

methodology was to set the probability of each of the competing hypotheses. However, our study design acknowledged from the beginning that our hypotheses were not mutually exclusive, and, thus, as Adkison et al. (Appendix SYN-01) found, additional interpretation was required. Therefore, as a group, we did not use this method to reach an analytical consensus, but to organize our thinking and allow investigators to approach the synthesis of the studies one element at a time. Thus, the enormity of information and lines of investigation could be more thoughtfully digested. While this method did not result in a single presentation of probabilities across species as was originally hoped, it did facilitate identification of evidence investigators felt was ambiguous or particularly strong. Areas of disagreement among investigators also were easily identified.

The need to conserve coastal biota and their critical habitats is broadly acknowledged. These communities face a constant array of anthropogenic challenges to their long-term viability. Growth of human populations and associated urban sprawl continue to cause broad degradation. Marine coastal zones form the heart of critical transportation corridors and are at risk from infrastructure development (e.g., harbors) and pollution (e.g., accidental spills and vessel discharge). At the end of the pipeline, any nearshore waters are the first to receive the effluent (e.g., sediment, agricultural nutrient) generated from inland river basins much larger in surface area than the receiving coastal habitat. Thus, the public and scientific need to detect ecological impacts here is clear (Schmitt and Osenberg 1996), but constrained by many issues typified within the *Exxon Valdez* oil spill experience. The final lesson gained from the NVP Study should be that we discovered definable spill-related effects at the population level some 6 to 10 years after the spill. This experience supports the conclusion that not only should studies be implemented quickly following a perturbation, but that they should be carried out over longer periods than first thought. Another critical lesson is that integrated, multispecies approaches can facilitate the development of a sufficient weight of evidence despite inherent system variability or data limitations. Thus, a better societal understanding of such pollution events can evolve and appropriate restoration efforts developed.

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Table 1. Injury and evidence for lack of recovery from the *1989 Exxon Valdez* oil spill at the start of the Nearshore Vertebrate Predator Study in 1995 in four nearshore vertebrate species as evidenced through demographic, bioindicator, and trophic evidence. Mortality estimates and status and recovery strategies are as per *Exxon Valdez* Oil Spill Trustee Council (EVOSTC 1994a,b).

Injured resource	Injury to nearshore ecosystem and lack of recovery as evidenced in four key species	Status/recovery strategy
Sea otter (*Enhydra lutris*)	**DEMOGRAPHIC** ! Up to 4,000 acute mortalities. ! Various surveys suggest abundance of sea otters has not recovered to pre-spill numbers. ! Significant differences in juvenile survival between oiled and nonoiled areas in 1990–1991 and 1992–1993. ! Proportions of prime aged animals among dead returning to pre-spill levels (Ballachey et al. 1994). **BIOINDICATOR** ! Hematological and serum chemistries suggest otters in oiled areas had higher incidence of inflammatory and/or infectious conditions. **TROPHIC** ! Primary foods include mussels, clams, and urchins, as well as other subtidal organisms. Sea otters feed in the lower intertidal and subtidal areas, areas that were especially contaminated by oil spilled from the *Exxon Valdez* (Wolfe et al. 1994) and may still be exposed to hydrocarbons through their feeding (EVOSTC 1994a). ! In areas where recovery has not occurred, increases in sea urchin densities (a preferred prey) have been observed (Jewett, University of Alaska Fairbanks, Fairbanks, personal communication).	! Stable, not recovered. ! Conduct research to find out why not recovering; hypotheses include continued hydrocarbon ingestion; and spill-caused changes in benthic prey. ! Recovery judged when population abundance and distribution are comparable to pre-spill and when all ages appear healthy.
Harlequin duck (*Histrionicus histrionicus*)	**DEMOGRAPHIC** ! 1,000 acute mortalities in harlequin ducks. ! Summer populations of harlequin ducks, which may be year-round residents, were lower than expected in the oiled area of Prince William Sound between 1989 and 1991 (Klosiewski and Laing 1994). **BIOINDICATOR** ! Patten et al. (1998) found hydrocarbon metabolites in seaducks collected in oiled areas and also suggested that reproductive effort and productivity of harlequin ducks were lower in oiled areas. **TROPHIC** ! Although harlequin ducks rely on benthic invertebrates that may continue to transport hydrocarbons through their food chain, no specific assessment evidence of the potential for trophic-related constraints to recovery exists.	! Unknown status. ! Conduct research to find out why not recovering; hypothesis related to oil-contaminated prey. ! Recovery judged for harlequin ducks when no difference between spill and nonspill areas.

Table 1. Continued

Injured resource	Injury to nearshore ecosystem and lack of recovery as evidenced in four key species	Status/recovery strategy
River otter (*Lontra canadensis*)	**DEMOGRAPHIC** ! Although some were killed, there was no catastrophic mortality—river otters continued to live in areas that were heavily oiled through 1990 (Testa et al. 1994). ! Initially modified their use of habitat by avoiding heavily oiled shorelines (Bowyer et al. 1995). Selected habitat differently on oiled versus nonoiled areas by concentrating their activities on steeper tidal slopes and using areas with greater exposure to wave action (Bowyer et al. 1994), where oil was less likely to persist (Wolfe et al. 1994). ! In 1990, home ranges in oiled areas were two times bigger than in nonoiled areas, suggesting a loss of habitat on oiled sites (Bowyer et al. 1995). ! Continued exposure has adverse health effects; lower body mass. Lower body mass often related to lower reproductive output in large mammals (Docktor et al. 1987). ! Throughout broad areas of Prince William Sound, latrine sites (an index of population density) were abandoned at a rate of three times greater on oiled versus nonoiled areas (Duffy et al. 1994a). **BIOINDICATOR** ! Continued exposure has adverse health effects; higher haptoglobin (an acute-phase protein indicator of damage) than otters in nonoiled areas (Duffy et al. 1993). **TROPHIC** ! Diets in oiled versus nonoiled areas were similar through 1990, but differed markedly by summer 1991 (Bowyer et al. 1994). A number of taxa were absent from the diet in oiled areas. ! Nearshore demersal fish, primary prey of this species, demonstrate a high incidence of hemosiderosis in oiled eelgrass beds of Herring Bay (Jewett et al. 1995). This suggests continued exposure to hydrocarbons.	! Unknown status. ! Rely on natural recovery; indications of recovery are when habitat use, food habitats, and physiological indices return to pre-spill conditions.
Pigeon guillemot (*Cepphus columba*)	**DEMOGRAPHIC** ! 1,500–3,000 killed by *Exxon Valdez* oil spill in 1989. ! Populations in Prince William Sound have declined from ca. 15,000 in the 1970s to ca. 3,000–5,000 in 1993 based on boat surveys. Declines have been greater in oiled versus nonoiled areas of Prince William Sound (Sanger and Cody 1994; Klosiewski and Lang, unpublished data).	! Stable or continuing decline. ! Conduct research to find out why not recovering; likely causes climatic/ oceanographic, prey limitations, and predation.

Table 1. Continued

Injured resource	Injury to nearshore ecosystem and lack of recovery as evidenced in four key species	Status/recovery strategy
	! Number of breeding pairs on Naked Island (largest guillemot breeding aggregation in Prince William Sound) has declined ca. 50% since the late 1970s and give no evidence of recovery (D. L. Hayes, U.S. Fish and Wildlife Service, personal communication).	! Recovery judged by stable or increasing populations.
	BIOINDICATOR ! Average growth rates of chicks have declined since the spill (Oakley and Kuletz 1994) and remained lower at Naked (oiled) versus Jackpot (nonoiled) Islands during the 1994 breeding season (D. L. Hayes, U.S. Fish and Wildlife Service, unpublished data).	
	TROPHIC ! No direct evidence collected. However, nearshore demersal fish, primary prey of this species, demonstrate a high incidence of hemosiderosis in oiled eelgrass beds of Herring Bay (Jewett et al. 1995). This suggests continued exposure to hydrocarbons. Nearshore demersal fish comprised ~half the diet of chicks on Naked Island. ! Sand lance, a schooling fish that burrows in nearshore sandy sediments, formerly comprised ca. a third of the diet of chicks on Naked Island. Since the spill, the proportion in the diet has declined.	

Table 2. Characteristics of four sentinel species examined for recovery status and continuing effects from the 1989 *Exxon Valdez* oil spill as part of the Nearshore Vertebrate Predator Study, 1995–1999 in Prince William Sound (PWS), Alaska. Assessment indicators reflect (1) the potential routes of oil exposure and (2) value of the species to assess food limitation theories. This information was based on the literature and investigators' professional opinion at the beginning of the study.

	Species			
Characteristics	Sea otter (*Enhydra lutris*)	Harlequin duck (*Histrionicus histrionicus*)	River otter (*Lontra canadensis*)	Pigeon guillemot (*Cepphus columba*)
Biological				
Phase of life cycle	Year-round PWS resident	Primarily winter resident, gather from dispersed breeding areas to PWS	Year-round PWS resident	Summer breeder, gather from dispersed wintering areas to PWS
Habitat	Intertidal to subtidal (in PWS most <40 m; Bodkin, U.S. Geological Survey, Anchorage, Alaska, personal communication)	Intertidal, shallow subtidal	Terrestrial, intertidal	Subtidal and pelagic
Home range-site fidelity	Few to >40 km	Both breeding and winter site fidelity high	20–40 km of shoreline	Nest site fidelity high. Forage range within 5 km of nest site
Trophic	Subtidal/intertidal invertebrates	Intertidal/shallow subtidal invertebrates	Fish, some invertebrates	Nearshore/pelagic fish
Assessment				
Oil exposure routes				
Food	High	High	Medium	Low
Contact	High	High	High	Low
Food-limiting evidence				
Feeding data/prey abundance	High/medium	Low/low	Low/medium	High/medium
Food structuring	High	Low	Low	Low
Link with production	Low	N/A	Low	High

Table 3. Summary of methods used to assess status of recovery and factors limiting recovery in four nearshore vertebrate predators in the Nearshore Vertebrate Predator Study listed by species and approach. More specific information is presented in the individual presentations in this volume (Chapter 2; Chapter 3 Part A, Part B, and Part C; Chapter 4; Chapter 5; Chapter 6).

	Species			
Approach	Sea otter (*Enhydra lutris*)	Harlequin duck (*Histrionicus histrionicus*)	River otter (*Lontra canadensis*)	Pigeon guillemot (*Cepphus columba*)
Demography	Aerial surveys of abundance	Assessment of habitat use and abundance	Latrine site abandonment as abundance index	Chick growth Reproductive success
	Surveys of annual reproduction rates	Overwinter survival of females	DNA-based mark-recapture	Adult attentiveness to chicks
	Recovery of carcasses to monitor mortality patterns		Age structure	Mean fledging size and food delivery rates
Health and oil exposure	Blood and immune function assays	Blood assays	Blood and immune function assays	Blood assays
	P450 assays	P450 assays	P450 assays	P450 assays
	Morphometrics condition	Body composition	Morphometrics condition	Chick body composition
Trophic interactions	Abundance, distribution, and size class structure of prey	Abundance and size class distribution of prey	Abundance of demersal fishes	Abundance of demersal fishes
	Prey selection and foraging success		Prey selection through remains in feces Stable isotope analysis	Chick feeding

Table 4. Recovery status in 1999 for four sentinel nearshore vertebrate predators examined in the Nearshore Vertebrate Predator Study and summary opinion of what major limiting factor(s) might still be in effect. Confidence in data reflect a combination of statistical confidence in estimates and strength of evidence that metrics relate to hypotheses. Key evidence resulting from the study is summarized.

		Factors assessed as limiting recovery Probability factor limit and confidence			
Species	Recovery status	Food limited	Health/oil	Demography	Key evidence
Sea otter (*Enhydra lutris*)	Not recovering (Chapter 3 Part A)	Low probability High confidence	Medium probability Medium confidence	Low probability High confidence	No population growth in oiled areas while growth in nonoiled areas (Chapter 3 Part A); temporary immigration of young males, but no residents established; reproduction and condition sufficient. Similar food resources supporting growth in nonoiled areas but not oiled areas (Chapter 3 Part B, but see Chapter 3 Part C). Mean cytochrome P450 (CYP1A) expression >18 times (reverse transcriptase polymerase chain reaction) at oiled sites (Chapter 2), but limited evidence in blood data to suggest reduced health (Appendix BIO-01); no population growth despite food and reproduction suggests mechanism.
Harlequin duck (*Histrionicus histrionicus*)	Not recovering (Chapter 4)	Low probability Medium confidence	High probability Medium confidence	Medium probability High confidence	Female survival lower at oiled sites and insufficient to support population (Chapter 4). Densities lower than predicted based on habitat for oiled area (Appendix HD-01). Mean CYP1A expression three times (ethoxyresorufin O-deethylase) at oiled sites (Chapter 2), but no evidence in blood data suggesting reduced health; but survival is impaired despite sufficient food, thus, mechanism is suggested.

Table 4. Continued

Species	Recovery status	Factors assessed as limiting recovery Probability factor limit and confidence: Food limited	Health/oil	Demography	Key evidence
River otter (*Lontra canadensis*)	Recovery likely (Chapter 5)	Low probability	Low probability	Low probability	No differences in home range, age, or survivorship; population growth suggested (Chapter 5).
		Medium confidence	Medium confidence	Medium confidence	Food resources abundant.
					Oiled-site effect in CYP1A expression variable among years (Chapter 2) and between sexes (Chapter 5), no evidence in blood data suggesting reduced health (Chapter 5).
Pigeon guillemot (*Cepphus columba*)	Not recovering (Chapter 6)	High probability	Low probability	Low probability	No population growth despite sufficient productivity and other demographic factors (Chapter 6).
		High confidence	Medium confidence	High confidence	Prey quality indicated, mean fledge weight lower suggesting potential food-related survivorship issues after fledging.
					Health/oil: Low and nonsignificant CYP1A expression between oiled or nonoiled sites for chicks (Chapter 2). Health parameters in chicks not indicative of clear acute or measurable chronic impacts. Significant but low CYP1A differences in adults (Golet et al. 2000).

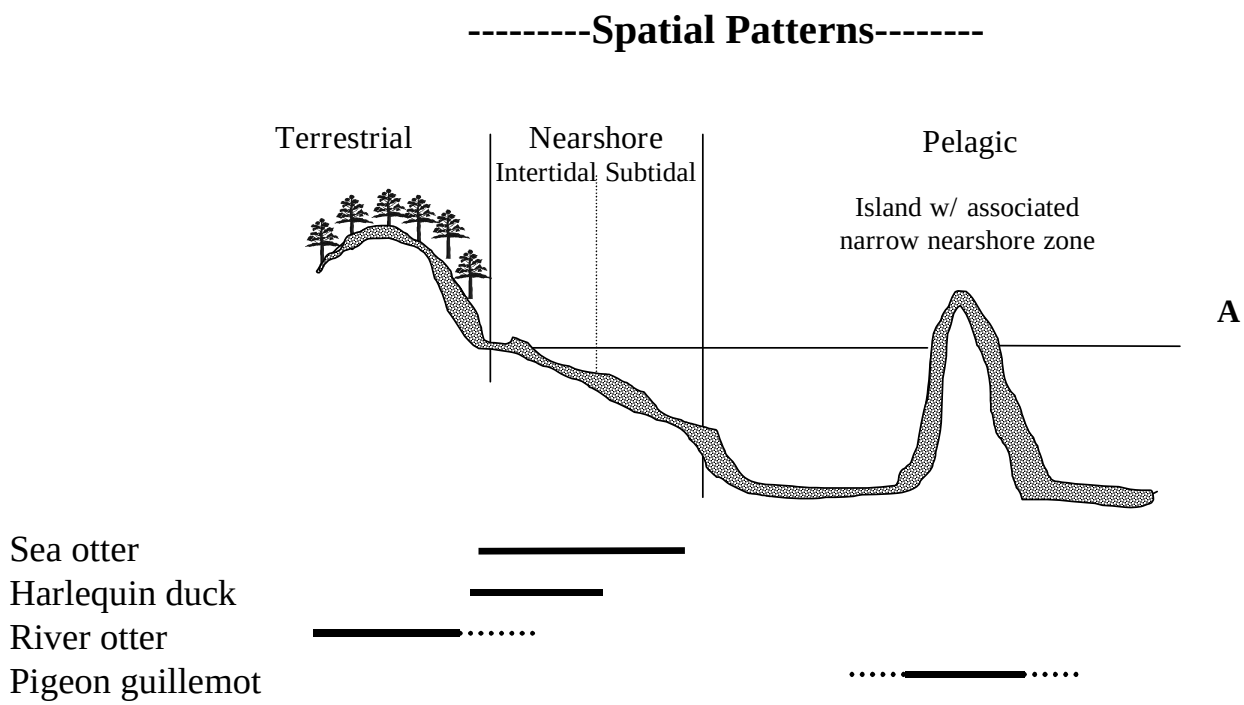

Figure 1. Variation in the use of Prince William Sound by four top predators used to assess the status of the nearshore ecosystem of Prince William Sound, Alaska, from 1995 to 1998. Spatial distribution (*A*) and annual pattern of general species presence (*black*), absence (*clear*), and primary life-history activities (*B*) in the Prince William Sound are presented relative to the timing of the March 24, 1989, *Exxon Valdez* oil spill (*hatched*).

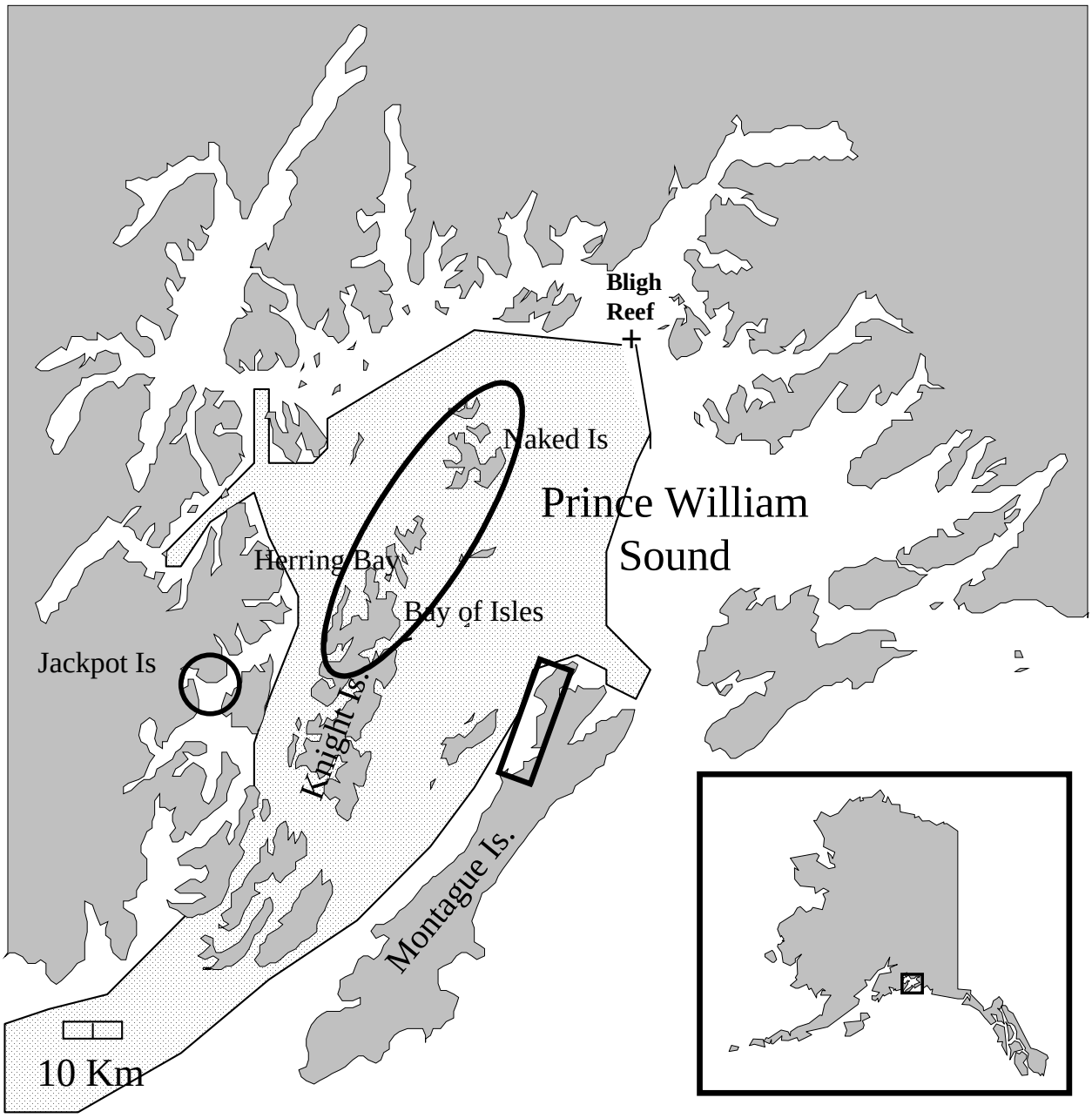

Figure 2. Locations of three focal areas in Prince William Sound, Alaska, studies during the Nearshore Vertebrate Predator Study. Shaded area represents the general extent of surface oil following the *Exxon Valdez* oil spill (modified from Short and Harris 1996).

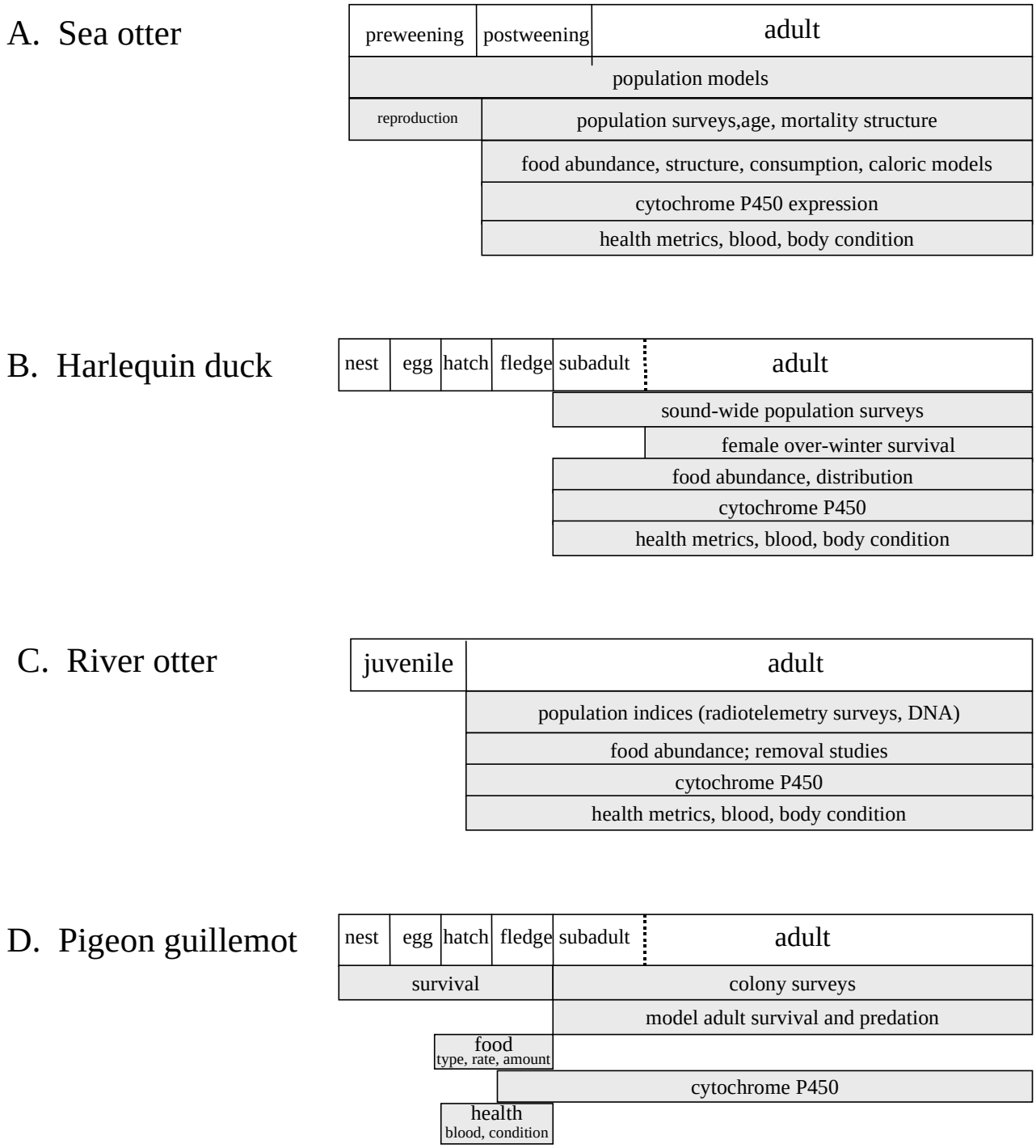

Figure 3. General demographic, trophic, and health and oil exposure data types collected by life-history component for the four focal species evaluated as part of the study to assess impact and recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill.

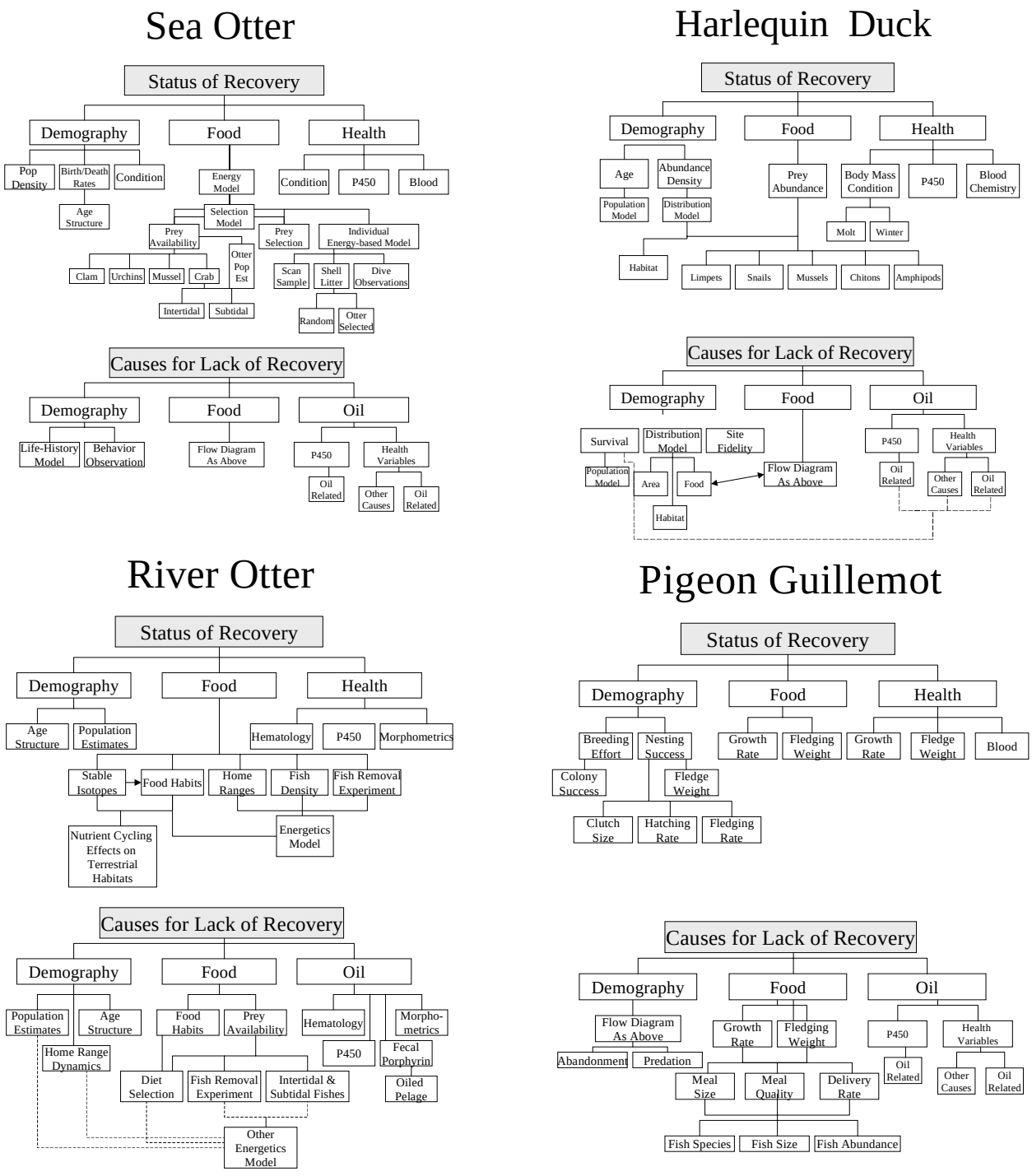

Figure 4. Flow chart depicting data type and use to assess status of recovery and various hypotheses for lack of recovery in four nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill.

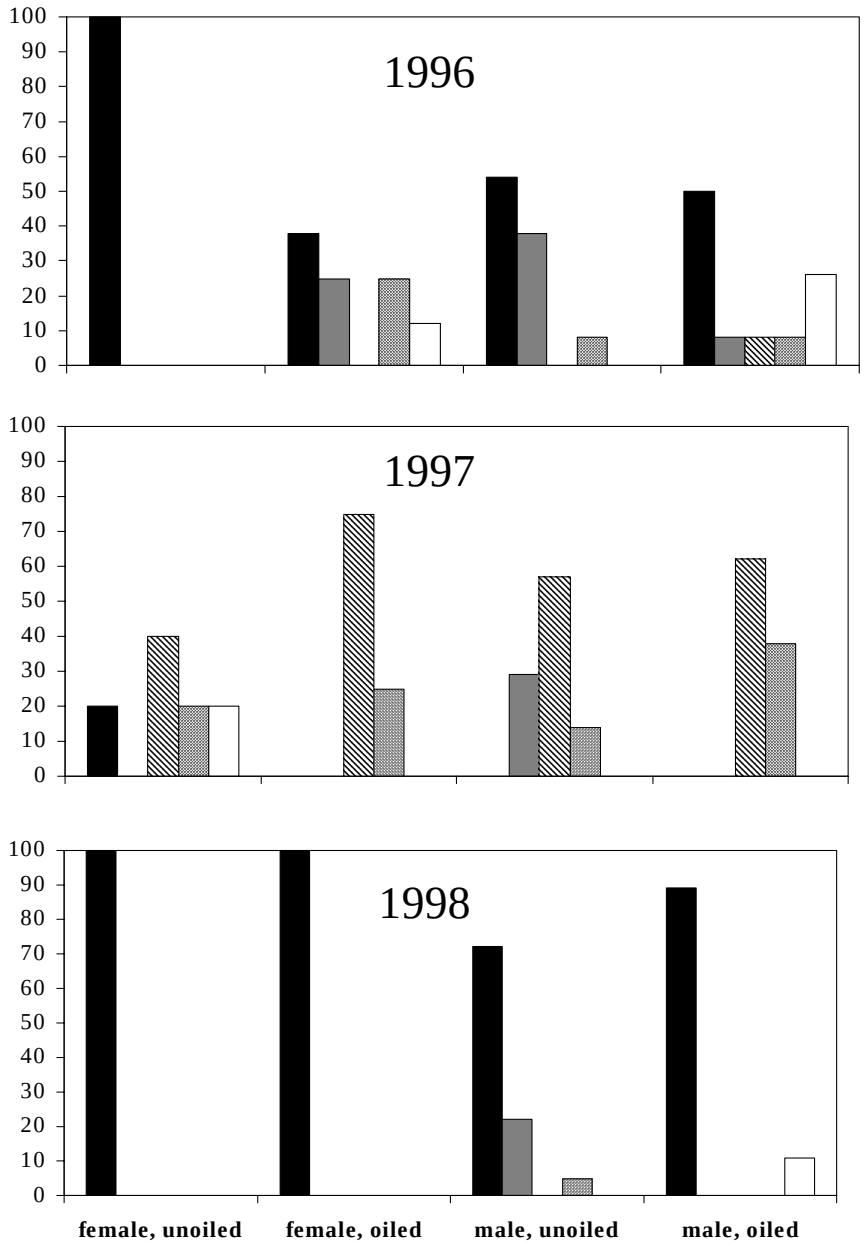

Figure 5. Percent distribution of Cytochrome P450 (CYP1A) expression in river otters (*Lontra canadensis*) as measured by immunohistochemistry (IHC) by sex and year. Bars are IHC scores 0–1: black; 2–3: grey; 4–5: hatched; 6–7: pattern; and 8–9: clear.

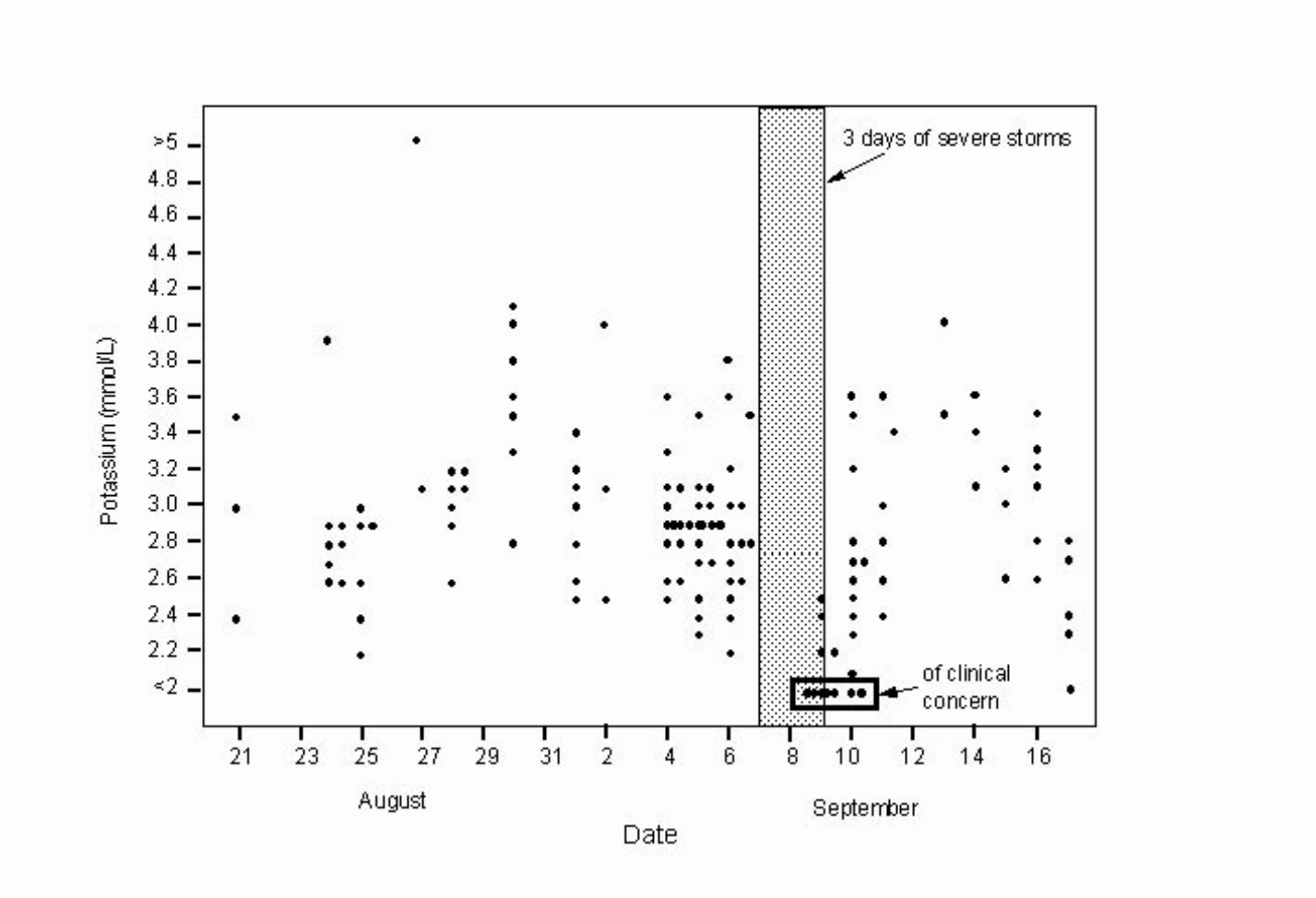

Figure 6. An example of the narrow temporal scale under which blood values of clinical concern can be expressed. Serum potassium levels for >60% of harlequin ducks (*Histrionicus histrionicus*) collected on September 9, 1997, were <2 mmol/L, a level of clinical concern. This was a period following 3 days of severe storm weather. Values deemed in the normal range were found before and after this event. Data provided by D. Mulcahy (U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska, USA).

Chapter 2. Biomarker Perspective

Oil Exposure and Health of Nearshore Vertebrate Predators in Prince William Sound Following the 1989 *Exxon Valdez* Oil Spill[1]

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ABSTRACT

A major component of the Nearshore Vertebrate Predator ecosystem study addressed the potential of continuing exposure to residual oil from the 1989 *Exxon Valdez* oil spill as a factor limiting recovery of top predators. To evaluate exposure, we measured induction of cytochrome P450 1A (CYP1A), a protein involved in metabolism of aromatic hydrocarbons, in harlequin ducks (*Histrionicus histrionicus*), pigeon guillemots (*Cepphus columba*), river otters (*Lontra canadensis*), and sea otters (*Enhydra lutris*) from oiled and unoiled areas of western Prince William Sound during 1995–99. We also assessed health of individual members of these species using hematologies, serum chemistries, and body condition as indicators. Two additional species were sampled for CYP1A: masked greenlings (*Hexagrammos octogrammus*) in 1996 and Barrow's goldeneyes (*Bucephala islandica*) in 1997. For all species, we found evidence of greater CYP1A induction in oiled areas compared to unoiled areas. Residual oil from the spill, rather than other petroleum or organochlorine contaminants in the environment, is the apparent source of the contaminant exposure. Species that prey on benthic invertebrates, including sea otters and seaducks, showed marked differences between areas, whereas species that consume primarily fishes showed either relatively small differences (river otters, pigeon guillemot adults) or no differences (pigeon guillemot chicks). These findings suggest that exposure resulted through

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[1]In preparation for submission to Environmental Toxicology and Chemistry

consumption of contaminated invertebrate prey or contact with sediments during foraging. Body condition generally was equivalent between animals in oiled and unoiled areas, and few differences in blood values were noted. Red blood cell counts were lower in both adult harlequin ducks and pigeon guillemots in oiled areas compared to unoiled areas, possibly indicating a mild anemia. Sea otters in oiled areas had higher concentrations of serum gamma glutamyl transferase, an enzyme indicating liver dysfunction, but differences were not large and were less than in previous post-spill studies, perhaps due to declining oil toxicity and loss of the most severely affected individuals from the population. A negative relation was found between body mass and CYP1A induction in harlequin ducks, but not for the other species. Continuing exposure to residual *Exxon Valdez* oil spill may be limiting recovery of harlequin ducks and possibly sea otters, but does not appear to be a factor in recovery of river otters or pigeon guillemots in western Prince William Sound.

Key words: Alaska, Barrow's goldeneye, cytochrome P450, *Exxon Valdez* oil spill, harlequin ducks, hematology, masked greenling, pigeon guillemots, river otters, sea otters, serum chemistry.

INTRODUCTION

The grounding of the T/V *Exxon Valdez* in March 1989 released approximately 42 million L of north slope crude oil into the waters of Prince William Sound. As the oil slick moved through the Prince William Sound and into the Gulf of Alaska, over 15 million L washed up on Prince William Sound shorelines (Galt et al. 1991; Wolfe et al. 1994). Although much of the beached oil was gone by 1992, oil that had penetrated into sediments was relatively persistent (Wolf et al. 1994) and 8 years post-spill, unweathered oil could still be found in subsurface sediments of Prince William Sound beaches (Broderson 1999; Hayes and Michel 1999). Clearance of residual oil from sediments is anticipated to be a slow process (Hayes and Michel 1999), and a further concern is that those fractions of the oil that are most persistent are also the most toxic to wildlife (Short et al. 1999).

A diverse variety of birds, mammals, fishes, and marine invertebrates inhabit the coastal areas affected by the *Exxon Valdez* oil spill (EVOS), and many species suffered extensive mortality from acute exposure during the initial months post-spill (Loughlin et al. 1996; Piatt and Ford 1996; Spies et al. 1996a). Injury from the spill, however, extended well beyond acute losses. Toxic effects of petroleum compounds on birds and fishes have been demonstrated (Leighton 1993; Spies et al. 1996b), although prior to the EVOS, few studies had been done on toxicity to marine mammals (Geraci and St. Aubin 1990). Potentially, both sublethal exposure to high oil concentrations in the initial post-spill period and prolonged exposure to lower levels of residual oil in the environment could result in reduced viability of individuals in oiled areas and delay population recovery. Although longer-term effects of oil have been difficult to document, continuing injury has been apparent for several species (Duffy et al. 1993, 1994; Ballachey et al. 1994; Bowyer et al. 1995; Bue et al. 1996; Agler and Kendall 1997; Rice 1999; Appendix BIO-03). By 1996, 10 species were still considered not to have recovered from spill-related injury, and recovery status of an additional eight species was unknown (EVOS Trustee Council 1996).

The Nearshore Vertebrate Predator ecosystem study was implemented in 1995 to examine the recovery status of four top predator species occurring in coastal areas of Prince William Sound (Chapter 1). Species selected for the study were sea otters (*Enhydra lutris*), river otters (*Lontra canadensis*), harlequin ducks (*Histrionicus histrionicus*), and pigeon guillemots (*Cepphus columba*), all of which had been designated as injured following the spill (EVOS Trustee Council 1996). The Nearshore Vertebrate Predator Study objectives were to assess whether or not these four species had recovered and, if they had not, to evaluate relative importance of demographic factors, food availability, and oil toxicity in limiting their recovery. The combination of species, which includes two (one mammal, river otter, and one bird, pigeon guillemot) that prey primarily on forage fish and two (one mammal, sea otter, and one bird, harlequin duck) that prey on benthic invertebrates, allows us to compare and contrast results providing insight into issues such as potential routes of exposure of oil. Summaries specific to each study species are presented separately (Chapter 3 Part A; Chapter 4; Chapter 5; Chapter 6). This chapter focuses on evidence pertaining to the question of continuing oil exposure affecting health and, consequently, delaying recovery of all four species. An additional seaduck, Barrow's goldeneye (*Bucephala islandica*), and a demersal fish, the masked greenling (*Hexagrammos octogrammus*), were also included in this component of the study. Our objectives were to (1) evaluate evidence for continued exposure of the study species to residual EVOS oil in the nearshore environment, and (2) determine whether or not there are differences in indices of health between animals in oiled and unoiled areas that may be related to continuing oil exposure or to residual effects of the initial exposure.

Cytochrome P450 1A (CYP1A) is a biomarker of exposure to aromatic hydrocarbons including the polycyclic aromatic hydrocarbons (PAH) found in crude oil and halogenated aromatic hydrocarbons including certain polychlorinated biphenyls (Stegeman et al. 1992). The CYP1A is a member of the cytochrome P450 family, a group of iron-containing hemoproteins involved in metabolism of a variety of endogenous and exogenous organic compounds. Because specific members of the P450 family are induced by different compounds or classes of compounds, their presence signals exposure (Payne et al. 1987; Stegeman et al. 1992). Induction of CYP1A messenger RNA (mRNA), elevated levels of CYP1A protein, and increased activity of ethoxyresorufin O-deethylase (EROD), an enzyme catalyzed by CYP1A, have been demonstrated in a variety of vertebrate species exposed to aromatic hydrocarbons (Lee et al. 1985; Stegeman et al. 1986; Gooch et al. 1989; Peakall et al. 1989; Rattner et al. 1993; Roos et al. 1996; Spies et al. 1996b; Marty et al. 1997; Woodin et al. 1997). When PAH are oxidized, the resulting intermediates may be highly reactive and more toxic than the original compounds (Nebert and Gonzales 1987; Fox 1993). Consequently, elevated levels of CYP1A indicate exposure to aromatic hydrocarbons and the potential for associated deleterious effects on health of the individual. Post-spill studies on fishes in oiled areas of Prince William Sound have utilized CYP1A as a biomarker of oil exposure (Carls et al. 1996; Collier et al. 1996; Wiedmer et al. 1996; Willette 1996; Woodin et al. 1997) but prior to our study, CYP1A had not been used as a biomarker of EVOS oil in birds or mammals.

In the Nearshore Vertebrate Predator Study, we assessed exposure of predator species to residual oil by measuring the expression of CYP1A and comparing populations in oiled (in the vicinity of northern Knight and Naked Islands) and unoiled areas (Montague Island, Jackpot Bay) of western Prince William Sound. We further evaluated the study species by assessing individual health through measurement of condition, hematology, and serum chemistry.

METHODS

Study Areas

The Nearshore Vertebrate Predator Study focused on animals inhabiting the coastal areas of northern Knight and Naked Islands, which were heavily oiled in the 1989 spill. Additionally, harlequin ducks were captured at Main Bay and Crafton Island, both of which were considered to be oiled. For unoiled reference samples, animals were caught at either Montague Island or in the vicinity of Jackpot Bay; these areas were lightly oiled or not oiled in the 1989 spill (Fig. 1). Additional adult pigeon guillemots were captured at Kachemak Bay in lower Cook Inlet.

Animal Capture and Sampling

Harlequin ducks were captured during wing molt in late summers 1995, 1996, and 1997 at Montague Island and in the areas of northern Knight Island, Main Bay, and Crafton Island (Chapter 4). Adult females were anesthetized for implantation of radiotransmitters (Appendix HD-04) and morphological, body mass and body composition measurements were taken (Chapter 4). Two mL of blood were collected from the jugular vein (Appendix HD-07), and a small biopsy of foot web was collected and fixed in 10% neutral buffered formalin for CYP1A analyses. Further captures of adult females and males were done in March and April 1998 at Montague Island, Main Bay, and Crafton Island (Chapter 4). From those birds, in addition to morphological, mass and condition measures and blood, a small biopsy (approximately 0.1 g) of liver for CYP1A assays was surgically removed following anesthesia. Biopsies were immediately placed in cryogenic vials and frozen in liquid nitrogen. Web tissue was not sampled from birds caught in 1998.

Pigeon guillemot chicks were captured on nests prior to fledging at Naked Island and at Jackpot and Icy Bays. Measures of growth (body mass and length of wing chord) were collected every 5 days until fledging. In 1997, 1 mL of blood was collected from the brachial vein when the chicks were approximately 20 days of age and again at approximately 30 days of age (Appendix PG-01). Adult birds were also captured in 1997 at Naked Island, Jackpot Bay, Icy Bay, and Kachemak Bay, and blood was collected from the medial metatarsal vein. Chicks on nests at approximately 30 days of age were captured in 1998 and adult birds in 1999 at Naked Island, Jackpot Bay, and Icy Bay for collection of liver biopsies for CYP1A bioassays (Chapter 6). Birds were anesthetized and liver biopsies weighing about 0.1 g were surgically collected, immediately placed in cryogenic vials, and frozen in liquid nitrogen. Body-condition estimates for adult birds were generated by Golet et al. (Chapter 6).

River otters were captured in May and June 1996 and 1997 from Herring Bay on Knight Island and from Jackpot Bay. Otters were also captured from Ewan and Paddy Bays, north of Jackpot Bay, to supplement the unoiled sample. In 1998, river otters were captured during April and May from three oiled areas: Herring Bay, Naked Island, and Eleanor Passage and three unoiled areas: Esther Passage, Unakwik Inlet, and Wells Bay (Chapter 5). Otters were anesthetized and samples collected at the capture site. Blood (approximately 22 mL) was drawn from the jugular vein. In all 3 years, skin biopsies were collected from the underarm area using a 3-mm disposable skin biopsy punch and preserved immediately in 10% neutral buffered formalin.

Sea otters were captured in July and August 1996, 1997, and 1998 at Knight, Naked, and Montague Islands using either tangle nets or diver-held Wilson traps (Chapter 3 Part A). They were anesthetized, weighed, and morphometric measurements were taken, and approximately 35 cc of blood were collected from the jugular vein. Color-coded plastic tags were placed on the flippers, and a premolar tooth was extracted for age determination (Bodkin et al. 1997). In 1996 and 1997, a small skin biopsy was collected from the flipper during the tagging process for CYP1A analyses. In 1997, a skin biopsy for CYP1A also was collected from the underarm using a 3-mm disposable skin biopsy punch. Biopsies were preserved in 10% neutral buffered formalin immediately after collection.

Barrow's goldeneyes were collected by shotgun in December 1996 and February 1997 from Knight and Montague Islands (Appendix HD-08) and tissues taken for CYP1A analyses. Liver samples (approximately 1 g) were obtained within 10 minutes of carcass retrieval, wrapped in aluminum foil, and frozen in liquid nitrogen. An additional sample of liver and a sample of foot web were placed in 10% neutral buffered formalin.

Masked greenlings were collected (speared by divers) at Herring and Jackpot Bays in July 1996 during the course of studies on forage fish availability (Chapter 5). Intact fish were placed in 10% neutral buffered formalin. Livers were later removed for CYP1A analyses.

Cytochrome P450 Assays

Three methods were used to assess CYP1A induction: (1) RT-PCR, a reverse-transcriptase-polymerase chain reaction assay that quantifies mRNA for CYP1A; (2) IHC, an immunohistochemical assay that quantifies CYP1A protein; and (3) EROD, an assay that quantifies activity of ethoxyresorufin-O-deethylase, an enzyme catalyzed by CYP1A (Table 1, Stegeman et al. 1992). These different methods were required because availability of tissue samples varied among the six species examined.

RT-PCR.—The RT-PCR assays were done at Purdue University on peripheral blood mononuclear cells (PBMC) isolated from river otters (1998) and sea otters (1996–98). The mRNA that codes for the CYP1A protein is quantified using specific cDNA probes and a quantitative reverse transcriptase polymerase chain reaction (Vanden Heuvel et al. 1993, 1994; Appendix BIO-02). Initially, the RT-PCR assays required the isolation, cloning, and sequencing of the polymerase chain reaction product and the development of otter specific primers for CYP1A (Appendix BIO-02).

Heparinized blood samples were used as the source of PBMC, which were isolated by a density gradient technique. Whole blood was mixed 1:1 with HBSS (Hank's buffered salt solution; Fischer MT-21-021-LV) with 1% antibiotic/antimycotic solution added (Sigma A-5955). Approximately 10 mL of diluted blood were layered over 4 mL of histopaque and centrifuged. The PBMC, which formed in a layer on top of the histopaque, were harvested, washed one time with HBSS, and resuspended in freezing medium (1:1 CSPR-2 [Sigma C-9030]: RPMI [Fischer MT-10-041-LV] with 10% DMSO added). Cells were aliquoted into 1-mL volumes and frozen in nalgene cryovials in liquid nitrogen and later shipped to Purdue University. Details of the RT-PCR procedures are presented in Appendix BIO-02. Results are reported as the number of molecules of mRNA for CYP1A per 100 ng of total RNA.

IHC.—Immunohistochemical staining of CYP1A protein, using an antibody specific to the CYP1A protein, was applied to skin samples from sea and river otters, foot web samples from harlequin ducks and Barrow's goldeneyes, and liver samples from Barrow's goldeneyes. Most studies of CYP1A induction have focused on liver, a major site of metabolism of aromatic hydrocarbons. However, at the onset of the study, we determined that collection of liver biopsies from study animals generally was not feasible. The endothelial cells that line blood vessels also are a significant site of induction of CYP1A (Smolowitz et al. 1991). We collected skin biopsies from sea and river otters and foot web punches from harlequin ducks and Barrow's goldeneyes as a potential alternative tissue for the IHC assay, hoping that sufficient endothelial cells could be visualized in these samples to allow detection of CYP1A if present.

Barrow's goldeneyes were lethally collected in winter 1996–97 primarily for other aspects of the Nearshore Vertebrate Predator Study. This provided an opportunity to compare results of IHC (on web punches and liver) and EROD (on liver; see below) approaches for measuring CYP1A induction in a seaduck. In addition, we were interested in whether goldeneyes showed evidence of oil exposure as a measure of their recovery and as a surrogate for harlequin ducks, which occur in the same habitats and forage on similar prey.

For IHC assays, tissues were fixed in formalin at the time of collection and shipped to Woods Hole Oceanographic Institute, Woods Hole, Massachusetts, for analyses. Tissues were embedded in paraffin and standard 5-μm sections cut and mounted on glass microscope slides. Prior to immunochemical staining, sections were deparaffinated and hydrated in 1% bovine serum albumin/phosphate buffered saline (BSA/PBS). During the hydration process, sections were incubated in 0.5% H_2O_2 in methanol for 45 minutes to block endogenous peroxidase (Polak and Van Noorden 1984). Hydrated sections were immunochemically stained using an indirect peroxidase stain (Universal Immunoperoxidase Staining Kit [Murine], Signet Laboratories, Inc., Dedham, Massachusetts, USA) with 1-12-3 monoclonal antibody against scup (*Stenotomus chrysops)* CYP1A as the primary antibody, as described below. Previous immunofluorescent studies have demonstrated the specificity of MAb 1-12-3 for CYP1A in tissue sections by immunoadsorption (Goksøyr et al. 1991).

After hydration in 1% BSA/PBS, sections were incubated in normal goat serum for 20 minutes to block any possible nonspecific attachment of the secondary antibody (goat antimouse IgG; Polak and Van Noorden 1984). Sections were washed once for 5 minutes and then incubated in 1/24,000 dilution (1.7 5g protein/mL) of MAb 1-12-3 in 1% BSA/PBS for 18 hours. Incubation in primary antibody was followed by washing with 1% BSA/PBS. This wash procedure follows all antibody incubations. Next, sections were incubated in a 1/200 dilution of goat antimouse IgG for 20 minutes, washed, and then incubated in a 1/600 dilution of peroxidase labeled nonspecific mouse IgG for 20 minutes. After another wash, sections were incubated for 30 minutes in 3-amino-9-ethylcarbazole (AEC) in acetate buffer to develop color. Sections were rinsed and then counterstained with Mayers hematoxylin and mounted in glycerol (Smolowitz et al. 1991). Two types of controls were used: (1) sections of liver from a fish (scup) with high and one with low content of CYP1A (as determined by EROD activity and immunoblotting) were included in every stained group as controls for the staining method, and (2) matching serial sections of all tissues were stained using a nonspecific IgG (purified mouse myeloma protein, UPC-10, IgG2A, Organon Teknika, West Chester, Pennsylvania, USA) at 1.55 g protein/mL of 1% BSA/PBS (Polak and Van Noorden 1984).

Specific staining by MAb 1-12-3 was evaluated by light microscopic examination of the stained sections. The occurrence of endothelial cells with stain and their staining intensity was recorded for each tissue section examined. At least two immunochemically stained sections were examined from each sample. Quantitative comparisons were made using a staining score that was the product of scaled values for intensity and occurrence.

EROD.—The activity of ethoxyresorufin O-deethylase, an enzyme catalyzed by CYP1A, was assayed in liver samples from harlequin ducks, Barrow's goldeneyes, and pigeon guillemot. Samples were cryopreserved in liquid nitrogen immediately after collection and subsequently shipped to Woods Hole Oceanographic Institute for fluorometric quantification of EROD catalytic rates. Methods are detailed in Trust et al. (Appendix HD-08); results are presented as pmol per minute per mg protein.

Blood Samples

Harlequin ducks: Two blood smears on glass microscope slides were made at the time of collection, and 1 mL of whole blood was placed into a heparinized plastic microtainer tube. The remainder of the blood was allowed to clot for between 1 and 12 hours and then centrifuged to separate the serum, which was divided into 2–3 aliquots and frozen. Blood smears, whole blood, and an aliquot of serum were shipped by air within 72 hours of collection to the Avian and Exotic Laboratory, Redondo Beach, California, for hematology and serum chemistry (Appendix HD-07). A second aliquot of serum was sent to the University of Alaska Fairbanks for haptoglobin analyses. Additionally, to assess the potential contribution of compounds other than PAH to induction of CYP1A, serum from ducks caught in 1998 was analyzed for total polychlorinated biphenyl (PCB) concentration and congener-specific concentrations of 93 congeners. Samples were pooled as necessary to obtain sufficient volume for analyses; detail on pooling and analytical methods is presented by Trust et al. (Appendix HD-08).

Pigeon guillemots: Two blood smears were made on glass slides at the time of collection, and two heparinized microhematocrit tubes were filled and capped. The remainder of the whole blood was placed in a heparinized microtainer tube and centrifuged within 2 hours of collection to obtain plasma, which was frozen in two aliquots. Smears, microhematocrit tubes, and one aliquot of plasma were shipped by air to the Avian and Exotic Laboratory within 48 hours of collection (Appendix PG-01).

River otters: Two blood smears were made on glass slides at the time of blood draw. Ten mL of blood were placed in a heparinized glass tube for transport back to the field camp or support vessel and later used for isolation of PBMC for CYP1A analyses. An additional 2 mL were placed in an EDTA tube for hematology analyses. Ten mL were collected in a glass tube, allowed to clot, and the serum separated into aliquots and frozen. Whole blood and blood smears were shipped by air to Quest Laboratories, Anchorage, Alaska, for hematology. In 1996 and 1997, serum samples were maintained in frozen storage and, when field work was complete, were submitted as a batch to Quest Laboratories for chemistries. In 1998, serum samples were shipped to the laboratory with the whole blood.

Sea otters: Approximately 10–15 mL of blood were drawn from the jugular by vacutainer. About 3 mL were placed in an EDTA tube and the remainder into 1 or 2 glass tubes and allowed

to clot for at least 30 minutes before centrifuging to separate serum. Serum was frozen in several aliquots. Two blood smears on glass slides were made from the whole blood. An additional 20–25 mL of whole blood were drawn into a 50-mL heparinized syringe for isolation of PBMC. Whole blood in the EDTA tube and blood smears were shipped to Quest Laboratories, Portland, Oregon, as soon as possible after collection. Only samples that arrived at the laboratory within 72 hours of collection were used in the data analyses. Serum samples were maintained in frozen storage until field work was complete and then submitted as a batch to Quest Laboratories, Portland, Oregon. Sea otter blood samples were sent to the Portland facility of Quest rather than the Anchorage facility to maintain consistency with samples submitted to the Portland facility in previous studies (Rebar et al. 1995, 1996).

From all four nearshore vertebrate predator species, aliquots of frozen serum (harlequin ducks, river otters, sea otters) or plasma (pigeon guillemots) were submitted to the University of Alaska Fairbanks for haptoglobin analyses (Duffy et al. 1994; Pritchard et al. 1997). Haptoglobins (Hp) are acute-phase proteins found in serum or plasma that are synthesized in response to a range of physical or physiological stresses and that bind free hemoglobin (Hb). Using a standard Hp assay, free Hb and the Hb-Hp complex were separated by electrophoresis, and the complex was quantified with densitometry. Results were expressed as mg Hb-bound per 100 mL of serum or plasma.

RESULTS

Cytochrome P450 1A

Harlequin duck.—The IHC scores on foot web samples from harlequin ducks captured in 1995 were consistently low (mean scores 0.55 and 0.40 for unoiled and oiled areas, respectively), and proportions of individuals in each staining category did not differ between areas (chi-square = 2.444; $P = 0.295$). Staining was generally faint and patchy and often was not certain to be cellular. In consideration of IHC data on web and liver samples from Barrow's goldeneyes (see below), we concluded that foot web of seaducks is not an appropriate tissue for IHC analyses, and no further web samples from harlequin ducks were assayed.

Harlequin duck liver samples from winter 1998 showed higher ($P < 0.001$) EROD activities in birds from the oiled area (mean = 204.6 pmol/min/mg protein) than from the unoiled area (mean = 70.7 pmol/min/mg protein; Fig. 2; Appendix HD-08). Analyses of PCB concentrations in serum samples indicated that this difference between areas was not accounted for by differences in PCB exposure of the ducks (Appendix HD-08). A number of specific PCB congeners, including some known to induce CYP1A in birds, were detected in the serum samples. However, there was no difference between areas ($P = 0.82$) in the proportion of samples with observations of PCB congeners above detection limits.

Barrow's goldeneyes.—The EROD activity of Barrow's goldeneye liver samples was higher ($P = 0.001$) at Knight Island (mean = 94.3 pmol/min/mg protein) than at Montague Island (mean = 49.5 pmol/min/mg protein; Appendix HD-08). Liver IHC scores for goldeneyes were marginally higher at Knight Island (mean = 5.38) than at Montague Island (mean = 4.26; $P = 0.083$). However, foot web IHC scores for goldeneyes did not differ between areas ($P = 0.34$);

means for Knight and Montague Islands were 3.59 and 4.36, respectively. Distribution of values from the three assay methods are presented in Figure 3.

Pigeon guillemot.—Liver biopsies from 14 chicks in the unoiled area and 12 chicks in the oiled area were assayed for EROD activity. Mean EROD values did not differ significantly by area (mean, sd: 4.1 ± 1.26 pmol/min/mg protein for oiled area versus 4.7 ± 1.85 pmol/min/mg protein for unoiled area; $P = 0.19$). Biopsies from 11 adult birds in the unoiled area and 12 in the oiled area were assayed; EROD activity was higher in the oiled area (mean, sd: 3.06 ± 1.32 pmol/min/mg protein for oiled area versus 1.93 ± 0.80 pmol/min/mg protein for unoiled area; $P < 0.02$). Distribution of values for chicks and adults are presented in Figure 4.

River otter.—Skin biopsies collected from the underarm in 1996, 1997, and 1998 were assayed by IHC (Chapter 5). Distribution of the staining scores are presented in Figure 5. Mean values for river otters from the oiled area were 3.2, 4.7, and 0.9 and from the unoiled area were 1.2, 4.0, and 0.7 for 1996, 1997, and 1998, respectively. Analysis of the data (two-way ANOVA with area and year as main effects) identified both area ($P = 0.02$) and year ($P < 0.0001$) as significant and no significant interaction between area and year ($P > 0.12$).

No data were obtained in the RT-PCR assays of PBMC collected in 1996 or 1997 due to procedural problems with the assay. In 1998, RT-PCR analyses were completed. The mean for the oiled area was higher than that for the unoiled area (36.8 versus 19.4×10^6 molecules of CYP1A mRNA per 100 ng total RNA, oiled and unoiled areas, respectively); however, this difference was not significant ($P < 0.34$). Distribution of values is presented in Figure 6.

Sea otter.—In all 3 years, RT-PCR results demonstrated greater induction of CYP1A in sea otters from the oiled area relative to otters in the unoiled area. Analysis of variance on ranks of values showed area to be a significant effect ($P < 0.001$), whereas age, sex, year, and capture method were not ($P > 0.05$; Appendix BIO-01). Distribution of RT-PCR values pooled across years is shown in Figure 7. The mean for sea otters in the oiled area was 27.3×10^6 molecules of CYP1A mRNA per 100 ng total RNA versus a mean of 1.5×10^6 for otters from the unoiled area.

Skin biopsies collected from the flipper in 1996 and 1997 and from the underarm in 1997 consistently showed no occurrence of staining (scores for all samples were zero) in the IHC assay.

Masked greenlings.—Fourteen fish from the oiled area and six from the unoiled area were sampled and livers assayed by IHC. Distribution of values is shown in Figure 8. Samples from the oiled area had higher levels ($P < 0.005$) of staining than those from the unoiled area (mean scores of 5.2 versus 1.9, respectively).

Hematology and Serum Chemistry

Detailed results of analyses on blood samples for each of the four nearshore vertebrate predator species are presented with the species' chapters (Chapter 5[2]; Appendix BIO-01; Appendix HD-07; Appendix PG-01). For each species, some differences in mean values of certain blood parameters were identified between oiled and unoiled areas. However, the differences are few, and no patterns of alteration in blood parameters are evident across species that suggest adverse effects of oil exposure. A subset of the blood parameters are listed in Table 2, allowing comparison of means by area (oiled versus unoiled) and direction of differences across species.

Both harlequin ducks and pigeon guillemots (adults but not chicks) in oiled areas had lower red blood cell counts than birds from unoiled areas (Table 2). Although no significant area differences were detected for the other hematology parameters for both species, mean corpuscular volume tended to be higher in the oiled areas (Appendix HD-07; Appendix PG-01). In concert with lower red blood cells counts, this may indicate a subtle macrocytic anemia for the two avian species. No differences in hematological parameters were identified for sea otters, and no differences between areas are apparent for river otters.

Serum chemistries also showed few differences between areas for any of the four species, and no differences that were consistent across species (Table 2). Higher levels of GGT, a serum enzyme, were noted for sea otters from oiled areas in all 3 years of sampling ($P < 0.001$), but not in any of the other species. Other serum enzymes (AST, ALT, AP, and LDH) were not elevated in sea otters from oiled areas ($P > 0.05$). Pigeon guillemots (adults) in the oiled area had levels of AST approximately twice as high as those in the unoiled area ($P < 0.05$), but no elevations were seen in the other serum enzymes. The GGT levels in adult pigeon guillemots from the oiled area were actually lower ($P < 0.05$) than in those from the unoiled area.

No area differences were detected for haptoglobin levels in harlequin ducks, pigeon guillemots, or river otters (Table 2). Haptoglobin data were available for sea otters captured in 1996 and 1997. Analysis of variance (on ranks) showed the area × year interaction to be significant, with relatively low levels in the oiled area the first year and somewhat higher levels the second year compared to the unoiled area. Overall, there was no pattern of significantly higher haptoglobins in oiled areas. Adult male sea otters in both areas generally had considerably higher haptoglobin concentrations than females (Appendix BIO-01).

Body Condition

In general, body condition of the predator species did not differ across areas in a manner that suggested toxic effects of oil on growth or health of the animals. Harlequin ducks showed no clear area differences in body mass or body composition (Chapter 4). In ducks sampled during winter 1998, however, body mass did show an inverse relation with EROD score (GLM with sex and EROD in model; t for EROD = 2.23, $P = 0.03$) suggesting deleterious effects of oil exposure

[2]Blood data from river otters were analyzed by principal components and MANOVA analyses and, thus, an area effect (and associated *P* value) was not computed for individual blood parameters.

on condition. For Barrow's goldeneyes, no inverse relation was detected between EROD activity and body mass.

Pigeon guillemot chicks in the oiled area had lower weights at fledging (Chapter 6). However, based on observations of forage fish delivered to the nests, these lower weights are thought to result from lower quality prey in the oiled area.

Body condition of river and sea otters in the oiled areas was equivalent to that of their counterparts in unoiled areas. For sea otters, subadult females actually were in better body condition ($P < 0.05$) in the oiled area, but a significant difference was not noted for older females or for males of any age.

DISCUSSION

The greater induction of CYP1A in oiled than unoiled areas in all six species examined is strong evidence of persistent contamination of nearshore areas with EVOS through at least summer 1998 and suggests that injury from oil toxicity could be much longer term than previously thought, extending well beyond acute effects from exposure at the time of the spill. Evidence of continued exposure is strongest for sea otters, harlequin ducks, Barrow's goldeneyes, and masked greenlings. For river otters, differences between oiled and unoiled areas are less striking and are minimal in the third year of study. Pigeon guillemot chicks on the nest, just prior to fledging, show very low induction in both areas. For adult guillemots, induction in both areas is also very low; however, it is greater in birds from the oiled area.

The contaminants inducing CYP1A in our study species are almost certainly PAH from the *Exxon Valdez* oil spill. Subsurface oil residues have been found under the cobble-boulder armor of beaches in Prince William Sound as late as 1997 and in at least one location, sheens were observed on the water at the falling tide (Brodersen 1999; Hayes and Michel 1999). These oil residues still contain toxic PAH (Hayes and Michel 1999), which could be released back into intertidal areas. Additional evidence on persistence and potential bioavailability of PAH from residual EVOS oil has been presented by Irvine et al. (1999), Short et al. (1999), Fukuyama et al. (2000), and Harris et al. (2000).

Background hydrocarbons and PCB have both been considered as alternate contaminants that might elicit a CYP1A response. Background PAH concentrations, however, appear unlikely to be the primary contributor to induction. Short and Babcock (1996) assayed sediments and mussels (*Mytilus trossulus*) and concluded other sources of PAH in intertidal regions of Prince William Sound were negligible prior to the spill. Detectable background hydrocarbons are found in benthic sediments in Prince William Sound, but the apparent source of these is coal deposits in the Gulf of Alaska (Short et al. 1999), and PAH in coal are not bioavailable (Chapman et al. 1996). The PCB are ubiquitous environmental contaminants (Loganathan and Kannan 1994) that also induce CYP1A (Boon et al. 1992; Stegeman et al. 1992) and which have been shown to bioaccumulate in top predators in the marine food web (Colburn and Smolen 1996). To address the possible contribution of PCB, we analyzed harlequin duck serum samples for total PCB and for specific PCB congeners (Appendix HD-08). The PCB were present at low levels, but areas did not differ in either proportion of samples with detectable PCB or in concentrations measured. One congener (138) was correlated with EROD activity. However, after accounting for its variation, birds in oiled areas had considerably higher EROD activity than did unoiled area birds

($P < 0.001$).Thus, we conclude that variation in CYP1A induction between harlequin ducks in oiled versus unoiled areas cannot be fully explained by PCB contamination and, presumably, that this finding extends to the other study species. Serum PCB also have been assayed in sea otters from oiled and unoiled areas (USFWS unpublished data). From both areas, many congeners were present at detectable concentrations, but based on preliminary examination of the data, concentrations of PCB are not elevated in samples from the oiled area relative to the unoiled area. Assays to compare serum PAH concentrations were not attempted because sea otter samples from earlier (1990–91) post-spill studies demonstrated serum PAH concentrations were below detection limits in sea otters from both oiled and unoiled areas (USGS unpublished data). Finally, spatial distribution of our data implicates oil from the 1989 spill as the contaminant, given that samples were collected from a relatively broad area of western Prince William Sound, including Main Bay, Naked Island, and northern Knight Island as oiled areas, and Montague Island and Jackpot Bay as unoiled reference sites. If other contaminants, either background PAH or PCB, were involved in the CYP1A response, it is difficult to envision why they would be distributed differentially among our study areas.

Route of oil exposure can be evaluated by consideration of life histories of the study species. Sea otters, harlequin ducks, and Barrow's goldeneyes all forage primarily on benthic invertebrates. The diet of sea otters consists largely of clams, which require excavating large amounts of sediment to obtain, with crab, sea urchins, mussels, and other invertebrates also taken (Chapter 3 Part A; Chapter 3 Part B). Seaducks forage on a broad array of benthic invertebrates, including amphipods, limpets, snails, chitons, and mussels (Goudie and Ankney 1986). In general, invertebrate prey lack the ability to metabolize hydrocarbons to any significant extent (Vandermeulen and Penrose 1978) and will bioaccumulate hydrocarbon contaminants (Roesijadi et al. 1978; Pruell et al. 1986; Short and Harris 1996). In contrast to the relatively similar results for the two areas observed for river otters and pigeon guillemots, both consumers of fish, highly significant area differences were seen in CYP1A for sea otters, harlequin ducks, and Barrow's goldeneyes implicating diet as a primary route of exposure. However, because sea otters and seaducks spend almost all their time in the water, groom or preen extensively, and may dig in sediments while foraging (particularly sea otters), inadvertent ingestion of contaminated sediments or residues on pelage or plumage is also a likely pathway of exposure. Masked greenlings are a benthic fish that live in close proximity to the bottom and consume a variety of benthic invertebrate prey (McConnaughey 1978) so induction of CYP1A in this species is consistent with observations on sea otters and seaducks.

Pigeon guillemot chicks were sampled while still on their nests where they were fed forage fish brought by adult birds. At this stage, they essentially would have had no direct contact with water or sediments, and their only avenue for contamination would have been through ingestion of contaminated prey. Because PAH are extensively metabolized by most fish species (Varanasi et al. 1989), the observation of low induction of CYP1A and no difference between areas was not surprising for chicks. Adult pigeon guillemots consume primarily fish, but also take invertebrates, particularly in winter (Oakley 1981; Ewins 1993), perhaps explaining the slightly higher induction in adults from the oiled area. River otters, which also consume a diet primarily of fish, did show CYP1A induction, particularly in the first 2 years of sampling (1996–97). However, because river otters forage to a limited extent on invertebrate prey (Larsen 1984; Stenson et al. 1984; Bowyer et al. 1994), they may have ingested hydrocarbons, although this

should have been a far less significant source of contaminants than for species which feed exclusively on invertebrates. River otters would have had opportunity for exposure through direct contact with hydrocarbons in the water and on intertidal shorelines. Because river otters also groom extensively, they would have ingested any PAH on the fur. Duffy et al. (1999) analyzed hydrocarbons on fur of river otters captured in oiled areas in 1996–97 and speculated that, although concentrations measured were very low, they may have been sufficient to induce CYP1A. However, the observation that the area difference for river otters is minimal in 1998, whereas sea otters and harlequin ducks still do differ, suggests that the most important routes of exposure are either through ingestion of invertebrate prey or in behaviors associated with gathering prey.

Decreasing induction of CYP1A would be anticipated over time, as residual oil dissipates from the intertidal areas. For only two species, sea and river otters, we have multiple years (1996 through 1998) of data. River otter IHC staining indices were lowest in the third year of study, when sample sizes were largest and a wider geographical area was covered. Mean scores at that point were relatively similar in both areas (the year x area interaction only approached marginal significance, at $P < 0.12$, but the power to detect the interaction was relatively low, at 0.43). Possibly, IHC values from the two areas were indeed converging by the third year of the study, which could have resulted from declining hydrocarbon exposure, combined perhaps with a low intake of invertebrate prey by river otters. Results of RT-PCR assays on river otter samples from 1998 (the only year that RT-PCR data were available) also did not demonstrate a difference between areas. In contrast through 1998, sea otters in the oiled and unoiled areas exhibited a marked difference in induction of CYP1A; however, there was some suggestion of a decline by the third year of sampling: mean values for sea otters in the oiled area were 28.9 ± 35.1, 38.6 ± 61.8, and $13.1 \pm 8.2 \times 10^6$ molecules of CYP1A mRNA per 100 ng of total RNA for 1996, 1997, and 1998, respectively. Whether this signals declining exposure for sea otters or is just a sampling issue (extremely high values were seen in less than 15% of the otters in the first 2 years and in no otters the third year) is unknown. Multiple years of data are also available for masked greenlings, which were resampled in oiled regions of Prince William Sound in 1998 and 1999 by Jewett et al. (2000). Through 1999, CYP1A was found to be elevated in fishes collected in the spill area, and there generally was no indication of a decline in CYP1A induction between 1996 and 1999 (Jewett et al. 2000).

Concentrations of PAH required to elicit the observed CYP1A responses in the study species are not known, as controlled studies relating oil dosage to induction in birds and mammals are limited. Ben-David et al. (2001) measured CYP1A by RT-PCR and IHC in captive river otters exposed to oil. Doses used were 5 or 50 ppm/day/kg body weight, and CYP1A induction generally appeared to be in the range of that observed in river otters captured during the Nearshore Vertebrate Predator Study. However, as discussed by Ben-David et al. (2001), other factors may be influencing the observed results for the captive otters. The RT-PCR assay is recognized to be extremely sensitive for detecting induction of CYP1A (Vanden Heuvel et al. 1994); EROD is also a sensitive technique, and low concentrations of PAH in the environment may be sufficient to induce CYP1A in animals in the wild. However, the general consistency of induction in almost all sea otters and harlequin ducks sampled from the oiled areas suggests that exposure, although it may be at a low level, is relatively continuous and widespread within the study area.

The extent of oil exposure undoubtedly has varied substantially among individual members of populations in contaminated areas. Animals alive in 1989 may have been exposed to large amounts of relatively unweathered oil. Post-mortem studies on sea otters that died in 1989 after the spill identified a suite of pathologies associated with oil exposure, including damage to liver, kidney, and lung (Lipscomb et al. 1993, 1994) and presumably surviving animals in the oiled areas also suffered some degree of injury. During 1990–92, significant quantities of oil remained in the environment (Wolfe et al. 1994) providing opportunities for continued exposure at relatively high levels and perhaps aggravating existing initial injury. Blood samples collected from river and sea otters during 1990–92 suggested that animals in the spill zone did suffer chronic organ damage (Duffy et al. 1994, 1996; Rebar et al. 1996). The likelihood of encountering oil would certainly have decreased over the last decade, and animals born after 1989 (or those that may have moved in from unoiled areas) would have been exposed to declining concentrations of residual oil. Furthermore, oil contamination was patchy initially (O'Clair et al. 1996) and likely became even more so with time. Thus, when we sample a population 6 or 8 years post-spill, the individual animals should encompass a wide range of exposures, depending on ages, size of home ranges, areas in which they have resided, and occurrence of chance events such as major storms that may mobilize oil in sediments. Induction of CYP1A in sea otters in oiled areas supports this pattern, as a 350-fold difference is seen between the low and high RT-PCR values.

A critical consideration is whether or not there are ramifications for health of individual animals that result from either acute and/or chronic exposure to residual oil. Direct effects of oil toxicity have been documented for many species (Geraci and St. Aubin 1990; Leighton 1993). However, few studies have looked at relatively low levels of oil exposure, particularly combined with other stressors. For seaducks, Holmes et al. (1979) showed that oil exposure had deleterious effects on health and survival of birds when they were also subjected to cold stress, which may be more representative of conditions faced by birds in the wild. A further health concern is that, although the CYP enzymes are involved in detoxification reactions, intermediate metabolites of these reactions may be mutagenic or carcinogenic (Nebert and Gonzales 1987; Fox 1993), thereby presenting an increased risk to the population, particularly when exposure is long term, as is the case in oiled areas of Prince William Sound.

Overall, we have little direct evidence of adverse health in individuals of our study species that show greater induction of CYP1A. Harlequin ducks do show a negative relation between CYP1A and body mass suggesting that oil toxicity is manifested on condition. However, preliminary analyses of blood data on those individual birds show no correlation between CYP1A and serum enzymes. The lower red blood cell counts in the oiled area, also noted for adult pigeon guillemots, combined with higher (but not significantly different) mean corpuscular volumes in both species may indicate a mild anemia that has been previously reported in birds acutely exposed to oil (Leighton 1993; Yamato et al. 1996). Survival rates of harlequins in oiled areas are lower than those of birds in the unoiled area (Chapter 4), and population surveys indicate declining abundance in the oiled areas (Rosenberg and Petrula 1998). Unfortunately, CYP1A data are not available for individual ducks from the survival rate study, thus, a direct link between CYP1A and survival cannot be evaluated. Further research on harlequin ducks, including controlled dosage studies, is under way to evaluate relations among oil exposure, health, and survival.

Sea otter populations in some of the most heavily oiled areas also have shown no increase over the last 6 years (Chapter 3 Part A) and remain well below estimated pre-spill abundance (Appendix SO-07). Resighting rates of tagged otters in 1997–99 suggest that either survival of sea otters in the oiled study area is compromised or otters are emigrating from that area at a higher rate compared to animals in the unoiled area (Chapter 3 Part A). Further evidence of relatively poor survival of sea otters in oiled areas is provided by Monson et al. (Appendix BIO-03) using age-at-death data on carcasses recovered in the spill zone from 1989 to 1998. However, no relations were seen among CYP1A induction and body condition and hematology or serum chemistry values. In fact, sea otters in the oiled area showed evidence of better body condition possibly because of greater availability of prey relative to otter numbers in that area (Chapter 3 Part B). Growth (or lack thereof) of the sea otter population could be influenced to a large extent by poor survival of older otters resulting from acute exposure in 1989 and continued heavy exposure in the early post-spill years. Sea otters rescued from the spill area in 1989 and subsequently placed in aquaria had relatively poor survival rates and at necropsy, organ pathologies were similar to those observed in oiled sea otters dying in 1989 (T. Williams, personal communication). Sea otters that survived initial exposure to oil but remained in the wild likely experienced similar sublethal pathologies and, as a consequence, were more susceptible to natural stressors leading to increased mortality and restricting population recovery. However, based on model results (Appendix BIO-03), chronic injury was not limited to otters alive at the time of the spill, but extends to those that were born in subsequent years as well.

Current levels of oil exposure, however, may be of little consequence for the sea otter population. Blood data collected between 1996 and 1998 show few area differences that seem to be biologically significant (Appendix BIO-01) suggesting organ damage is no longer prevalent. However, serum GGT, an enzyme associated with liver disease or injury (Hanigan 1998), was elevated in about 12% of the otters from oiled areas. From a clinical perspective, the GGT elevations are slight; nevertheless, area differences are statistically significant ($P < 0.002$) over the 3-year study period. These observations are consistent with results from a 1992 study that compared serum chemistries of sea otters in eastern (unoiled) and western (oiled) Prince William Sound (Appendix BIO-01) and with earlier observations of liver pathology in oiled otters (Lipscomb et al. 1993, 1994) and suggest that GGT may be a sensitive indicator of hepatocellular damage in this species. By 1996–98, both the mean GGT level and the proportion of animals with elevated values had declined in the oiled area compared to 1992. We conclude that individuals with liver damage are gradually being lost from the population, and exposure to oil is diminishing. Increases in GGT (and other liver enzymes) were also noted for mink *Mustela vison* exposed to oil (Mazet et al. 2000), but river otters captured in oiled areas of Prince William Sound in 1991 did not have elevated GGT values (Duffy et al. 1996). No GGT differences were found between areas for river otters or harlequin ducks in the present study, and adult pigeon guillemots actually had lower mean GGT in the oiled area suggesting that GGT is not a sensitive indicator for these species or that liver damage is not present.

No correlation was found between serum GGT and CYP1A induction in sea otters. This may result because CYP1A induction reflects recent exposure (and our method is highly sensitive), whereas GGT elevations presumably reflect sublethal initial exposure in addition to longer-term exposure, the cumulative effects of which have been sufficient to cause organ damage. (However, no correlation was seen between GGT and age of sea otters, which suggests

long-term exposure may be of less importance, relative to the variability in exposure experienced by individual animals.) Finally, higher GGT values in only a few (less than 15% by 1998) sea otters sampled in the oiled area, with normal values for most animals, suggest that residual oil in quantities sufficient to cause toxicity is highly patchy in distribution, and that animals with liver dysfunction from earlier exposure are being lost from the population and are not available for sampling.

For river otters, no adverse signs of health were seen in animals from the oiled areas. Body mass, which was lower for river otters in oiled areas in earlier post-spill studies (Duffy et al. 1993), was similar between areas and in 1998, animals from oiled areas did not exhibit higher haptoglobins or serum enzymes in contrast to earlier observations (Duffy et al. 1994). Furthermore, in 1997–98, survival rates of river otters in oiled and unoiled areas were similar and relatively high (Chapter 5).

Pigeon guillemots in oiled areas have not recovered from the spill (Chapter 6); however, our interpretation of recovery of this species is complicated by a decrease in the abundance of high-lipid forage fish, herring, and sand lance (Hayes and Kuletz 1997). A regime shift in ocean climatic parameters in the late 1970s and 1980s may have contributed to these declines (Piatt and Anderson 1996), but oil-related injury may also have been a factor at least for herring (Brown et al. 1996a,b). The impact of the spill on sand lance has not been investigated.

Fledgling weights of pigeon guillemots in the oiled area were lower, likely due to lower quality prey (Chapter 6). The lack of CYP1A induction in chicks suggests oil toxicity is not a factor limiting recovery. Adults have greater potential for exposure given that they are in direct contact with the water and also may consume a low proportion of invertebrates (Ewins 1993), which may account for the slightly elevated EROD activities in adult birds from the oiled area. Further, adults may also be subjected to greater stress as the chicks grow and require increased amounts of food. The EROD activities measured in adults and chicks from both areas were low, relative to those in the seaducks, but this may reflect, in part, a species difference rather than simply a difference in exposure. Blood testing of adult guillemots found higher levels of AST, a serum enzyme associated with liver, heart, or muscle tissue damage, in birds from the oiled area, but lower levels of GGT in the same group. A further finding was the lower red blood cell counts in oiled area as discussed above. None of these differences, however, were seen in chicks at either 20 or 30 days of age.

Additional insight into feasibility of CYP1A measurement was gained by application of different approaches in our study. The opportunity to compare the three methods on samples from Barrow's goldeneyes was extremely useful as it enabled us to determine that web samples from harlequin ducks were not an appropriate tissue for IHC assays. The RT-PCR and IHC values measured on river otters did not correlate, perhaps because they are measuring different aspects of CYP1A induction (mRNA production versus EROD activity) that may vary in level or timing between the two tissues. Further, a general lack of concurrence in RT-PCR results was seen between river and sea otters, given that, for sea otters, the mean RT-PCR value in the unoiled area was much lower than that obtained for river otters from the unoiled area. The IHC assays on skin from river and sea otters also provided different results: staining was observed in a high proportion of river otter samples, particularly in 1996 and 1997. In contrast, sea otters showed no staining, and all samples scored zero. This may reflect low (below detection) levels of CYP1A in sea otter skin, or that skin tissue is not appropriate for the assay, or, perhaps, that a procedural

artifact is interfering with staining in these samples. Further efforts are needed to assess within and between-species differences in CYP1A induction measured in different tissues and by different methods.

CONCLUSIONS

In consideration of the generally similar results on blood and body conditions (i.e., lack of area differences) for all four study species, differential conclusions regarding the status of recovery need to be made cautiously. Where available, data on survival rates are extremely valuable in determining population status. For river otters, CYP1A and survivorship data both support recovery of the species by 1998. For harlequin ducks, the determination that oil exposure continues to limit recovery can be made because radiotelemetry studies demonstrated lower survival rates in the oiled area. Without survival rate information, conclusions about the role of oil toxicity in limiting recovery of harlequin ducks might be similar to those tentatively drawn for sea otters as other evidence is generally similar. For sea otters, however, the indirect evidence of relatively poor survival in the oiled area generates concern and indicates further population monitoring is needed to ascertain recovery status. Survival rates have not been directly assessed for pigeon guillemots, which limits our ability to evaluate oil toxicity as a factor constraining their recovery.

Based on the CYP1A biomarker, we conclude that exposure to residual oil from the 1989 spill continues in western Prince William Sound. Consumption of contaminated benthic invertebrate prey and direct contact with oil in the sediments or water (and subsequent inadvertent consumption during foraging or grooming) are possible routes of exposure. However, the CYP1A results must be considered in concert with data for each species on individual health. By 1998, the apparent impact of oil on river otters is negligible. Further data are required before drawing conclusions about pigeon guillemots although preliminary evidence does not implicate oil toxicity as a primary factor limiting recovery. Harlequin ducks are the species that appears to be at greatest risk from continued exposure, and oil toxicity is implicated as a primary factor limiting their recovery. Similarly, based on a synthesis of results, recovery of sea otters also has been limited by chronic exposure to oil, although there is some indication that oil concentrations are diminishing and, consequently, that the severity of oil-related injury is declining.

RECOMMENDATIONS

The Nearshore Vertebrate Preadators (NVP) Study design, utilizing four nearshore predator species captured across several different oiled and reference sites and looking simultaneously at demographic, trophic, and health aspects of the populations, provided a solid foundation for comparisons to aid in the interpretation of our data and assess continued injury to and recovery status of these species following the 1989 spill. We realized tremendous benefit by integration of a variety of research objectives on individual species into a larger, coordinated project.

The NVP Study was the first post-EVOS study to gather extensive data on continuing oil exposure using the CYP1A biomarker. At the initiation of the project, 6 years post-spill,

essentially nothing was known about the expression of CYP1A in our study species. As has been pointed out numerous times in other discussions of EVOS research findings, baseline data on the expression of CYP1A would have been of enormous value in interpreting our results. Further, collection of biomarker data much earlier, in the months and years immediately following the spill, would have been useful to evaluate variability and temporal patterns of CYP1A expression. In the event of a similar disaster, we recommend biomarker studies be implemented as rapidly as possible to help define the temporal pattern of exposure and biomarker expression. Controlled studies of exposure can also be implemented to understand the relations among oil exposure, CYP1A induction, and changes in individual health, including blood parameters, and to assess relations among the expression of CYP1A measured at varying biochemical stages, using different methodologies.

Our knowledge of normal ranges of hematology and serum chemistry values for the study species was also relatively limited at the time of the spill. Between 1989 and 1995, baseline data had been collected for river otters and sea otters, but not for the avian species. By the conclusion of the NVP Study, we had obtained baseline data on all four species, providing a basis for initial health assessment of exposed animals. However, our knowledge of the correspondence between blood values and oil toxicity to organ systems with chronic exposure is still limited as we have had few opportunities for direct observation and histological sampling of tissues from live animals. Blood variables measured in our study may not necessarily be a sensitive indicator of organ damage. Opportunities to directly examine organ systems for pathological changes in wild-caught animals or in laboratory studies with controlled exposure are valuable and should be furthered.

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Table 1. Matrix of cytochrome P450 1A assays and tissues and years sampled for Nearshore Vertebrate Predator Study species.

	Method		
Species	RT-PCR[a]	IHC[b]	EROD[c]
Sea otter (*Enhydra lutris*)	Blood lymphocytes 1996, 1997, 1998	Skin (flipper and underarm) 1996, 1997	
River otter (*Lontra canadensis*)	Blood lymphocytes 1998	Skin (underarm) 1996, 1997, 1998	
Harlequin duck (*Histrionicus histrionicus*)		Foot web 1996	Liver 1998
Pigeon guillemot (chicks; *Cepphus columba*)			Liver 1998
Barrow's goldeneyes (*Bucephala islandica*)		Foot web, liver 1997	Liver 1997
Masked greenlings (*Hexagrammos octogrammus*)		Liver 1996	

[a]RT-PCR=reverse-transcriptase-polymerase chain reaction
[b]IHC=immunohistochemical
[c]EROD=ethoxyresorufin O-deethylase

Table 2. Comparison of selected blood variables for the nearshore vertebrate predator species in oiled and unoiled areas.

	Harlequin duck[a]		Pigeon guillemot[b]		River otter[c]		Sea otter[d]	
Variable[e] (units)	Unoiled Median (N)	Oiled Median (N)	Unoiled Mean, SD (N)	Oiled Mean, SD (N)	Unoiled Mean, SE (N)	Oiled Mean, SE (N)	Unoiled Mean, SE (N)	Oiled Mean, SE (N)
WBC (10^3/µL)	13.7 (80)	12 (87)	8 ± 1 (7)	8 ± 2 (10)	11.9 ± 0.8 (46)	11.0 ± 0.7 (50)	10.7 ± 0.26 (52)	9.3 ± 0.37 (63)
RBC (10^6/µL)	3.17 (48)	2.85 (51)	3.76 ± 0.59 (6)	3.01 ± 0.35 (10)	9.0 ± 0.1 (46)	8.7 ± 0.1 (50)	4.82 ± 0.05 (52)	4.85 ± 0.04 (63)
Hematocrit (%)	54 (79)	55 (86)	58 ± 6 (7)	53 ± 5 (10)	44.7 ± 0.7 (46)	44.4 ± 0.7 (50)	57.8 ± 0.58 (52)	58 ± 0.39 (63)
Eosinophils (%)	1 (79)	2 (87)	0 ± 1 (7)	0 ± 0 (10)	0.4 ± 0.3 (46)	1.3 ± 0.3 (50)	14.8 ± 0.77 (52)	16.4 ± 1.15 (63)
Sodium (mEq/L)	155 (37)	161.5 (42)	143.8 ± 9.3 (4)	138.6 ± 17.1 (7)	150.4 ± 0.4 (55)	150.7 ± 0.4 (59)	152.45 ± 0.32 (91)	152.55 ± 0.55 (80)
Potassium (mEq/L)	2.7 (39)	2.45 (44)	—	—	4.1 ± 0.05 (55)	4.1 ± 0.05 (59)	4.23 ± 0.03 (91)	4.10 ± 0.04 (80)
ALT (IU/L)	—	—	—	—	177 ± 10.9 (55)	150 ± 10.6 (59)	203 ± 12.1 (91)	204 ± 16.6 (80)
AST (IU/L)	65.5 (80)	58 (88)	461 ± 199 (7)	979 ± 816 (10)	579 ± 67.7 (55)	377 ± 65.3 (59)	235 ± 13.6 (91)	239 ± 23.8 (80)
AP (IU/L)	316 (39)	305.5 (44)	137 ± 102 (6)	93 ± 70 (8)	150 ± 7.8 (55)	163 ± 7.5 (59)	136 ± 8.1 (91)	133 ± 5.8 (80)
GGT (IU/L)	9.0 (39)	11.0 (44)	10.8 ± 8.2 (7)	3 ± 5 (9)	32.4 ± 3.9 (55)	27.0 ± 3.7 (59)	14.0 ± 0.87 (89)	17.8 ± 1.2 (80)
LDH (IU/L)	363.5 (80)	349 (88)	915 ± 143 (7)	892 ± 296 (10)	281 ± 23.2 (55)	189 ± 22.4 (59)	462 ± 23.3 (91)	335 ± 15.1 (80)

Table 2. Continued

Variable[e] (units)	Harlequin duck[a] Unoiled Median (N)	Harlequin duck[a] Oiled Median (N)	Pigeon guillemot[b] Unoiled Mean, SD (N)	Pigeon guillemot[b] Oiled Mean, SD (N)	River otter[c] Unoiled Mean, SE (N)	River otter[c] Oiled Mean, SE (N)	Sea otter[d] Unoiled Mean, SE (N)	Sea otter[d] Oiled Mean, SE (N)
Haptoglobin (mg Hp-Hb per 100 mL)	97 (76)	102.8 (83)	93 ± 50 (7)	122 ± 28 (8)	22.3 ± 6.0 (55)	27.6 ± 5.7 (59)	43.3± 7.43 (57)	37.8 ± 8.01 (57)

[a]*Histrionicus histrionicus* from Mulcahy et al. (Appendix HD-07).
[b]*Cepphus columba* adult birds from Seiser (Appendix PG-01).
[c]*Lontra canadensis* from Bowyer et al. (Chapter 5). Note that data were collected over 3 years, and for several variables, significant year differences were detected. See Bowyer et al. (Chapter 5) for means presented by year.
[d]*Enhydra lutris* from Bodkin et al. (Chapter 3 Part A).
[e]Abbreviations: WBC white blood cells; RBC red blood cells; ALT (SGPT) alanine aminotransferase; AST (SGOT) aspartate aminotransferase; AP alkaline phosphatase; GGT gamma glutamyl transferase; LDH lactate dehydrogenase; Hp haptoglobin; Hb hemoglobin.

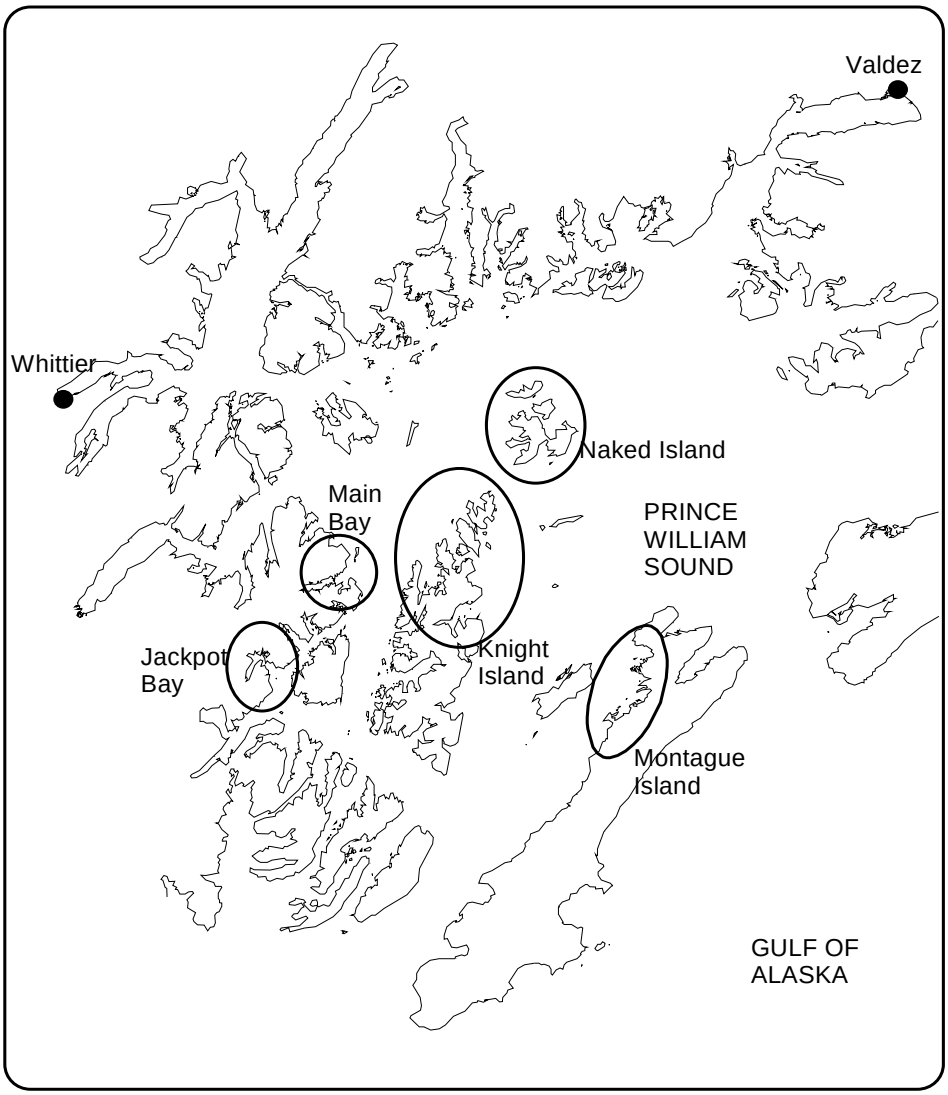

Figure 1. Map of Prince William Sound, Alaska, showing Nearshore Vertebrate Predator Study areas (*within circles*). The areas at Knight and Naked Islands were the primary oiled areas; additionally, for harlequin ducks *(Histrionicus histrionicus)* only, Main Bay was an oiled study area.

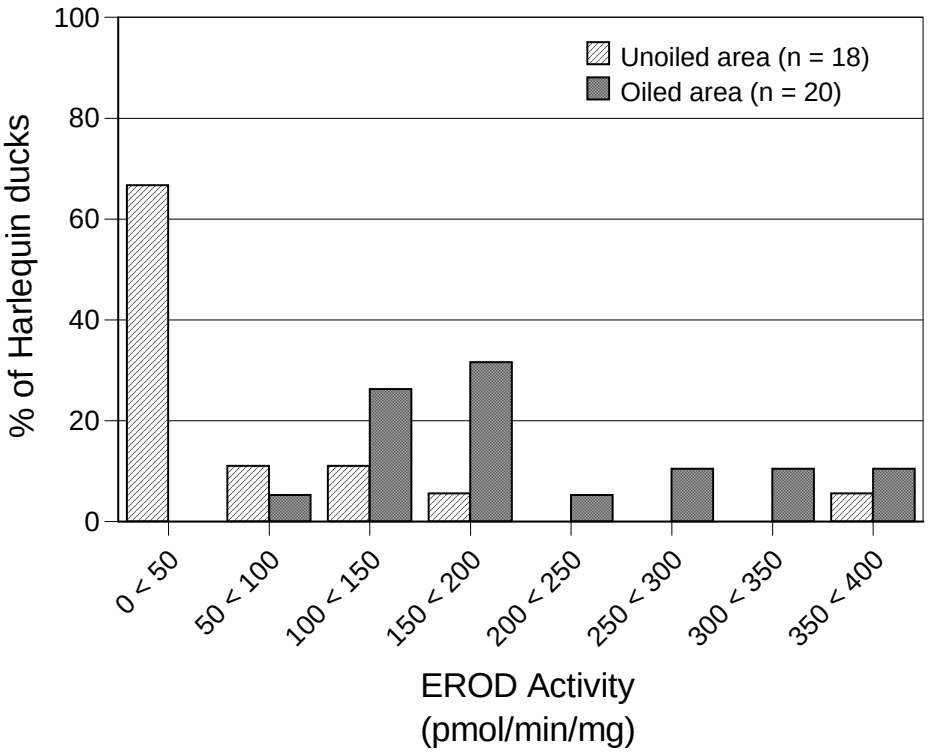

Figure 2. Distribution of ethoxyresorufin O-deethylase (EROD) activities for liver samples collected from harlequin ducks *(Histrionicus histrionicus)* in oiled and unoiled areas, March–April 1998.

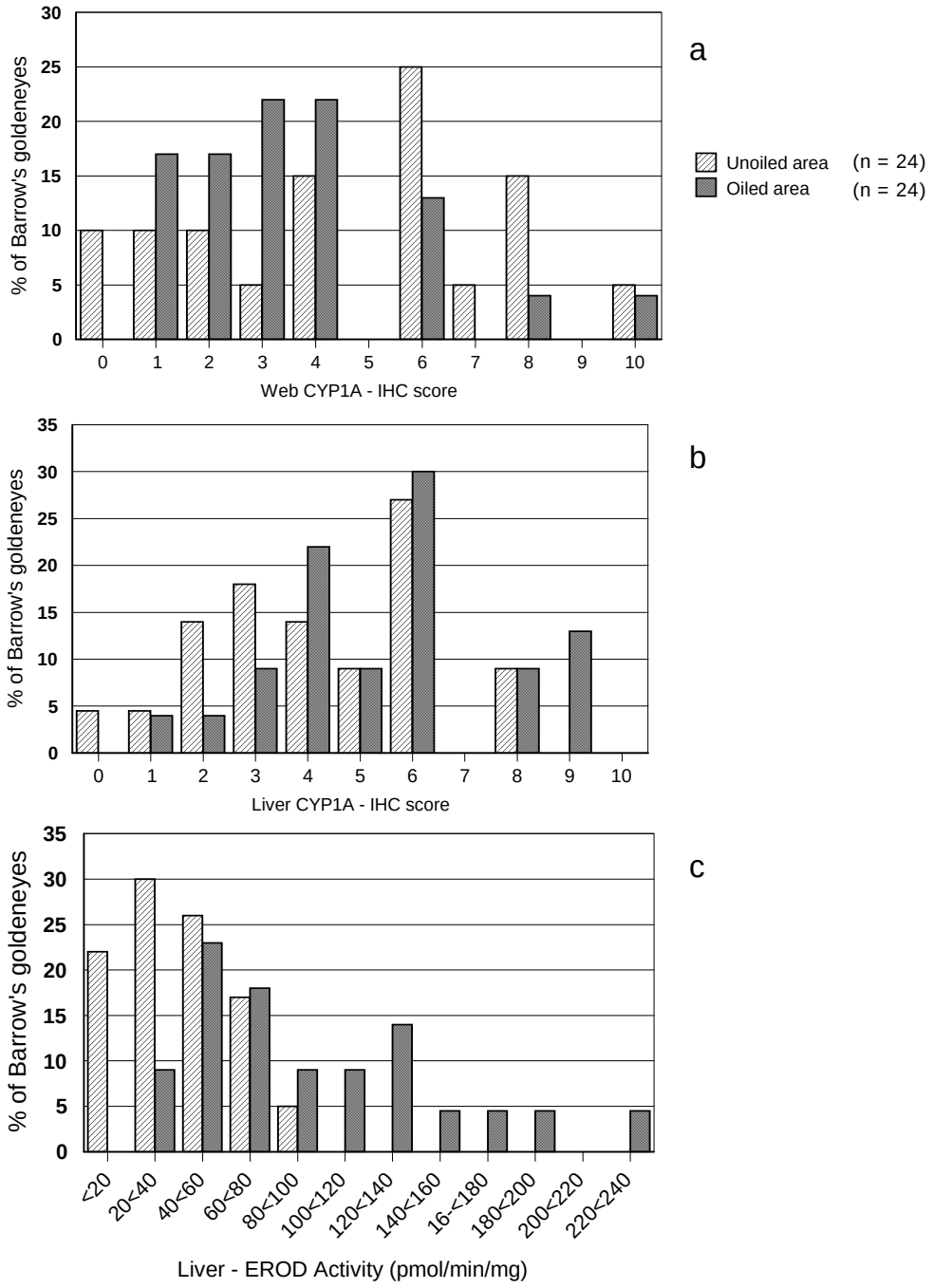

Figure 3. Distribution of (*a*) immunohistochemical (IHC) scores on web, (*b*) IHC scores on liver, and (*c*) ethoxyresorufin O-deethylase (EROD) activities on liver samples collected from Barrow's goldeneyes (*Bucephala islandica*) in oiled and unoiled areas, winter 1996–1997.

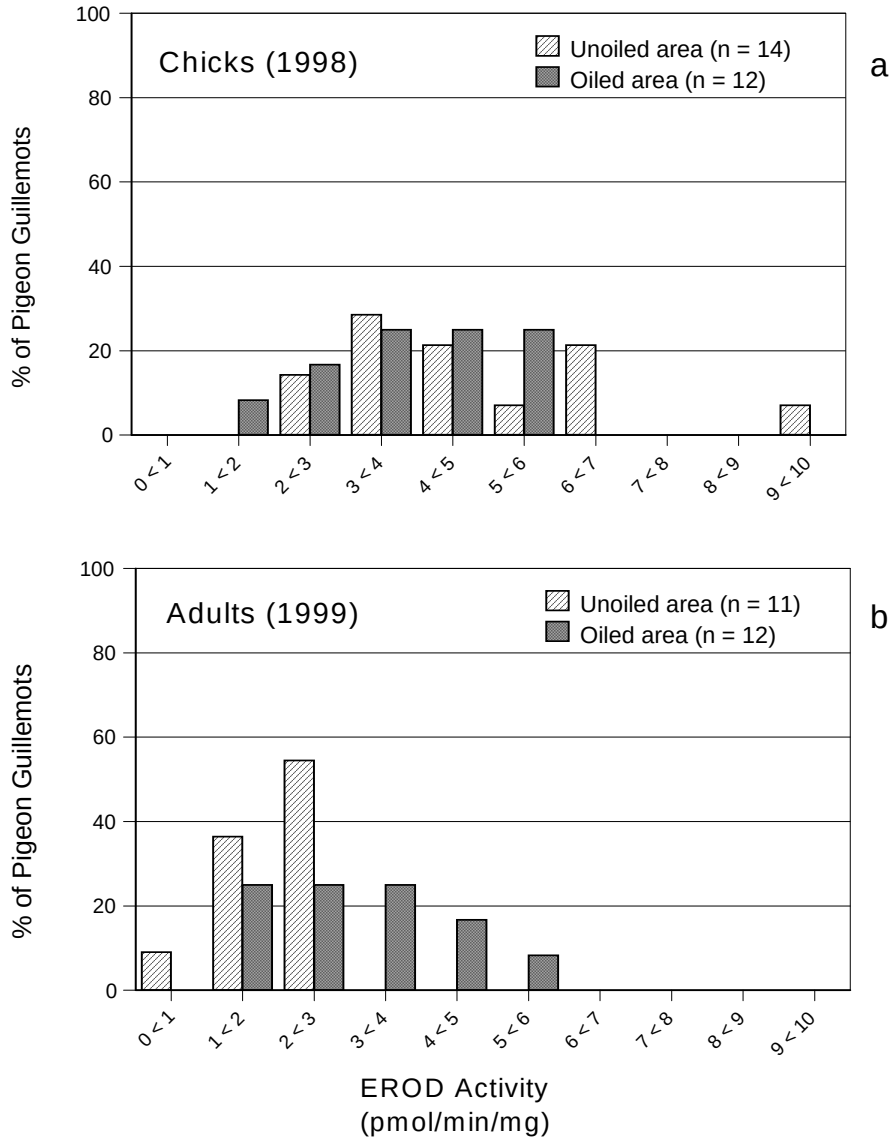

Figure 4. Distribution of ethoxyresorufin O-deethylase (EROD) activities for liver samples collected from (*a*) pigeon guillemot *(Cepphus columba*) chicks in summer 1998, and (*b*) pigeon guillemot adults in early summer 1999 in oiled and unoiled areas.

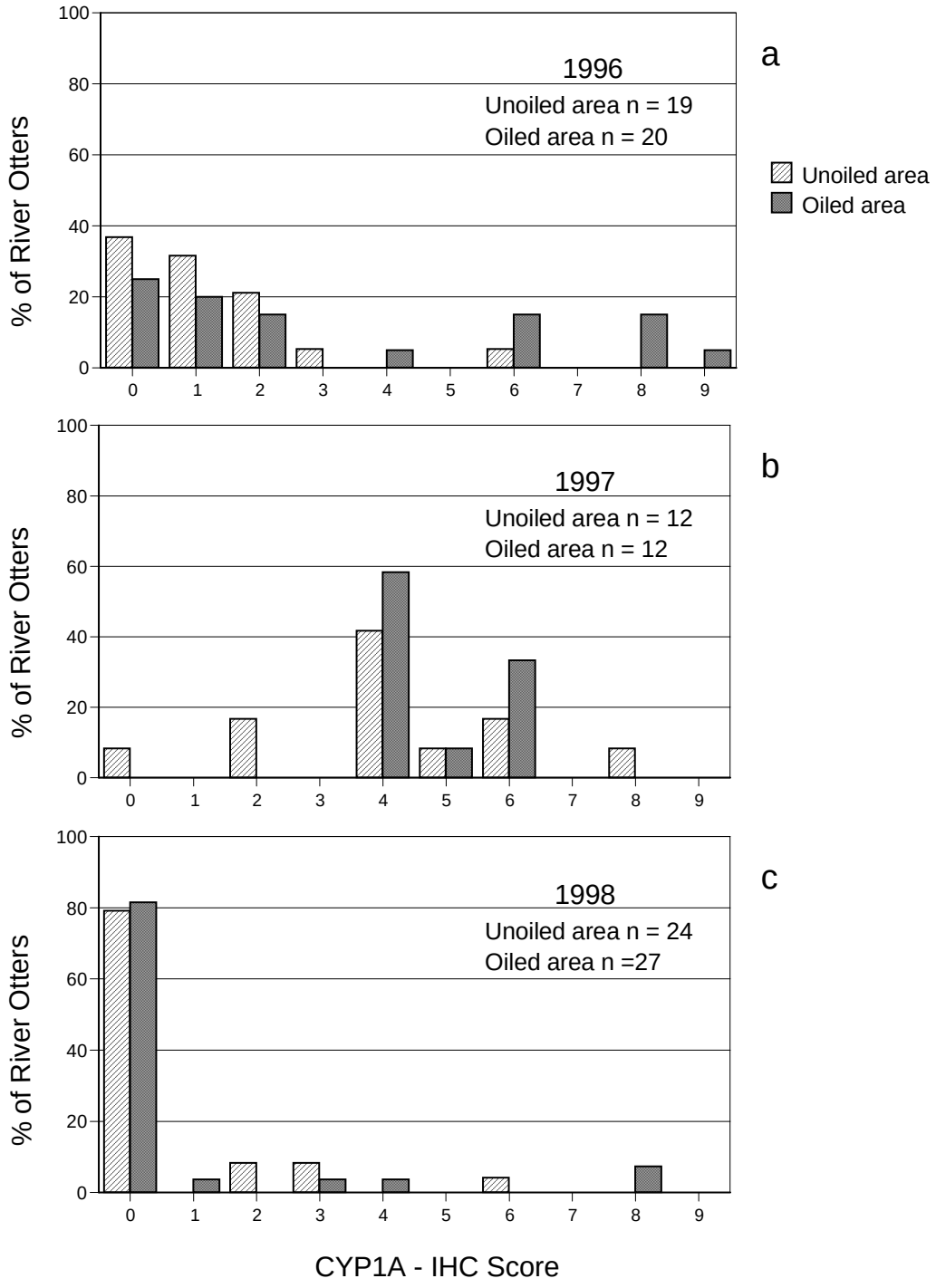

Figure 5. Distribution of immunohistochemical (IHC) scores on skin samples collected from river otters (*Lontra canadensis*) in oiled and unoiled areas in (*a*) late spring 1996, (*b*) late spring 1997, and (*c*) spring 1998.

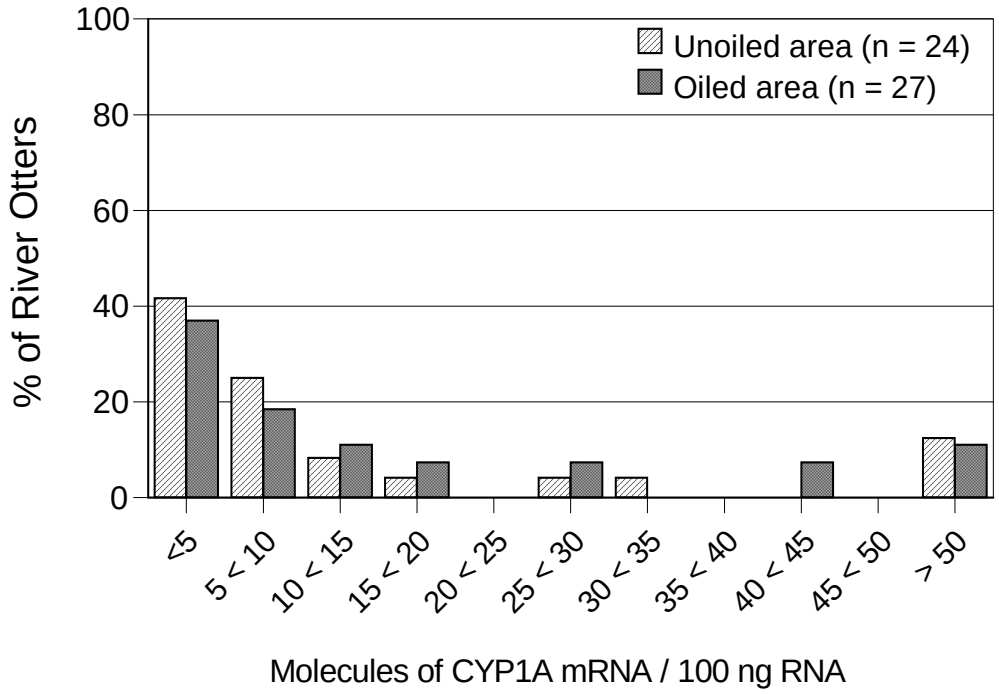

Figure 6. Distribution of reverse-transcriptase polymerase chain reaction (RT-PCR) values on peripheral blood mononuclear cells collected from river otters (*Lontra canadensis*) in oiled and unoiled areas in spring 1998.

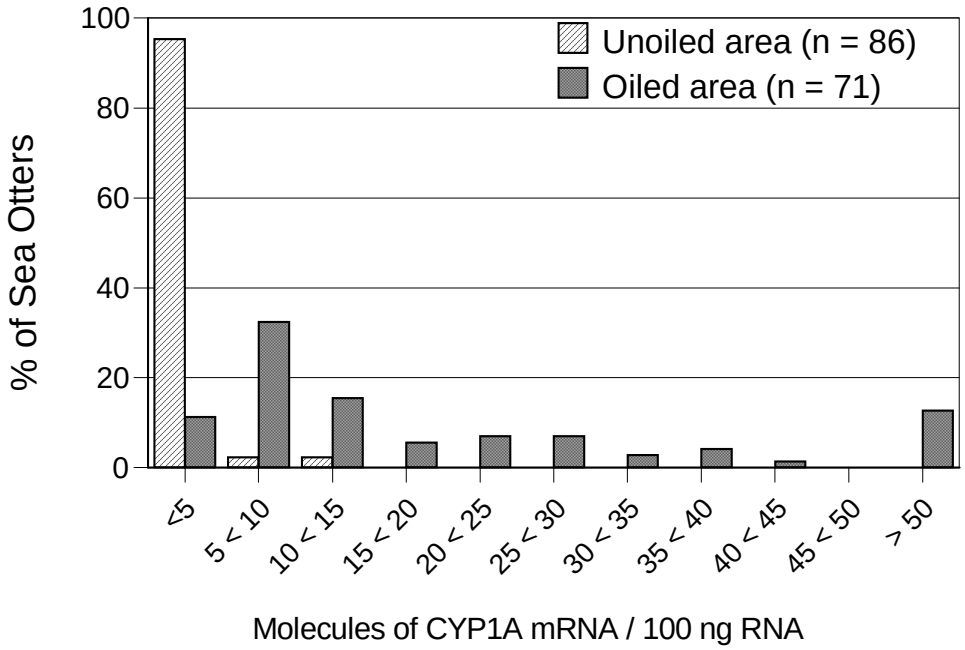

Figure 7. Distribution of reverse-transcriptase polymerase chain reaction (RT-PCR). Values on peripheral blood mononuclear cells collected from sea otters (*Enhydra lutris*) in oiled and unoiled areas in summers 1996–1998.

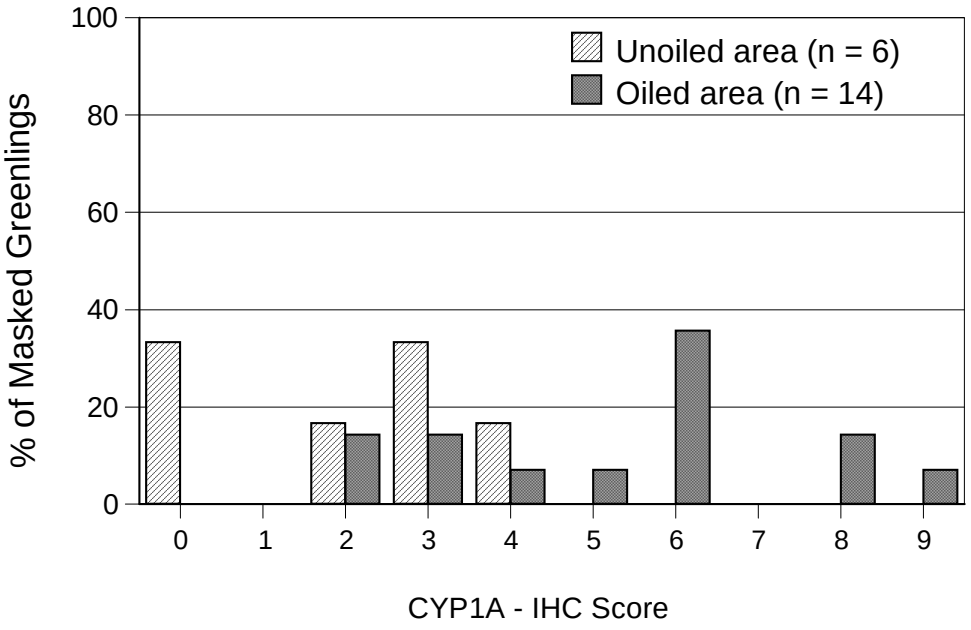

Figure 8. Distribution of immunohistochemical (IHC) scores on liver samples from masked greenlings (*Hexagrammos octogrammus*) collected in oiled and unoiled areas in summer 1996.

Chapter 3. Sea Otter (*Enhydra lutris*) Perspective

Part A. Sea Otter Population Status and the Process of Recovery from the 1989 *Exxon Valdez* Oil Spill

Part B. Food Limitation and the Recovery of Sea Otters Following the *Exxon Valdez* Oil Spill

Part C. Trophic Linkages among Sea Otters and Bivalve Prey in Prince William Sound, Alaska, in the Aftermath of the *Exxon Valdez* Oil Spill: Implications for Community Models in Sedimentary Habitats

Sea Otter (*Enhydra lutris*) Perspective: Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

Part A. Sea Otter Population Status and the Process of Recovery from the 1989 *Exxon Valdez* Oil Spill[1,2]

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ABSTRACT

Sea otter (*Enhydra lutris*) populations were severely affected by the 1989 *Exxon Valdez* oil spill in western Prince William Sound, AK, and had not fully recovered by 2000. Here we present results of population surveys and incorporate findings from related studies to identify current population status and factors affecting recovery. Between 1993 and 2000, the number of sea otters in the spill-area of Prince William Sound increased by about 600 to nearly 2700. However, at Knight Island, where oil exposure and sea otter mortality in 1989 approached 0.90, no increase has been observed. Sea otter reproduction was not impaired and the age and sex structure of animals captured are consistent with both intrinsic reproduction and immigration contributing to recovery. However, low resighting rates of marked animals at Knight Island compared to an unoiled reference area, and a high proportion of young animals in beach cast carcasses through 1998, suggest that the lack of recovery was caused by relatively poor survival or emigration of potential recruits. Significantly higher levels of cytochrome P4501A (CYP1A), a biomarker of hydrocarbons, were found in sea otters at Knight Island in 1996-98 compared to unoiled Montague Island, implicating oil effects in the lack of recovery at Knight Island. Delayed recovery does not appear to be directly related to food limitation. Although food availability was relatively low at both oiled and unoiled areas, we detected significant increases in sea otter abundance only at Montague Island, a finding inconsistent with food as a principal limiting factor. Persistent oil in habitats and prey provides a source of continued oil exposure and, combined with relatively low prey densities, suggests a potential interaction between oil and

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[2]Document citation has not been revised to reflect overall final restoration report citation.

food. However, sea otters foraged more successfully at Knight Island and young females were in better condition than those at Montague Island. We conclude that progress toward recovery of sea otters in Prince William Sound is evident, but that in areas where initial oil effects were greatest, recovery may be constrained by residual spill effects, resulting in elevated mortality and emigration. It is evident that internal reproduction and immigration of juveniles has been the primary means of population recovery, as opposed to broad scale redistribution of adults from outside affected areas. The result is a recovery period protracted by long-term spill effects on survival and emigration and intrinsic limits to population growth.

Key words: abundance, biomarker, *Enhydra lutris*, food, mortality, reproduction, survival.

INTRODUCTION

Sea otters (*Enhydra lutris*) are coastal marine carnivores of the North Pacific Ocean (Kenyon 1969) spending their entire life cycle in nearshore habitats (Wild and Ames 1974, Estes 1980, Riedman and Estes 1990). They utilize both rocky and unconsolidated habitats, and in Prince William Sound (PWS) forage primarily on burrowing clams, crabs, and mussels (Calkins 1978, Kvitek and Oliver 1988, Doroff and Bodkin 1994, Dean et al. 2001). Sea otters lack the insulating blubber of other marine mammals, and have instead a dense, water resistant pelage and an elevated metabolic rate that enable them to survive in a cold aquatic environment (Kenyon 1969, Costa and Kooyman 1984, Riedman and Estes 1990). Using their mouth and forepaws, they groom their pelage for up to several hours each day to maintain its insulating quality (Estes et al. 1982). Because both the habitat they forage in and the prey they consume serve as repositories for spilled oil, and the fur they rely on for insulation is sensitive to contamination, sea otters are particularly susceptible to the effects of oil spills.

Sea otters are long lived (Kenyon 1969, Bodkin et al. 1997), with relatively low annual reproductive rates (females produce single offspring) (Siniff and Ralls 1991, Bodkin et al. 1993, Jameson and Johnson 1993, Riedman et al. 1994, Monson and DeGange 1995, Monson et al. 2000a) and high annual adult survival (Siniff and Ralls 1991, Monson and DeGange 1995, Monson et al. 2000a, Monson et al. 2000b). These life history traits resulted in a long-term annual growth rate of about 10% in PWS following the end of the fur harvest in 1911 (Bodkin et al. 1999). Mechanisms ultimately limiting sea otter density are not completely understood, but likely include limits imposed by prey availability and some form(s) of territoriality (Kenyon 1969, Bodkin et al. 2000). Factors affecting sea otters, such as contaminants or predation, that result in either reduced reproduction, increased mortality, or increased emigration will eventually lead to reduced population growth rates (Riedman and Estes 1991).

The grounding of the T/V *Exxon Valdez* in March 1989 resulted in a spill of approximately 42 million L of crude oil (Spies et al. 1996), with acute mortality among a diverse number of marine organisms (Peterson 2001), including the sea otter (Ballachey et al. 1994). Prolonged effects of the spill on populations have been more difficult to measure but are evident across a wide range of taxa, including invertebrates (Fukuyama et al. 2000), fishes (Jewett et al. 2002), birds (Irons et al. 2000, Esler et al. 2002, Golet et al. 2002), and mammals (Bowyer et al. 1995, Ben-David et al. 2001) and are reviewed in Peterson (2001). To assess the recovery status of the nearshore ecosysytem in western Prince William Sound (WPWS), a comprehensive study

of nearshore bird and mammal predators, including harlequin ducks (*Histrionicus histrionicus*), pigeon guillemots (*Cepphus columba*), river otters (*Lontra canadensis*), sea otters, and their invertebrate prey, was conducted during 1995-1999 (Holland-Bartels 2000). Here we report on the sea otter component of that study; results of other components are presented in accompanying papers in this volume (Dean et al. 2002, Esler et al. 2002, and Golet et al. 2002).

Accurate and defensible estimates of acute sea otter mortality from the spill, beyond the number of carcasses recovered (nearly 1,000 throughout the spill area), could not be made, largely because accurate and recent pre-spill population estimates were not available (Ballachey et al. 1994). While several widely disparate estimates of sea otter mortality resulting from the spill in PWS have been published, all include recognized uncertainties (Garrott et al. 1993, Bodkin and Udevitz 1994, DeGange et al. 1994, Garshelis 1998, Dean et al. 2000). Although acute mortality estimates generated controversy (Eberhardt and Garrott 1997, Garshelis and Estes 1997, Garshelis 1998), it is clear that sea otter mortality was extensive and widespread regardless of the particular estimate.

Oil exposure and acute sea otter mortality were not distributed evenly throughout PWS (Bodkin and Weltz 1990, Bodkin and Udevitz 1994). Generally, shoreline oiling decreased as distance from the spill origin increased. Along the spill trajectory in WPWS, bays and shorelines oriented between north and east were subjected to high oil exposure and persistence (Fig. 1), while more distant shores or those with different orientation may have received little or no oiling (Neff et al. 1995). Exposure to oil and sea otter mortality in 1989 were particularly high along the shores of the northern Knight Island archipelago in WPWS (Figs. 1 & 2), where mortality in one large bay was estimated at 0.88 (Bodkin and Udevitz 1994).

Immediate impacts of oil spills on sea otters occur through contamination of pelage, ingestion, and inhalation, and are well understood (Costa and Kooyman 1982, Siniff et al. 1982, Geraci and Williams 1990, Williams et al. 1995), but longer-term effects have not been well documented. Chronic effects of initial oil, continued exposure to persistent oil (through prey or physical contact) and reduction in prey caused by direct oiling all may result in long-term injury. Oil-related damage to liver, kidney, and lung was documented in sea otters that died in 1989 after being exposed to oil (Lipscomb et al. 1993, 1994; Williams et al. 1995). Presumably, the health of animals that survived initial exposure could have been compromised and exposed individuals may exhibit reduced long-term survival (Monson et al. 2000b). Further, sea otters have a high potential to encounter residual oil while excavating infaunal and epifaunal prey such as clams and mussels.

The importance of spill related effects on sea otter prey populations to sea otter recovery are not well understood. Oil in nearshore habitats persisted through at least 1997 in WPWS, although at greatly reduced levels from 1989 (Hayes and Michel 1999, Carls et al. 2001) and through 1994 along the Alaska Peninsula (Irvine et al. 1999). Projected recovery times for hydrocarbon levels in mussel beds to return to background range to 30 years (Carls et al. 2001). The spill and related clean-up activities resulted in reductions in densities of some sea otter prey along oiled shorelines, including intertidal clams (Driskell et al. 1996), mussels (Gilfillan et al. 1995, Highsmith et al. 1996), and the helmet crab *Telmessus cheiragonus* (Dean et al. 1996). Elevated levels of hydrocarbons were found in some surviving sea otter prey populations, including intertidal mussels from 1990 through at least 1995 (Short and Babcock 1996, Babcock et al. 1996, Carls et al. 2001) and some mollusks from northern Knight Island in 1991

(Armstrong et al. 1995). Residual oil at northern Knight Island through at least 1996 resulted in elevated tissue burdens of hydrocarbons in clams (*Protothaca staminea*) that reduced their growth and survival (Fukuyama et al. 2000). However, in subtidal clams collected from southern Knight Island, where oiling was less, elevated hydrocarbon levels were not detected in 1991 (Doroff and Bodkin 1994).

One measure of recovery after a population decline is simply replacement of the number of animals removed from the affected population. Two independent modeling efforts projected recovery times for spill-affected sea otter populations (Garrott et al. 1993, Udevitz et al. 1996). Both sea otter recovery models implicitly assume that all otters remaining in the area of interest will contribute equally to the replacement of animals removed and that the study population is geographically closed. Garrott et al. (1993) applied an estimated pre-spill annual population growth rate of 1.09 to the entire PWS 1989 post-spill sea otter population of about 13,000 and estimated a minimum recovery time of 3 years, but recognized that population growth was not evident, based on post-spill surveys through at least 1991. An age-specific reproductive and survival rate recovery model for only the oiled WPWS population of about 2,000, produced by Udevitz et al. (1996), projected recovery times ranging from 10 to 23 years, dependent on assumptions regarding survival rates. Although Garrott et al. (1993) and Udevitz et al. (1996) calculated similar growth rates (about 1.10/yr), recovery times differed primarily because the number of animals assumed to contribute to recovery differed (13,000 vs. 2,000). However, neither recovery model incorporated long-term spill related effects in projecting recovery times.

There are relatively few data available to evaluate how a sea otter population may recover from the removal of a proportion of its population, but except for human aided translocations only two mechanisms are possible. One is intrinsic growth, resulting from births that exceed deaths and emigration from within the affected population. The second is successful immigration of surplus animals from outside the affected area. The latter assumes an increased survival probability of individuals that immigrate (relative to the area they came from), and not simply a large-scale redistribution of the population. As the potential source of replacements into a reduced population increases beyond the area of reduction, recovery time will decrease if growth rates are held constant. The relative contribution of intrinsic growth and immigration to recovery of depleted sea otter populations is unknown. The reduction in sea otter abundance resulting from the spill provides a unique opportunity to observe and describe the processes contributing to replacement of lost animals.

The primary purpose of this work is to assess the status of the oil-affected sea otter population in PWS, and if the population is not recovering, determine if growth is constrained by toxicological effects of oiling, indirect effects of food limitation, or remnant demographic consequences from the spill (e.g. changes in age and sex composition). We present results of population surveys conducted between 1993 and 2000 in WPWS that identify the current status of the affected population. Additionally, we review related studies on sea otter exposure to residual oil and on sea otter prey populations available to support recovery, and integrate the results of those studies with the population studies to provide a synthesis of the state of sea otter recovery and factors apparently affecting recovery. We also discuss our findings relative to the conservation and recovery of other reduced or depleted sea otter populations.

Because the 1989 spill was accidental, it was not replicated, making it difficult to disassociate the potentially confounding effects of area from those of the spill. Much of the work

we report on here was designed to contrast a single oil-affected area and an unoiled reference area. We recognize the limits imposed by the sampling design (lack of replication of the oil spill treatment and selected study areas) relative to assigning cause to observed differences and extrapolating beyond study area boundaries. However, we make no inference to areas outside our intensive study areas, except where we have direct observations, such as the WPWS survey area. Because of limits imposed by study design we recognize that our findings relative to potential spill effects and constraints to sea otter recovery are subject to interpretation.

STUDY AREA

The WPWS study area includes all shorelines within the PWS spill area that were oiled, and some areas along the boundary of the spill area that may not have been oiled (Fig. 1). The area included approximately 2,358 km^2 of sea otter habitat (defined by the area between the shoreline and the 100 m depth contour or 0.4 km from shore, whichever is greater).

For comparison between oiled and unoiled habitats, we selected two intensive study areas within WPWS. The oiled site was in the area of northern Knight Island, including 198 km of shoreline from the northwest Pt. of Herring Bay to the southeast Pt. of Bay of Isles, including the smaller islands in the northern archipelago (Fig. 2). This area received heavy oiling and sea otter mortality approached 90% (Bodkin and Udevitz 1994). An estimated 165 sea otters were removed from the heavily oiled area around northern Knight Island as a result of the spill (Dean et al. 2000) and few if any sea otters remained there following the spill in summer 1989 (Bodkin and Udevitz 1994, J.Bodkin & D. Monson, unpublished data). Oil persisted for at least 6 years in some nearshore sediments and in some invertebrate populations (Babcock et al. 1996, Boehm et al. 1996, Fukuyama et al. 2000, Carls et al. 2001). Our unoiled reference area included 72 km of shoreline along Montague Island southwest from Graveyard Pt. and extended to Green Island (Fig. 2). Our two study areas are separated by a minimum of 24 km of open water and we observed no movement of marked animals between areas.

RECOVERY STATUS

Aerial survey methods

Aerial survey methods follow those described in detail in Bodkin and Udevitz (1999) and consisted of two components: (1) strip transects and (2) intensive search units to estimate the probability of detection of otters along strips. Sea otter habitat was sampled in two strata, a stratum characterized by high sea otter densities between the shore and 40 m depth contour, and a deeper water stratum offshore between the 40 and 100 m depth contours, where sea otter densities are usually lower. Survey effort was allocated proportional to expected sea otter abundance by systematically adjusting spacing of transects within each stratum. Transects 400-m wide were surveyed by a single observer at an airspeed of 65 mph (29 m sec^{-1}) and an altitude of 300 ft (91 m). Strip transect data included location, group size, and group activity (diving or not diving). A group was defined as one or more otters separated by less than 4 m. Transect end points were identified by latitude/longitude coordinates in ARC INFO and displayed visually in the aircraft global positioning system (GPS). Intensive searches, made by flying five 400-m

diameter circles within the strip transects, were conducted systematically to estimate the proportion of animals not detected during strip counts. Population estimates were generated by adjusting strip counts for areas not surveyed and for animals not observed using the intensive searches within strips.

From 1993 to 2000, we conducted an annual summer survey of WPWS (Fig. 1). The area surveyed included approximately 1,003 km^2 in the nearshore stratum and 1,355 km^2 in the offshore stratum.

From 1995 to 2000, we surveyed our northern Knight and Montague study areas (Fig. 2). Because those areas are relatively small, precision in individual estimates was limited by the number of transects in each area (sample sizes). Therefore, we replicated the surveys in each study area up to six times within each year, within a two-week period in mid summer. In 1993 and 1994, only a single estimate was obtained for our Knight and Montague study areas, using strip transect and intensive search unit data collected in our larger WPWS survey area. The Knight and Montague Island areas we surveyed for sea otters was larger than, but encompassed all the area sampled for their prey (Dean et al. 2002).

Trends in population estimates over time were calculated by regressing the natural logs of survey counts (Ln [x]) over time. The slope of the line was back transformed by the antilog to yield a discrete growth rate. Because of apparent non-linearity in population estimates at Montague Island, we did not calculate an average annual growth rate, but simply report annual population number and proportional change.

Aerial survey results and discussion

Between 1993 and 2000, there was a significant increase (P = 0.03) of about 600 sea otters in WPWS (Fig. 3). The population appeared generally stable from 1993 to 1996, with most of the increase apparently occurring after 1996. The minimum estimate was 2,054 (se = 698) in 1993 and the maximum was 3,119 (se = 494) in 1998. The annual growth rate was estimated at 0.04, and the rate from 1996 to 2000 was 0.05 (Fig. 3). The observed rate of increase in WPWS is about half the predicted rate used in recovery models (Garrott et al. 1993, Udevitz and Ballachey 1998) and half the long-term rate during recovery of the PWS sea otter population during much of the 20^{th} century (i.e. something is constraining the rate of recovery).

Between August 1995 and July 2000, we completed four to six replicate sea otter surveys at our Knight and Montague Island intensive study areas each year (Fig. 4). We also estimated abundance (without estimated precision) within our intensive study areas in 1993 and 1994, using data from the WPWS surveys. At Knight Island, the mean population size over the 8 years was estimated to be 77 (se = 2.4), and there was no significant trend in sea otter abundance. In 2000 we estimated the Knight Island study area population to be 79 (se = 6). This estimate remains slightly less than half of a minimum prespill estimate in this same area of 165, based on the number of carcasses recovered (Dean et al. 2000, Fig. 4) and about a third of a 1973 estimated population size of 237 (Dean et al. 2000). In contrast, at Montague Island, sea otter abundance appeared relatively stable between 1993 (N = 335) and 1995 (N = 297) but increased by about 300 animals between 1995 and 1998 (N = 622; Fig. 4).

The primary conclusion from 8 years of population surveys is that sea otter recovery in most of WPWS is under way, although at a rate less than expected, with an increase of about

600 sea otters between 1993 and 2000. However, we found no comparable increases in sea otter abundance at our northern Knight Island study area, where oil exposure and persistence were high and sea otter mortality approached 90% immediately after the spill. The estimated number of sea otters at northern Knight Island from 1993 to 2000 remains about half the number removed from our study area because of the spill in 1989 (77 vs. 165) (Dean et al. 2000, Fig. 4).

Reproductive survey methods

Indices of annual reproduction, as indicated by ratios of dependent (pups) to independent (non-pup) sea otters, were obtained in each of our intensive study areas (Fig. 2) from small boat surveys in August 1995, 1996, and 1997. Sample units corresponded to coastline transects, 200-m long with widths extending offshore out to the 100-m depth contour or 1/2 the distance to the opposing shoreline, whichever was less. The entire coastline of each study area was surveyed. The survey vessel maneuvered about 200 to 300 m offshore and out to the offshore boundary in an attempt to observe all otters within each sample unit. Two observers used high-resolution 10x binoculars to classify and record otters as either dependent or independent. Proportions of dependent sea otters were calculated for each group of otters within an area, and the proportions within areas were compared using continuity adjusted Chi-Square analysis.

Reproductive survey results and discussion

Ratios of dependent pups to independent animals ranged from 0.29 to 0.48 at Knight Island and from 0.37 to 0.51 at Montague Island (Table 1), with mean ratios of 0.38 (Knight) and 0.42 (Montague). There were no differences among years or between areas ($P > 0.1$). In 1996 we observed a group of 26 young independent male sea otters at Knight Island (see capture-recapture) that resulted in a relatively low independent to dependent ratio of 0.29. This group was not observed after 1996, despite intensive searches around northern Knight Island.

The equivalent and high ratios of dependent to independent sea otters at our study sites suggest several biological processes relevant to sea otter population recovery. First, similar rates of pup production in the oiled and unoiled areas indicate no reproductive impairment. Second, reproduction as indexed by this ratio, equaled or exceeded values reported for sea otters elsewhere in Alaska, and Russia (Riedman and Estes 1990, Johnson and Garshelis 1995, Bodkin et al. 2000). Third, although we noted differences in female age composition (higher proportion of females age 0-3 years captured at Knight, see capture-recapture, Fig. 5), the equivalent ratios suggest that a relatively large proportion of females age three are successfully raising pups at Knight Island. This observation is consistent with sea otters at Knight being in good physical condition (Bodkin et al. 1993, Monson et al. 2000a).

It is possible that immigration of sea otters from unoiled to oiled areas could contribute to recovery of depleted populations (but only to the extent that immigrant survival increased as a consequence of immigration, as otherwise there would be no overall net gain). Immigrants would likely consist of dispersing juveniles of both sexes and older males, as adult females are the most sedentary component of the population, with the smallest home ranges (Garshelis and Garshelis 1984, Riedman and Estes 1990). If immigration were widespread, we would expect a lower dependent to independent ratio in the oiled area, than in unoiled areas. However, similar

dependent to independent ratios between oiled and unoiled areas were observed shortly after the spill (0.46 at Knight and 0.47 at Montague in 1991; Johnson and Garshelis 1995) and during our study (with the exception of 1996, when the group of young males was observed at Knight Island). These results are inconsistent with widespread immigration of sea otters as a principal means of recovery. Further, the high ratio of dependents to independents at Montague Island is consistent with the observed growth being supported, at least in part, through intrinsic reproduction. However, at our oiled area, similar reproduction did not result in population increases and apparently was offset by either increased post-weaning mortality or emigration, evidenced in part by the absence after 1996 of the young male immigrants that we marked at Knight in 1996 (see below).

Capture-recapture methods

In 1996, 1997, and 1998, we captured and tagged sea otters in our intensive study areas. The primary method of capture was tangle nets, supplemented by diver-operated Wilson traps (Ames et al. 1986). Measurements taken from sedated sea otters (Monson et al. 2001) included mass (to the nearest 0.5 lb [230g], 100 lb spring scale) and total length (dorsal, from tip of tail bone to nose in supine position, measured to the nearest cm). A pre-molar was collected from independent animals for aging (Bodkin et al. 1997). Adults and juveniles were tagged with color-coded plastic ear tags (Temple Tag, Temple, Texas) in the interdigital webbing of the hind flipper (Ames et al. 1986). Different colors and tag locations allowed individual identification of all marked animals. Up to 35 cc of blood was collected for blood chemistries and bioindicator analyses (Ballachey et al. 2000). We directed our capture efforts to areas where sea otters were most abundant based on prior skiff, shore, and aerial surveys. We assume that the two capture methods resulted in random samples of the populations.

We used the SAS GENMOD procedure and the Chi-Square statistic to make comparisons of the age and sex composition of sea otters we captured between areas and among years. Each animal captured was assigned to one of 36 categories based on year; capture location, sex and age. Age categories were 0-3 years (juvenile), 4-9 years (adult), and >9 years (aged) (Bodkin et al. 2000). We assumed that capture of individual sea otters was independent of age and sex.

In 1999, we conducted two comprehensive visual surveys of our intensive study areas to search for animals marked in 1996-1998. Surveys were conducted in both April and July. During these surveys, teams of two observers systematically searched the entire study areas, attempting to locate and observe as many otters as possible. Observations were made from shore vantage points that were accessed by small (3 or 4 m) skiffs. High resolution 50-80X Questar telescopes were used to identify each sea otter observed as either marked or unmarked. Each animal was observed until both rear flippers were determined to be with or without tags. Occasionally, otter activity or distance from observer precluded certainty in determining the presence or absence of tags. In those cases, the animal was not included in the analysis. We used estimated population sizes, the expected number of marked animals in the population and the observed number of marked and unmarked animals to estimate retention or survival of marked animals in our two study areas. We assumed that re-sighting followed a binomial distribution with re-sighting probability equaling:

$$\hat{P} = \frac{(m_{96}s_{96}s_{97}s_{98}) + (m_{97}s_{97}s_{98}) + (m_{98}s_{98})}{\hat{N}}$$

Where:

m_i = *number marked in year i*

$\hat{N}$ = *total population size in 1999 (aerial survey est.)*

S^i = *survival rate for year i to i + 1*

The survival rate estimates were based on age-specific survival rates for the PWS sea otter population (Udevitz and Ballachey 1998). Age-specific female survival rates were 0.92 for ages 2-4, 1.00 for ages 5-9, 0.81 for ages 9-15 and 0.00 for ages 16-20. Male survival rates were estimated as the female rate minus 0.05 to account for the generally lower survivorship of males (Siniff and Ralls 1991, Monson and DeGange 1995). Survival of post-weaning juveniles, ages 0-1, was estimated at 0.75.

The tag retention rate was calculated as:

$$\hat{R} = \frac{r\hat{N}}{n(m_{96}s_{96}s_{97}s_{98} + m_{97}s_{97}s_{98} + m_{98}s_{98})}$$

Where:

n = total number of otters sighted in 1999

r = number tags re - sighted in 1999

The variance of the tag retention rate was estimated using a combined bootstrap/Monte Carlo routine. We bootstrapped the 1999 replicate population counts from each study area to estimate a new $\hat{N}$, and used Monte Carlo simulation to provide new estimates of s_i in the calculation of retention rates. We do not derive an estimate of tag retention from recapture rates from our study sites in 1997 and 1998 because of potential biases against recapturing previously handled sea otters.

Capture-recapture results and discussion

During July and August 1996-98, we captured 180 sea otters, with approximately equal numbers captured each year (Table 2). In both areas, most animals were caught with tangle nets (137 of 180, including 68% at Knight Island and 83% at Montague Island). Females were captured at a much higher frequency in all years and in both areas (Table 2), except in 1996 at Knight Island, when the number of males and females captured were equal. We captured males at a higher frequency at Knight Island, compared to Montague Island, each year (Table 1), and the sex ratio of captured animals differed between areas and among years (X^2_{area} = 6.75, P = 0.0094, X^2_{year} = 10.49, P = 0.0053).

Because sex composition differed between areas and among years, we examined age composition by sex. For female sea otters captured at our two study sites, the age class distributions were similar among years but differed significantly between areas (X^2_{area} = 4.58, P = 0.03, X^2_{year} = 0.09, P = 0.96) (Fig. 5). At Knight, we caught a higher proportion of young females aged 0-3 (0.48 at Knight vs. 0.22 at Montague), whereas at Montague we caught more adult females (0.35 at Knight vs. 0.66 at Montague). Nearly equal proportions of older females, >9 years, were caught at both areas (0.16 at Knight vs. 0.13 at Montague). There was no difference in the age class distributions of captured males between areas or years (X^2_{area}= 0.64, P=0.42, X^2_{year}=1.02, P=0.60). In 1996, we captured a relatively large number of young male sea otters from a male group observed at Knight Island. However, this male group was not present at Knight Island during later years and primarily older males, presumably holding territories, were captured in both areas at all other times.

In April 1999, we visually re-sighted 14 marked animals at Knight Island and 26 at Montague Island (Table 3), and, in July, we observed 9 marked animals at Knight Island and 19 marked animals at Montague Island. Average retention rate estimates of marked animals from visual recaptures were three times higher at Montague Island (1.86) than at Knight Island (0.59), with broad, but non-overlapping confidence intervals (Table 3). No otter tagged at one of our study areas was recaptured or re-sighted at the other study area.

Several generalities relative to movements of sea otters are required to provide a framework for evaluating the sex and age differences that we observed among sea otters in our study areas. Adult sea otter home ranges are relatively small and stable, commonly including a few to tens of kilometers of coastline (Jameson 1989, Ralls et al. 1996). Generally, male sea otters exhibit greater movements than females, and juveniles exhibit greater movements than adults and are more likely to disperse from natal areas (Riedman and Estes 1990, Ralls et al. 1996). If population recovery resulted from reproductive recruitment from within the affected population, the sex ratio in the recovering population should favor females, because young males exhibit greater movements and are more likely to be excluded from reproductive areas by territorial males. Alternatively, if recovery resulted from immigration, the sex ratio in the recovering population should favor males, for the same reasons. At Knight Island, we observed consistently higher proportions of male sea otters in our annual samples, compared to Montague Island, particularly in 1996 when the sex ratio was 1:1 (Table 2). The 1996 sex ratio resulted in part from a group of 26 young males (sex assumed based on 12 captures) found near Knight Island (south-east of Eleanor Island; Fig. 2). Because male groups are commonly associated with the initial recolonization of habitat (Riedman and Estes 1990), this finding is consistent with an immigration pathway of recovery. However, this male group was not observed in subsequent ground, skiff, or aerial surveys of northern Knight Island, through 2000. The higher proportion of young animals of both sexes that we captured at Knight Island, compared to Montague Island, remains consistent with recovery through either reproductive recruitment within the area or immigration of young animals of both sexes from outside the study area.

Our aerial survey data describe a population increasing in abundance throughout much of the spill-affected areas of WPWS, although no increase is evident in the northern Knight Island area where oil was persistent and mortality had been high (Bodkin and Udevitz 1994, Dean et al 2000). The high proportion of pups, the large proportion of young females, and the presence of a large group of young males at Knight Island suggest potential population growth could result

from both intrinsic reproduction and immigration. However, the lack of population growth observed at northern Knight Island suggests that losses (due to high mortality, emigration or both) were equivalent to the birth plus immigration rate at Knight Island, thus constraining population recovery. The difference in retention rates estimated from the re-sighting of marked individuals from our study areas is consistent with this conclusion.

Mortality and population trend

Between 1976-1985 and 1989-98, beach-cast sea otter carcasses were systematically collected from the shores of WPWS each spring (Monson et al. 2000b). Ages of individuals dying each year were estimated from teeth collected. Collections prior to the spill, during the spill in 1989, and after the spill provide an annual description of the age distribution of dying otters. Monson et al. (2000b) used time-varying population models in combination with maximum-likelihood methods to evaluate hypotheses about changes in sea otter survival rates in the years following the spill that would result in the observed age distributions after the spill. The model best fitting the data indicates sea otter survival after the spill was generally lower than before the spill (Fig. 6A & 6B) and survival declined rather than increased after the spill, particularly for older animals. Furthermore, the data indicate that animals born after the spill also exhibited reduced survival. The effects of the spill on survival and population abundance appear to be moderated largely by time as those animals affected by the spill eventually die (Fig. 6B & 6C). The divergent population trends at heavily oiled Knight Island, compared to the larger WPWS (Fig. 6C) suggest that effects of the spill on survival reported by Monson et al. (2000b) may persist longest where initial oil impacts were greatest.

Predation-related mortality likely is contributing to the observed population patterns at our two study areas, although the specific predators and magnitude of the effect is largely unknown. At least some losses can be attributed to killer whales and subsistence harvest. There were nine reported cases of killer whale (*Orcinus orca*) predation on sea otters between 1992 and 1996. Of these, three were at Knight Island, including two in our northern Knight Island study area (Hatfield et al. 1998). Another possible attack by a killer whale was reported from Montague Island in 1998 (C. Gorbics and J. DeGroot, personal communication). One human subsistence harvest of a sea otter was reported from our Knight Island study area in 1995, although an additional 25 sea otters were reported as harvested elsewhere at Knight Island and 11 were reported from Naked Island between 1992 and 1998 (U.S. Fish and Wildlife Service, Anchorage, Alaska, USA, unpublished data). During this same period, 11 sea otters were reported as subsistence harvested from Montague Island.

Killer whales have been proposed as agents of decline in Aleutian Islands sea otter populations during the 1990s (Estes et al. 1998). Given the increase in sea otter abundance in WPWS and at Montague Island since 1993, any effect of predation must be localized to the northern Knight Island area, which is inconsistent with the nature of widespread declines attributed to killer whale predation in the Aleutian Islands. In addition, predation events should not result in beach-cast carcasses. The estimates of increased mortality are based on beach-cast carcasses (Monson et al. 2000b), suggesting that predation would be additive to estimates of decreased survival, rather than explaining it. However, any predation occurring in the northern Knight Island area may be expected to have a comparatively large effect because of the relatively

small sea otter population existing there. For example, given the population size of 77 at northern Knight Island, an annual loss of 3 additional animals would offset the expected growth increment of 0.04, the growth rate observed elsewhere in WPWS since 1993. In contrast, an increase in annual mortality of 3 otters at Montague Island would result in only a slight reduction in annual population growth from 52 to 49 animals per year, assuming a similar growth rate of 0.04, and a population size of 586.

CONTINUED EXPOSURE TO OIL

It is possible that residual effects of spilled oil could be limiting recovery of sea otter populations. Exposure to oil following the spill in 1989 resulted in organ damage among lethally exposed animals (Lipscomb et al. 1993, 1994). Presumably sub-lethal exposure also causes similar pathologies among surviving otters, eventually contributing to long-term reduced survival rates such as reported by Monson et al. (2000b). Additionally, residual oil sequestered in nearshore habitats becomes available through disturbances such as storms and excavation by foraging animals such as sea otters. From 1996-98, 157 of the 180 sea otters captured were tested for exposure to oil, hematology, serum chemistries and body condition (Ballachey et al. 2000). Cytochrome P450 1A (CYP1A) is a protein involved in the metabolism of aromatic hydrocarbons. Using a reverse transcriptase polymerase chain reaction (RT-PCR) to quantify mRNA for CYP1A production (Vanden Heuvel et al. 1993), Ballachey et al. (2000) measured mRNA for CYP1A peripheral blood mononuclear cells, and found significantly higher levels in sea otters at Knight Island compared to Montague Island (Fig. 7). Mean CYP1A values at Knight were 27.3×10^6 vs. 1.5×10^6 at unoiled Montague. Ballachey et al. (2000) also report higher CYP1A findings at Knight Island for other species that are residents of nearshore communities, including the harlequin duck, pigeon guillemot, Barrow's goldeneye (*Bucephala islandica),* and the masked greenling (*Hexagrammos octogrammos*). One common feature of these species is a strong behavioral or trophic link to the nearshore marine habitats that were repositories for residual oil. Ballachey et al. (2000) found greater differences in CYP1A levels between oiled and unoiled areas in consumers of nearshore invertebrates (i.e. sea otters and sea ducks), as compared to consumers of fish (i.e. river otters and pigeon guillemots). Because invertebrates do not metabolize hydrocarbons as vertebrates do (Vandermeulen and Penrose 1978), they are capable of accumulating hydrocarbon burdens (Roesijadi et al. 1978, Pruell et al. 1986, Short and Harris 1986).

Many of the nearshore invertebrates that sea otters prey on (e.g. clams and mussels) occur in habitats that serve as repositories for residual oil and they accumulate hydrocarbons in their tissues. Because sea otters consume invertebrates that sequester hydrocarbons and they excavate large volumes of sediments to recover prey (Hines and Loughlin 1980, Kvitek and Oliver 1988), they are potentially exposed to residual oil through two pathways (i.e. in sediments and in prey). Although the levels of exposure that lead to the differences in CYP1A among areas reported by Ballachey et al. (2000) are unknown, the elevated CYP1A levels occurred in the same regions where reduced survival was observed among sea otters (Monson et al. 2000b) and harlequin ducks (Esler et al. 2002) and where sea otter populations have not increased (Figs. 3 & 4). In addition to CYP1A, significantly higher levels of the serum enzyme GGT, associated with liver disease or injury, were found in sea otters from Knight Island from 1996-1998 compared to

Montague Island (Ballachey et al. 2000). Elevated GGT levels are consistent with the liver pathologies observed during the spill (Lipscomb et al. 1993, Lipscomb et al. 1994) and with observations of captive mink (*Mustela vison)* exposed to oil (Mazet et al. 2000). However, high variation in CYP1A and GGT levels led Ballachey et al. (2000) to speculate that residual oil sufficient to cause toxicity is patchily distributed. The convergence of GGT values from oiled and unoiled areas between 1989 and 1998 likely reflects mortality among individuals with chronic organ damage and their removal from the population being sampled. Their conclusion is consistent with the independent results of Monson et al. (2000b) indicating the convergence of survival estimates to pre-spill values largely as a result of mortality within spill-affected cohorts.

FOOD LIMITATION

Sea otter population size at equilibrium density is generally considered to be limited by available food resources. Sea otter prey populations were reduced directly by oiling and shoreline treatments (including physical modifications to habitats through the removal of fine sediments) that persisted for years (Lees et al. 1996). The initial perturbation caused by the spill and subsequent clean-up efforts resulted in cascading effects through shoreline habitats (Peterson 2001). Both direct spill-related reductions in prey and cascading community effects would likely delay recovery of affected sea otter populations through limiting food availability. Further evidence suggests that some biological components of the nearshore community, including some important sea otter prey, had not fully recovered several years after the spill (Jewett et al. 1999, Fukuyama et al. 2000, Dean and Jewett 2001, Peterson 2001), leading Dean et al. (2002) to evaluate in a comprehensive fashion the potential role of food limitation in constraining sea otter recovery at Knight Island. Because of difficulty in directly measuring the diverse array of sea otter prey, uncertainty in energy content and the cost to sea otters in recovering different prey, both direct (energy/area and energy/otter) and indirect (foraging efficiency and body condition) measures of food availability were made at northern Knight Island and at the Montague site (Fig. 2). Dean et al. (2002) found prey availability to be variable, relatively low, but approximately equivalent between areas, while foraging efficiency and young female sea otter condition were significantly greater at Knight Island (Table 4). Dean et al. (2000) also found increasing densities and sizes of sea urchins at Knight Island between 1996 and 1998, where sea otter densities had been reduced since 1989. These findings are consistent with at least a partial relaxation of the predation pressures sea otters are known to exert on their preferred prey (Estes and Palmisano 1977, Estes and Duggins 1995), and suggestive of prey resources sufficient to support some level of sea otter population growth at Knight Island. Although all prey did not demonstrate consistent responses to reduced sea otter densities, preferred clam species (*Protothaca* and *Saxidomus*) were larger in size at Knight Island (VanBlaricom et al. 2001). During the course of this study, we found significant increases in sea otter abundance at Montague Island that apparently were supported by prey availability that was approximately equivalent to prey availability at Knight Island. Therefore, we concur with the conclusion of Dean et al. (2002) that prey populations at Knight Island were capable of supporting a growth rate approximately equal to that observed at Montague Island, and that food limitation may be acting to constrain growth only above those rates observed at Montague Island.

While we do not have strong evidence to suggest that food availability is limiting recovery at Knight Island, it is possible there are important interactions between food availability, chronic exposure to oil contamination, and sources of sea otter mortality that contribute to the lack of recovery we have observed (Fig. 4). Annual population growth rates in PWS averaged about 0.10 throughout much of the 20th century, a level well below the 0.21 observed in some other recovering sea otter populations (Bodkin et al. 1999). Causes for differences in growth rates among recovering populations are unclear but may be explained, at least in part, by potential differences in food availability as well as human sources of mortality (Bodkin et al. 1999). In the decade following the spill in PWS, the surviving sea otter population at Knight Island encountered food resources that were negatively influenced by spill effects (i.e. population reductions and persistent oil) (Jewett et al. 1999, Fukuyama et al. 2000, Dean and Jewett 2001, Peterson 2001). During this same period some prey populations were experiencing the positive effects of reduced sea otter densities (i.e. increasing densities and mean sizes) (Dean et al. 2000, Dean et al. 2002). However, residual oil in their food and environment may lead to additional metabolic costs and reduced foraging efficiency for sea otters (Davis et al. 1988, Ben-David et al. 2000), potentially offsetting benefits gained through increasing prey densities or sizes. Our results support the hypothesis that long-term spill effects may be dominating the process of sea otter recovery, and despite equal or higher levels of prey, otters at Knight Island may be more susceptible to other stresses (e.g. environmental conditions) than otters that do not encounter similar contamination. This interaction may contribute to the elevated levels of mortality observed after the spill (Monson et al. 2000b).

CONCLUSION

Sea otter populations declined precipitously following the *Exxon Valdez* oil spill of 1989. While populations are recovering throughout much of WPWS, in the area most heavily impacted by the spill, we found no evidence of population growth through 2000, and recovery remains incomplete. Accumulating data from sediments and across a broad suite of taxa (including sea otters) that occupy and utilize nearshore habitats indicate residual oil persists and has been transferred through the nearshore food web for up to a decade after the spill. Elevated mortality in, and emigration from, the oiled area appear to be contributing to the lack of population growth. It appears likely that continued exposure to residual oil or persistent sub-lethal effects are linked to mortality and emigration. However, our study design precludes assigning cause to effect. While spill-related reductions in prey populations may be limiting growth below maximum, estimated prey availability at Knight Island should be capable of supporting some level of growth, as indicated by population growth at Montague Island where prey resources are comparable, and elsewhere in WPWS. Based on our findings from Knight Island, recovery of the WPWS sea otter population apparently resulted from intrinsic reproduction and immigration of juveniles as opposed to broad-scale redistribution of adults. The limited reproductive potential of sea otters, coupled with apparent chronic spill related effects on survival, has resulted in a protracted period of recovery, particularly where oiling was extensive and persistent and mortality was greatest.

CONSERVATION AND MANAGEMENT IMPLICATIONS

Our view of sea otters during this century has been one of widespread recovery of both remnant and reintroduced populations. This view has provided unique opportunities to study the community-level consequences of a "keystone" predator recolonizing habitat after long (decades to a century) periods of absence, during which time the community changed dramatically, generally including large increases in sea otter prey populations (Estes and Palmisano 1974, Estes and Duggins 1995). Our understanding of sea otter population dynamics and influences on community ecology has been strongly influenced by this situation of recovering populations with largely unexploited prey resources to support growth rates that reached their theoretical maximum (Estes 1990, Bodkin et al. 1999, 2000). As sea otters increased their range and abundance, we are afforded a very different view, one where populations may achieve a dynamic equilibria (long-term growth $\cong$ 1.00) with their prey resources and exist in a state that may be more representative of their pre-exploitation status in nearshore marine communities. However, as sea otter populations return to their pre-exploitation status, they face increasing threats that may result in population reductions. The 1989 oil spill in PWS provides an opportunity to address new issues relating to recovery processes following large declines in sea otter abundance, but where community structure and prey populations do not have the long periods of relaxed predation that were characteristic of earlier periods of sea otter recovery. It may be unrealistic to expect growth rates to approach the theoretical maximum in cases where prey populations experience only a partial relaxation of the effects of sea otter predation. Further, it is necessary to consider how recovery may be constrained by effects other than simply the reduction in abundance. In the case of oil spills, the potential biological consequences of sub-lethal initial oil exposure, exposure to residual oil over a longer period, and effects of prey reductions are demonstrated in a recovery period extending more than a decade following the *Exxon Valdez* spill. The potential role of predation (both human and other) in constraining recovery of depleted populations also warrants consideration. Additional knowledge of relations between sea otter social organization and behavior and depleted populations may be important in understanding processes regulating population recovery.

Our results identify several issues relevant to the conservation and recovery of species reduced or depleted by similar catastrophic events. First, how large is the pool of survivors that will contribute to recovery within the depleted area, and how will intrinsic growth and immigration contribute? Second, is there potential for residual effects of the event on critical life history attributes such as fecundity and survival, and will residual effects influence emigration and immigration? Third, are there direct or indirect effects of the event on critical resources required for recovery, such as food? And finally, are there sources of mortality that can be reduced to facilitate recovery? Answers to these questions will benefit from a thorough knowledge of the natural history of the species and ecosystem in question.

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Table 1. Ratio of independent to dependent sea otters at Knight and Montague study areas, 1995-1997, Prince William Sound, AK.

Area	Year	# Independents	# Dependents	Independents/Dependents
Knight	1995	44	21	0.48
Knight *	1996	78	23	0.29
Knight (w/o 26 males)	1996	53	22	0.42
Knight	1997	55	24	0.44
Montague	1995	134	68	0.52
Montague	1996	158	58	0.37
Montague	1997	126	50	0.40

* Includes a group of 26 young males near SE Eleanor Island in 1996.

Table 2. Age class composition and sex ratios of sea otters captured at Knight[1] and Montague Islands, 1996-1998, in Prince William Sound.

		Age Class			
Year	Area (N)	0-3 yrs (%)	4-7 yrs (%)	8+ yrs (%)	#?F: #?M
1996	Knight (30)	17 (0.57)	7 (0.23)	6 (0.20)	15:15
	Montague (31)	5 (0.16)	17 (0.55)	9 (0.29)	24:7
1997	Knight (19)	8 (0.42)	8 (0.42)	3 (0.16)	15:4
	Montague (29)	4 (0.14)	16 (0.55)	9 (0.31)	22:7
1998	Knight (22)	8 (0.36)	7 (0.32)	7 (0.32)	18:4
	Montague (35)	12 (0.36)	12 (0.36)	11 (0.28)	33:2
1996-98	Knight (71)	33 (0.46)	22 (0.31)	16 (0.23)	48:23
	Montague (95)	21 (0.22)	45 (0.47)	29 (0.31)	79:16

[1] Excludes 9 animals captured at Naked Island (8 in 1997 and 1 in 1998).

Table 3. Retention rates of marked sea otters at Knight and Montague Islands, based on visual resighting of marked individuals.

Area & Time	Population size[1]	# Observed	# Expected[2]	# Re-sighted	"Retention" R	95% CI of R
Knight						
1999 April	81	32	20	14	0.71	0.49-0.92
1999 July	81	31	19	9	0.47	0.33-0.62
Montague						
1999 April	586	120	16	26	1.66	1.17-2.26
1999 July	586	71	9	19	2.05	1.43-2.73

[1] Population size estimates from aerial surveys.
[2] Based on estimated survival of marked animals and the number of animals observed.

Table 4. Summary of energy availability, energy consumption, foraging time and young female sea otter condition at Knight and Montague Islands (95% confidence interval) (From Dean et al. 2000a).

Metric	Knight Is.	Montague Is.	Significance at 0.05
Energy/area (kj/m^{-1})	74.2 ($\pm$ 91.6)	149.3 ($\pm$ 144.0)	N.S.
Energy/otter	4.8 x 10^6 ($\pm$ 6.0 x 10^6)	1.1 x 10^6 ($\pm$ 1.2 x 10^6)	N.S.
Prey consumption (kj/hr)	2260 ($\pm$ 280)	1900 ($\pm$ 270)	Significant
Hrs/day foraging	9.9 $\pm$ (1.2)	11.8 ($\pm$ 1.5)	Significant
Wt/total length (g/cm)	170	160	Significant

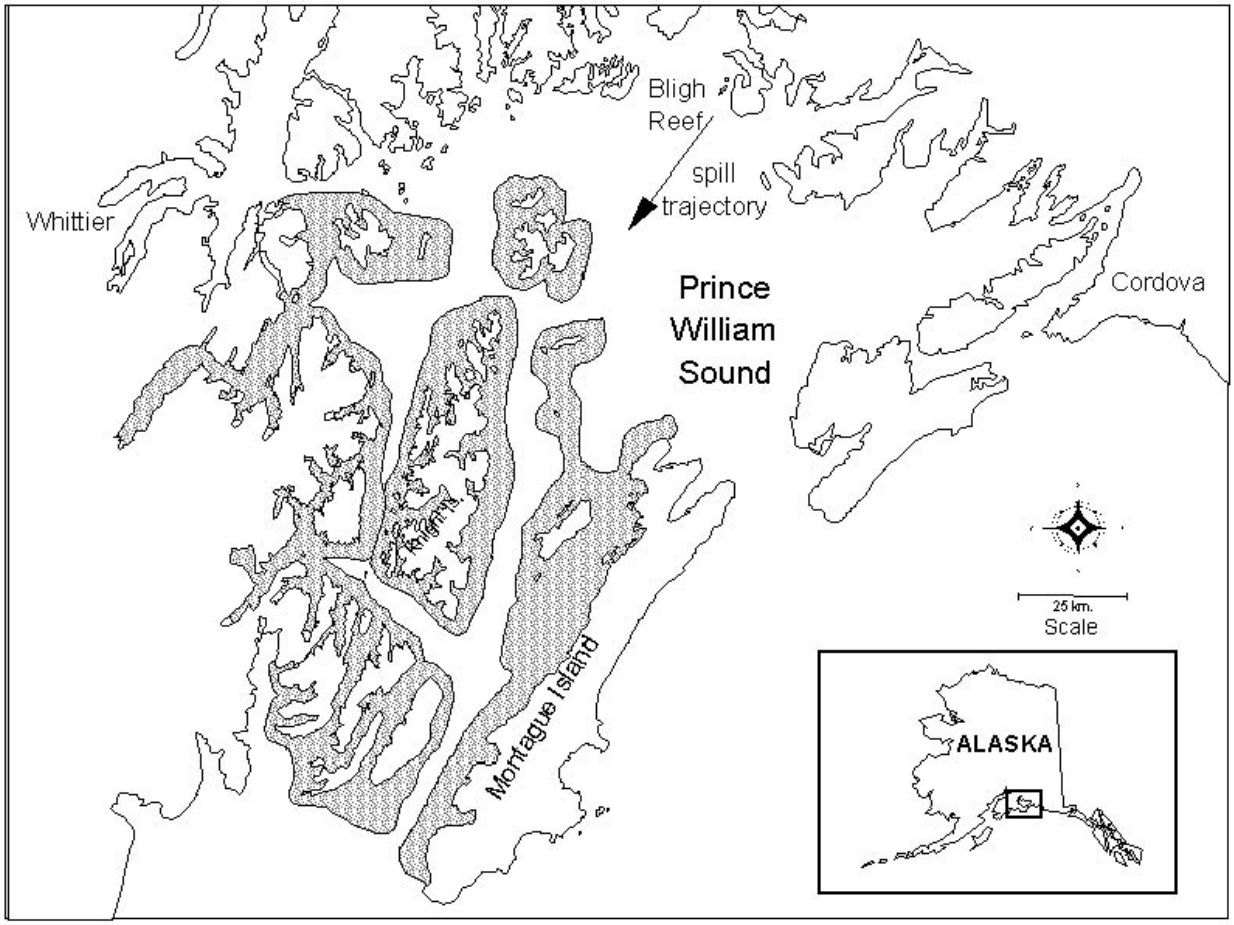

Figure 1. Western Prince William Sound sea otter survey area (shaded).

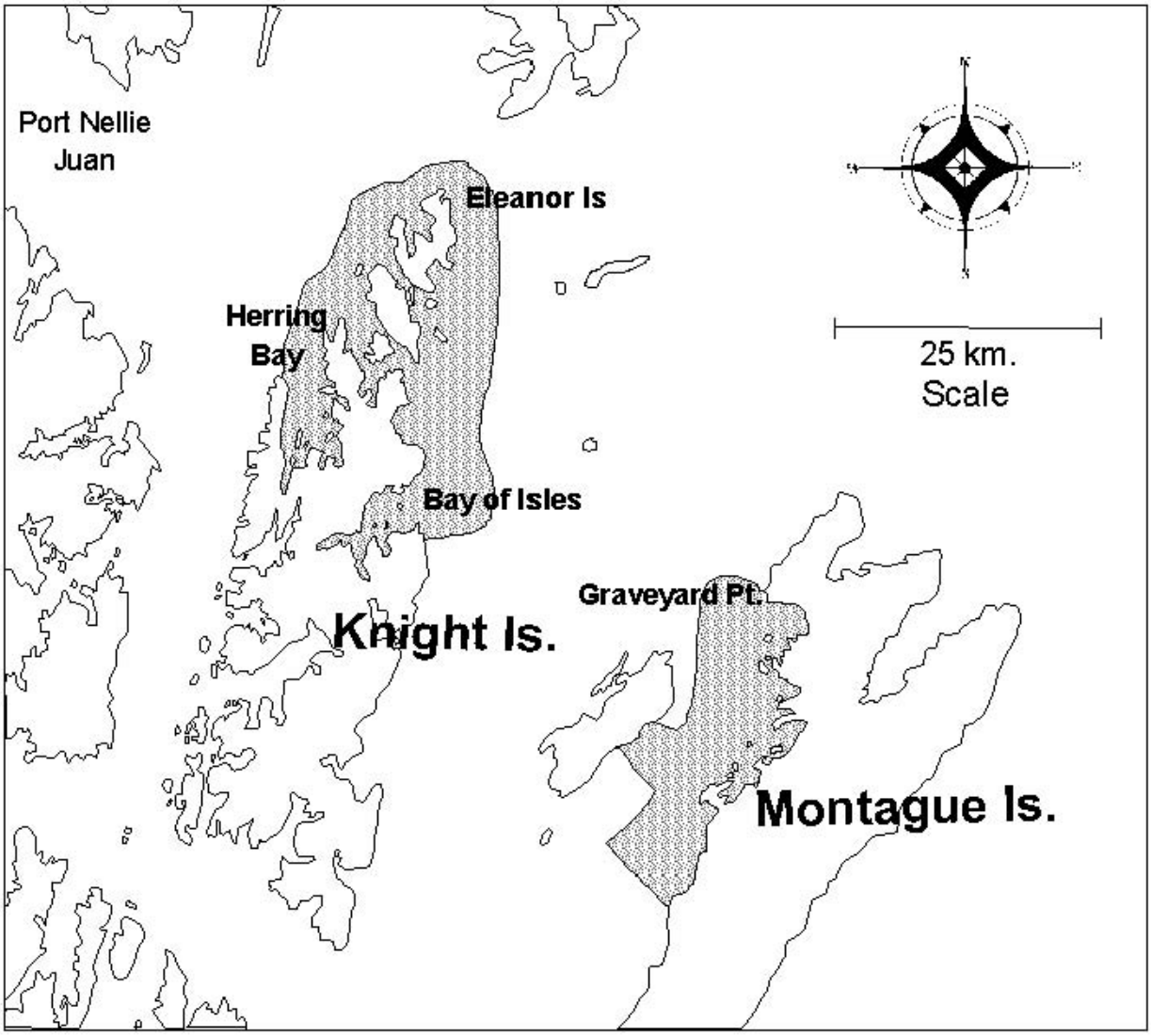

Figure 2. Intensive sea otter study areas for surveys (shaded), capture, food habits, and prey measures at northern Knight Island (oil affected) and Montague Island (unoiled).

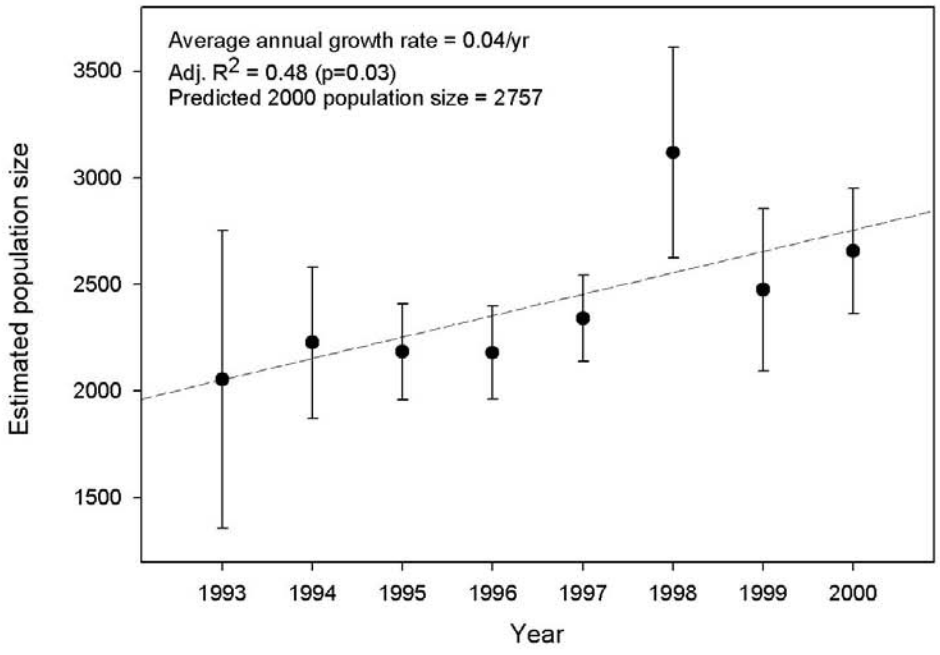

Figure 3. Estimates of sea otter abundance (± se) in western Prince William Sound, 1993-2000.

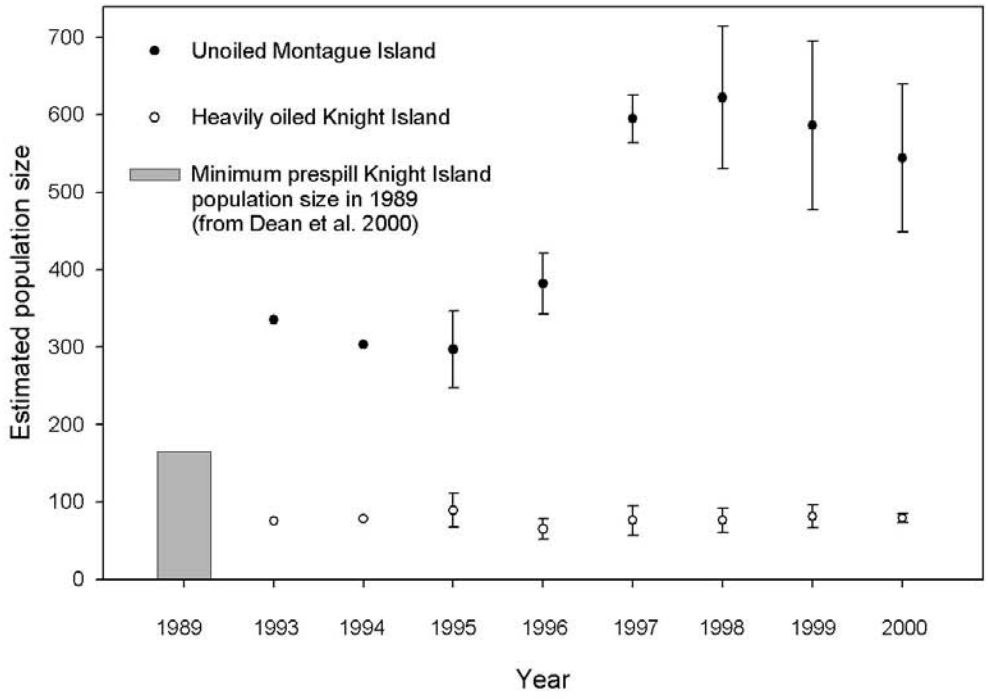

Figure 4. Estimates of sea otter abundance (± se) at northern Knight and Montague Island intensive study areas, 1993-2000 (no estimates of precision acquired in 1994-94). 1989 pre-spill estimate based on actual and estimated carcasses recovered from the study area and assuming no survivors (from Dean et al. 2000).

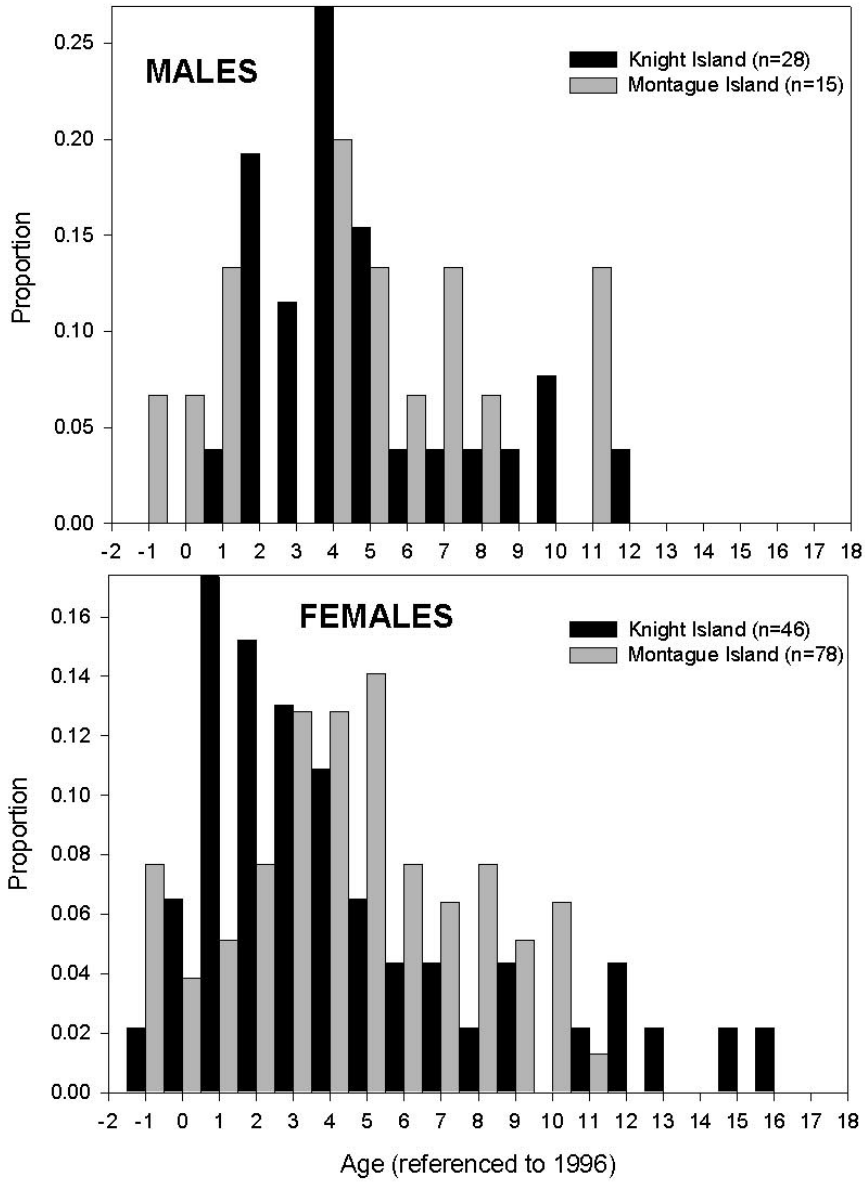

Figure 5. Age distribution of sea otters captured at intensive study areas at northern Knight and Montague Islands, 1996-1998.

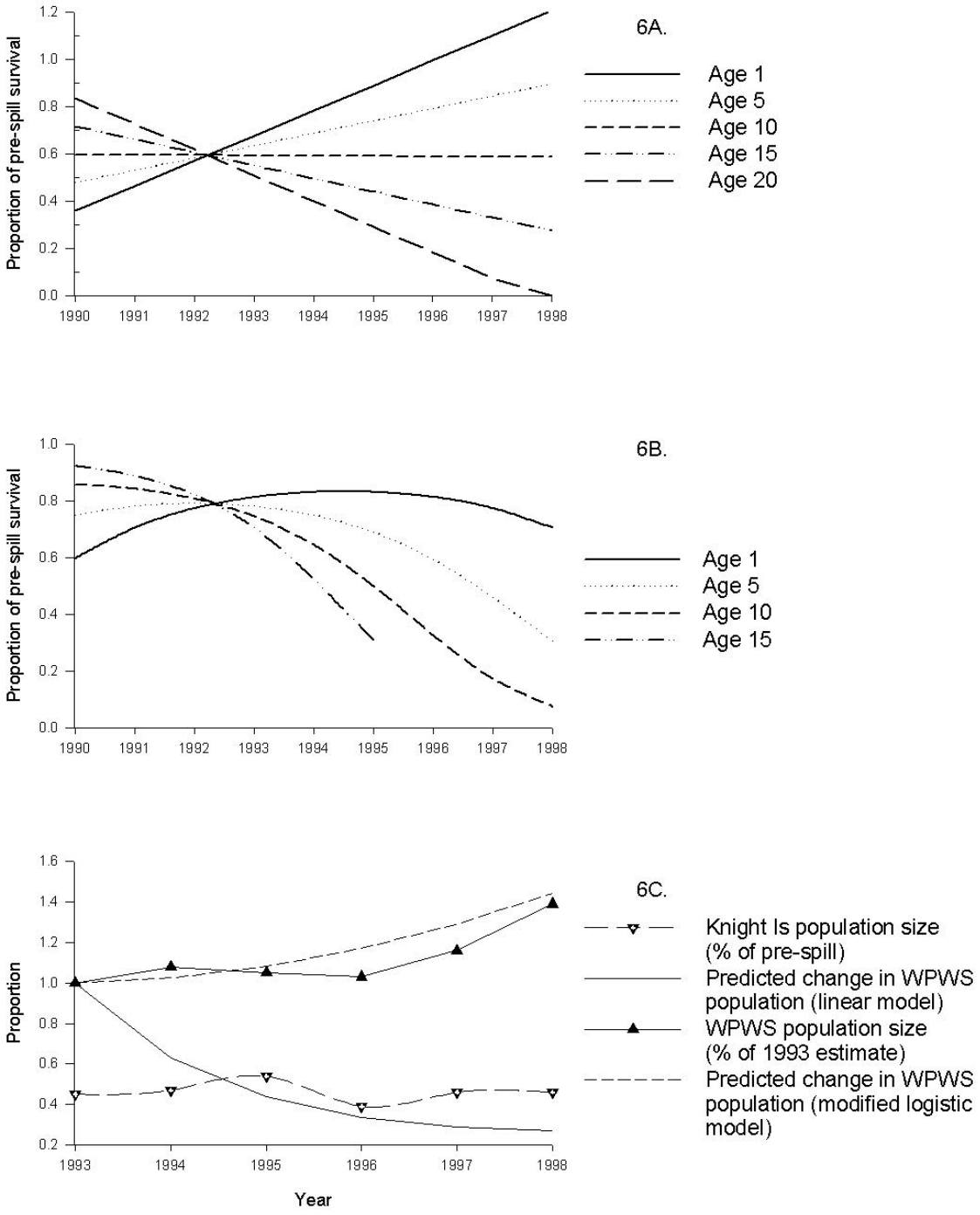

Figure 6. Estimated post-spill effects on age-specific survival rates (linear model) 6A, and for cohorts of a given age, 6B, expressed as a proportion of pre-spill survival, and predicted vs. observed population trends (6C) in western Prince William Sound (from Monson et al. 2000b).

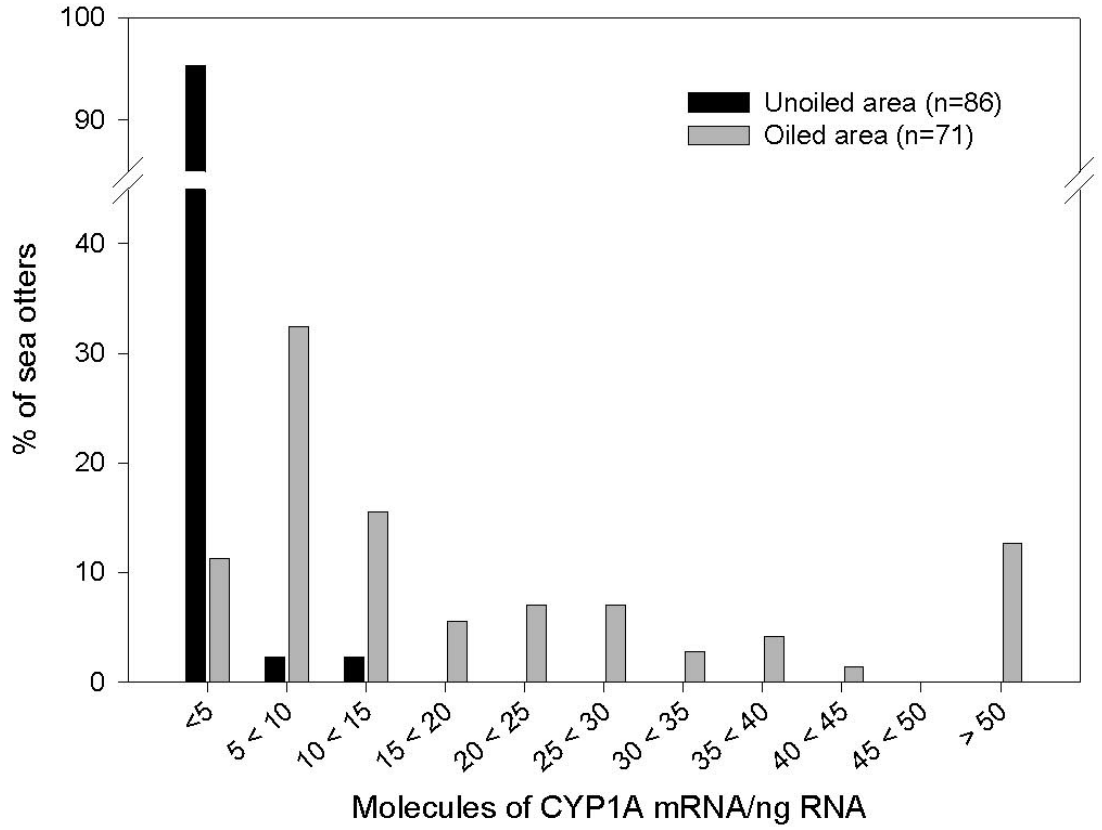

Figure 7. Distribution Cytochrome P4501A (CYP1A) mRNA values in peripheral blood mononuclear cells collected from sea otters in oiled and unoiled areas of western Prince William Sound (from Ballachey et al. 2000).

Sea Otter (*Enhydra lutris*) Perspective: Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

Part B. Food Limitation and the Recovery of Sea Otters Following the *Exxon Valdez* Oil Spill[1,2]

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ABSTRACT

We examined the potential role of food limitation in constraining recovery of sea otters in Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. The spill resulted in the removal of a large number of sea otters in 1989, and as of 1998, the portion of the population in the heavily oiled northern Knight Island region had not fully recovered. Between 1996 and 1998, prey consumption rate was higher and the condition of sea otters was better at northern Knight Island than in an unoiled area of the sound (Montague Island). Estimates of prey energy available per unit mass of sea otter were about 4 times higher at Knight than Montague Island, albeit not significantly different between the two areas. Over this same period, the number of sea otters remained constant at northern Knight Island but increased at Montague Island. These data suggest that food was at least as abundant at Knight than at Montague Island, and that recovery of sea otters via intrinsic population growth was limited by factors other than food. However, the availability of food, the prey consumption rate, and the condition of sea otters were all much lower at both Knight and Montague Islands than in areas newly occupied by sea otters where the population growth rate was near the theoretical maximum. It is possible that the relative short supply of food (compared to areas where sea otter population growth rate was high) may have inhibited immigration or interacted with other factors (e.g., oil-induced mortality or predation) to restrict sea otter population growth. Nonetheless, these data suggest that impacts of anthropogenic disturbances on large, often food-limited vertebrate predators can persist in spite of the availability of food resources that are sufficient for intrinsic population growth.

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[1]2002. Marine Ecology Progress Series 241:255–270.

[2]Document citation has not been revised to reflect overall final restoration report citation.

Key words: *Enhydra lutris*, Prince William Sound, Alaska, predator-prey interaction, clams, sea urchins, mussels, prey availability, prey consumption rate, condition indices

INTRODUCTION

The *Exxon Valdez* ran aground in Prince William Sound (PWS), Alaska, in March 1989 and the estimated 42 million liters of crude oil that were spilled from the tanker had severe adverse impacts on the nearshore ecosystem (Paine et al. 1996, Spies et al. 1996, Peterson 2001). One effect of the spill was the removal (either via death or for the purposes of rehabilitation and permanent placement in captivity) of a significant proportion of the sea otter population in heavily oiled parts of PWS (Garrott et al. 1993, Bodkin & Udevitz 1994, Garshelis 1997, Dean et al. 2000). An estimated 165 sea otters were removed from the heavily oiled area of the western sound around northern Knight Island as a result of the spill (Dean et al 2000), and few if any sea otters remained there following the spill in summer 1989 (Bodkin & Udevitz 1994, JLB & DMM unpublished data). As of 1998, the sea otter population in this area had not fully recovered (Dean et al. 2000, Bodkin et al. 2001). From 1993 to 1998, yearly aerial surveys at northern Knight Island found fewer than 90 sea otters and there was no significant increase in sea otter density over this period. In 1997 and 1998, there were an estimated 76 sea otters, far fewer than the 237 sea otters found in a pre-spill census of the area in 1973, and fewer than half the number of sea otters removed in 1989 following the spill. In contrast, along Montague Island, an area within PWS unaffected by the spill, sea otter density increased between 1993 and 1998, and was 27% higher in 1998 than in 1973.

Models using age-distribution of carcasses collected from beaches indicate that survival of sea otters in oiled areas was lower after the spill than before (Monson et al. 2000a). However, the causes for poorer survival and lack of recovery of sea otters in the northern Knight Island region have not been identified. Determining these causes is important in managing and conserving sea otter populations, evaluating the overall health of the nearshore system during recovery following the oil spill, and predicting patterns and rates of recovery following environmental perturbations of similar scope and type (Bodkin et al. 2001). We hypothesize that the lack of recovery was the result of: 1) a slow rate of increase in sea otter populations, even in the absence of chronic effects of the spill, 2) continued exposure to oil and concomitant effects on survival, immigration, or emigration rates, or 3) a lack of food (resulting from reductions in prey abundance caused by the spill or from natural causes). In this paper, we examine the evidence regarding the food limitation hypothesis by comparing the prey availability, prey consumption rate, and condition of sea otters in an oiled vs. unoiled area in PWS.

The diet of sea otters in PWS consists mostly of clams, primarily *Saxidomus gigantea*, *Protothaca staminea*, *Humilaria kennerleyi*, *Macoma* spp., and *Mya* spp. (Calkins 1978, Estes et al. 1981, Garshelis et al. 1986, Doroff & Bodkin 1994.) Crabs (primarily *Telmessus cheiragonus*) and mussels (*Mytilus trossulus*) are taken somewhat less frequently, although mussels may be an important food resource for juvenile sea otters (VanBlaricom 1988). Occasional prey in PWS include echiurid and polychaete worms, sea urchins, and sea stars. Densities of some sea otter prey were reduced at sites adjacent to heavily oiled beaches in PWS following the oil spill in 1989. These included *Mytilus trossulus* (Gilfillan et al. 1995, Houghton et al. 1996, Highsmith et al. 1996), *Protothaca staminea* (Driskell et al. 1996, Trowbridge et al. 1998, Fukuyama et al.

2000), and *Telmessus cheiragonus* (Dean et al. 1996). The recovery status of these populations has not been fully evaluated. There is some evidence that communities in the rocky intertidal and rocky subtidal habitats had recovered by 1992 or 1993, 3 to 4 yr after the spill (Coats et al. 1999, Dean & Jewett 2001). However, for *Protothaca staminea*, mortality were higher and growth was slower in oiled areas through 1996 (Fukuyama et al. 2000) and adverse impacts of the oil spill to some species of infauna in subtidal, soft-sediment eelgrass habitats persisted through 1995, and perhaps longer (Jewett et al. 1999, Dean & Jewett 2001).

In the two decades prior to the oil spill, sea otter densities in our oiled (northern Knight Island) and reference (Montague Island) study areas were relatively stable (reviewed in Bodkin et al. 2000) and further population growth was considered limited by food (Garshelis et al. 1986, VanBlaricom 1988). This was based on observations of a relative reduction in several key food items (crabs and mussels) coincident with sea otter expansion and on sea otter feeding observations (Estes et al. 1981, Garshelis et al. 1986). In the early 1980s, sea otters from (or near) our study areas spent approximately twice as long foraging than otters in parts of PWS where the population had only recently expanded (Estes et al. 1981, Garshelis et al. 1986). The fact that the pre-spill population of sea otters was apparently food limited, coupled with evidence of reductions in sea otter prey as a result of the spill, suggested that food may be limiting recovery of sea otters.

Over the course of our study (from 1996 through 1998) there was an increase in sea otter density in unoiled portions of western PWS, but no increase in the heavily oiled region around northern Knight Island (Dean et al. 2000). Therefore, demonstration of less food in the oiled area would suggest that food was limiting recovery there. On the other hand, equal or greater abundance of food at the oiled site would indicate that factors other than food were responsible. We relied on both direct and indirect measures of food availability because it is difficult to measure precisely the abundance of the diverse group of sea otter prey, and because prey abundance does not account for factors such as quality of food or the cost to the predator of acquiring its prey. In the absence of other factors that can influence prey abundance, a reduction in the density of food-limited mammalian predators generally leads to an increase in either quantity or quality of food available, and an increase in the condition of the remaining (especially younger) animals (Bobek 1977, Sinclair 1977, Skogland 1983, 1985, Bayliss 1985, Sinclair et al. 1985, Fryxell 1987, Freeland & Choquenot 1990, Choquenot 1991, Messier 1994). Therefore, in addition to prey abundance, we examined prey consumption rate and condition of young sea otters as further indicators of food limitation. Prey consumption rate and condition of animals are often better indictors of food resources than direct measures of prey abundance, especially for large marine mammals (Eberhardt & Siniff 1977).

METHODS

Design. As is the case for most large motile predators, it is not practical to test experimentally the food limitation hypothesis (Estes 1996). Instead, we will rely on three separate lines of indirect evidence concerning: 1) The availability of food, in terms of both prey energy per unit area and prey energy per unit mass of sea otter, 2) the rate of consumption of food by sea otters, and 3) morphometric characteristics (age-adjusted mass and mass to length ratio) for sea otters that might be expected to be affected by the availability of food. Evaluation

of these three relatively independent data sets (prey availability, prey consumption rate, and condition of sea otters) provides a more rigorous test of the food limitation hypothesis than evaluation based on any single line of evidence.

For all three factors, we compare a heavily oiled area in the vicinity of northern Knight Island with an unoiled area at Montague Island. The Montague site was relatively unaffected by the spill (ADEC 1989, ADNR 1991, Galt et al. 1991, Wolfe et al. 1994, O'Clair et al. 1996, Jewett et al. 1999) and there were no detectable impacts to sea otters (Ballachey et al. 1994) or nearshore benthic communities (Dean et al. 1996, Jewett et al. 1999). Based on the history of sea otter recolonization in PWS following their near extinction in the late 1900s, observations of sea otter movements, and phenotypic and genotypic characteristics of individuals from throughout PWS, it is clear that sea otters at Knight and Montague Islands are subsets of a larger metapopulation (Gorbics & Bodkin 2001). However, mark-recapture studies indicated little if any movement of sea otters between our Knight and Montague study areas between 1996 and 1999 (Bodkin et al. 2001). Between 1996 and 1998, a total of 66 and 91 sea otters were tagged at northern Knight and Montague Island respectively, and a total of 47 tagged sea otters were observed between 1997 and 1999 in each area. None of the sea otters tagged at Knight Island were observed at Montague Island or vice versa. Population densities of sea otters were likely lower at northern Knight Island than at Montague Island at the time of the spill in 1989 (Dean et al. 2000), but sea otters in both areas were considered food limited and at or near equilibrium prior to the spill (Estes et al. 1981, Garshelis et al. 1986, Bodkin et al. 2001).

For each metric (prey energy per unit area, prey energy per unit mass of sea otter, consumption rate of prey, age-adjusted mass, and mass to length ratio) we tested the hypothesis that there was no difference at northern Knight Island vs. Montague Island against the alternative that values for these metrics were greater at Knight Island. Equal or higher values at Knight Island would indicate that recovery of sea otters at Knight Island was limited by factors other than food.

When possible, we also compared post-spill values for each metric for the northern Knight and Montague Island with pre-spill PWS values, and with similar data for sea otter populations outside of PWS. Histories of sea otter colonization in these areas are known, and the status of the populations with respect to food limitation is generally acknowledged (Lensink 1962, Kenyon 1969, Estes et al. 1986, Garshelis et al. 1986, Kvitek et al. 1992).

We recognize that this is a pseudoreplicated design in that we primarily rely on comparisons between a single oiled area with a single unoiled reference area (Hurlbert 1984, Stewart-Oaten et al. 1986). Therefore, statistical inference can be made only to those two areas and not to other areas within PWS that were impacted by the spill, to spill impacted areas outside of the sound, or to other oil spills. However, our northern Knight Island study area represents one of the most heavily oiled parts of the sound where sea otters were not recovering, and we were primarily interested in evaluating why recovery of sea otters in this particular area was slow. We did not replicate reference areas (e.g. other unoiled parts of PWS) primarily because of cost constraints. However, the increase in sea otters that we observed in our Montague study area over the course of the study was also observed in other unoiled portions of PWS (Bodkin et al. 2001). Thus, patterns observed at Montague are reflective of sound-wide patterns, at least with respect to this one important aspect. We also recognize that our design relies largely on post-spill comparisons (especially with respect to food availability) and that interpretations of results with

respect to potential food limitation rest on assumptions regarding food resources and the status of food limitation in sea otter populations prior to the spill. However, based on the long history of sea otter occupation in our study areas (Lensink 1962), and the widely recognized impact of sea otters on their food resources (e.g. Estes & Duggins 1995, Kvitek et al. 1992) the assumption that sea otters in both our study areas were food limited prior to the spill seems reasonable.

Food Availability. We used two metrics to assess prey availability because of uncertainties as to how sea otters perceive their prey base, uncertainties as to whether recovery of sea otter populations may be dependent on immigration or intrinsic growth (i.e. growth resulting from births in the resident population), and known differences between our two study areas. First, we examined the energy of prey available per unit area. We assume that immigrating sea otters might assess the suitability of a particular habitat based on the prey that can be obtained in a few foraging sessions, and that the average prey density (i.e. mean prey energy per unit area) is a reasonable index of what an immigrating sea otter might encounter. This especially may be the case for young sea otters that have little knowledge of preferred feeding sites where prey densities may be higher than average. Younger sea otters (especially young males) are the most likely immigrants (Reidman & Estes 1990).

However, energy available per unit area might not be a reasonable means of assessing the status of a particular area with respect to its carrying capacity and its potential with respect to intrinsic population growth. Our Montague Island study area supported higher densities of sea otters than our northern Knight Island study area prior to the spill, and there are known differences between the two study areas that suggest that Montague Island might support a more productive prey base. In a pre-spill census conducted in 1973, densities of sea otters were 5.4 and 1.4 per km^2 at Montague and Knight Island study areas respectively (Dean et al. 2000). The Montague study area is generally shallower and has a higher proportion of soft sediment (Holland-Bartels 1996) suggesting that it may be a more suitable habitat for clams, a preferred sea otter prey in PWS. Thus, it is likely that the energy of sea otter prey per unit area was substantially lower at Knight Island than at Montague Island prior to the spill, when food was presumably limiting at both locations. Furthermore, it is possible that prey energy per unit area would remain lower at Knight than at Montague Island, even if the sea otter population at Knight Island was below carrying capacity and food was no longer limiting with respect to the potential for intrinsic population growth. Therefore, we assessed availability of food in terms of energy available per unit mass of sea otter to account for possible differences in carrying capacity of the two study areas. We assume that, while prey energy per unit area may differ between the two areas when at carrying capacity, the energy available per unit mass of sea otter would be roughly equivalent. Thus, food limitation at northern Knight Island, especially with respect to the potential for intrinsic population growth, would be indicated by lower or equal energy of prey available per unit mass of sea otter.

Prey items evaluated were clams, crabs, mussels and sea urchins. Independent estimates of sea otter diets made both prior to (Calkins 1978, Estes et al. 1981, Garshelis et al. 1986) and subsequent to the oil spill (Doroff & Bodkin 1994 and section on energy consumption below) suggest that these prey comprise the vast majority of food consumed by sea otters in PWS. The density and size distributions of prey were estimated from stratified random sampling within each of two study areas: Knight and Montague Islands (Figure 1). Sampling was stratified by depth and was conducted between 1996 and 1998 (Table 1). We collected prey by hand along

intertidal strata sampled at low tide or from subtidal strata using SCUBA. Subtidal clams were sampled using a diver operated suction dredge (Fukuyama 2000). Not all species or strata were sampled in each year, and we used combined estimates for all years, ignoring possible year to year differences.

The prey energy available per unit area within each study area and depth stratum was calculated based on abundance and size distribution of prey. In most instances, all sampled individuals for a particular prey were measured, and sizes of individuals were converted to energy units using size to dry-tissue mass regressions, and estimates of energy per unit dry-mass. For crabs (*Telmessus cheiragonus)*, we did not measure sizes but only counted crabs larger than 44 mm carapace length (about 50 mm carapace width), and made conversions from abundance to energy by assuming the average size (carapace width) of crabs was 44 mm. This is a reasonable approximation based on crab size selection in sea otter feeding observations (DH Monson, personal observation).

For all prey, we assumed that sea otters were size-selective predators, and that only prey above a given size were available. This assumption is supported by direct observations of sea otter foraging, and by comparisons of sizes of prey eaten vs. sizes available in a wide variety of sea otter prey, over a range of habitat types (Estes et al. 1978, Simenstad et al. 1978, Ostfeld 1982, Kvitek & Oliver 1988, VanBlaricom 1988, Kvitek et al. 1992, Estes & Duggins 1995). A size cutoff of 20 mm was used for clams and mussels (length) based on the sizes of clams in collections of sea otter-cracked shells (Kvitek et al. 1992, Fukuyama 2000), the sizes of mussels available to otters (VanBlaricom 1988), and direct foraging observations (JL Bodkin & DH Monson, personal observation). For crabs, we used a 44 mm carapace length (approximately 50 mm carapace width) lower limit based primarily on feeding observations. A size of 15 mm test diameter was used for sea urchins based on the lower limit of sea urchins in sea otter scat (Estes & Duggins 1995).

For the more abundant prey species, we developed dry mass to size relationships, using a subset of animals collected (Table 2). In other cases (all of which were clams) we substituted dry mass to size regressions using similarly shaped species. Energy conversions were based on our calorimetry of a subsample or values reported in the literature.

Weighted mean values for energy of prey per unit area (kj m^{-2}) were computed based on the calculated mean energy of prey per unit area in each stratum in each study area and the size (km^2) of each stratum in each study area (Table 3). For strata to a depth of 10 m, the stratum size was determined based on sampling of distances between stratum boundaries at systematically selected shoreline sites and the total shoreline length. The area within the 20 to 100 m depth stratum was determined from a GIS analysis of bathymetric charts. Area of the 10 to 20 m depth stratum was determined by subtraction. Not all species were sampled in each depth stratum, either because we had some prior knowledge of the depth distribution of species, (e.g., *Mytilus trossulus* occurs almost exclusively in the upper intertidal region in PWS) or because of logistical considerations. None of the species were sampled at depths of greater than 20 m because of our inability to safely sample these depths using SCUBA. Extrapolations to depths up to 100 m using data from the 10 to 20 m depth stratum may have introduced bias. There are no quantitative estimates of densities of various sea otter prey at depths greater than 20 m, and we cannot evaluate the direction or extent of these potential biases. Assumptions regarding the abundance estimates for unsampled strata are given in Table 4. It was assumed that otters seldom

feed at depths greater than 100 m, as confirmed by feeding observations (JL Bodkin & DH Monson, unpublished data).

Prey energy available per unit mass of sea otter was estimated as:

> prey energy per unit mass of sea otter (kj kg^{-1} of otter) = [prey energy density (kj m^{-2}) x sea otter sampling area (m^2) x the proportion of the sea otter sampling area that is less than 100m depth]/[sea otter abundance x avg. mass of a sea otter (kg)]

Sea otter abundance was the mean of 1996, 1997, and 1998 aerial survey estimates (replicated in each year) at Knight and Montague Islands (Dean et al. 2000). The area over which otters were surveyed (168 and 90 km^2 at Knight and Montague Islands respectively) was larger than the prey sampling area (27 and 73 km^2). We assumed that prey abundance within the smaller prey sampling area was representative of the larger sea otter sampling area. The average mass of a sea otter (22.85 kg) was determined from a sample of 145 individuals captured between 1996 and 1998. This estimate, based on a pooled sample from northern Knight and Montague Islands, was used in the calculation of prey energy available per unit mass of sea otter in each area.

Variances and confidence intervals for both prey energy per unit area and prey energy per unit mass of sea otter were calculated using formulae for estimating the variances of products of an independent variable and a constant, and of ratios of two independent means (Goodman 1970). The null hypotheses that prey energy per unit area and prey energy per unit mass of sea otter at Knight Island was equal to that at Montague Island were tested using a one-tailed Z test (Snedecor & Cochran 1969).

Energy requirements of sea otters at Knight and Montague Islands were determined based on sea otter abundance, the average energy requirement of a sea otter, and the average mass of a sea otter. The energy requirement of 1,019 kj kg^{-1} d^{-1} was an average of several published values (Kenyon 1969, Fausett 1976, Costa 1982). Estimates of yearly energy requirements were compared to estimates of yearly production of prey. The latter assumed that the ratio of yearly net production to standing stock (the P:B ratio) for prey was 2.0. This was based on values given for several benthic invertebrates in PWS (Feder & Jewett 1987).

There are no comparable pre-spill estimates of prey availability for PWS, and no estimates for areas outside PWS. However, Kvitek et al. (1992) gave standing stocks (wet meat mass) of bivalves from areas where sea otters were feeding at locations around Kodiak Island with various histories of sea otter colonization. We converted these values to energy units assuming that dry mass was 18.7% of wet meat mass (based on the average for clams collected in our study) and an energy density of 18.8 kj g^{-1} dry mass (based on our data and on values for energy density of *Saxidomus gigantea*, the numerically dominant clam). We compared these values to similar estimates of prey energy per unit area from sea otter feeding sites at northern Knight Island (five sites) and Montague Island (three sites) sampled in 1997. Sampling and estimation of prey energy per unit area were as described above for systematic sites.

Rate of consumption of food by sea otters. The average prey consumption rate by sea otters in each study area was calculated based on measurements of: 1) the time of an average dive plus the time interval between dives, 2) the proportion of dives that were successful in obtaining food, 3) the type, number, and size of prey obtained on each successful dive, and 4) the average energy content of each prey. One through three above were based on direct foraging observations made from sites along the shoreline using a 50 to 80 power spotting scope while four was based

on estimates from sea otter cracked shells from sea otter foraging sites (see below). Observations were made during daylight hours in June through August 1996 through 1998. A total of 117 foraging observation sessions were conducted at Knight Island and 113 were conducted at Montague Island. An average of eight dives per session was observed in each area. Energy conversions were made based on expressions given in Table 3, or from values given in Cummins & Wuycheck (1971) or Wacasey & Atkinson (1987).

Observers could distinguish prey type (clam, mussel, crab, sea urchin, etc.) and the size class (< 4, 4 to 8, or > 8 cm in length) of each prey, but could not accurately estimate size or, in the case of clams, species. Therefore, we estimated the species composition of clam prey and average size of each species of clam based on collections of sea otter-cracked shells from sea otter foraging sites. This method is based on the unique way in which sea otters feed and the ability of divers to distinguish otter-cracked shells from others (Kvitek et al. 1988, 1992, Fukuyama 2000). A total of 33 and 30 foraging sites were sampled at Knight and Montague Islands respectively in summer 1996 and 1997. An average of 11 and 20 otter-cracked clam shells was collected and measured at each site respectively. Only newly deposited shells (based on color and degree of epifaunal growth) were included.

We tested the hypothesis of no difference in consumption rate between Knight and Montague Islands using a Monte-Carlo re-sampling method (Manly 1991). We used the mean and variance estimate for each of the observable foraging attributes used in the calculation of consumption rates (dive times, number and size of prey, etc.) to estimate a statistical distribution for each attribute. Initially data from both study sites were combined to represent a null distribution of no difference between populations at Knight and Montague Islands. A sample size of 117 (Knight) and 113 (Montague) was randomly selected (representing the number of foraging sessions observed in each area) from the distribution of each attribute, the averages of these were computed, a consumption rate calculated for each area, and a difference in consumption rate found. This process was repeated 1,000 times to create a Monte Carlo simulation of the null distribution of differences. The *observed* difference in consumption rates was estimated using the *site-specific* mean values for each attribute to derive one consumption rate for each area. The statistical significance of the difference in consumption rate was estimated by the proportion of the null distribution of differences that was greater than the observed difference. This can essentially be interpreted in the same manner as the probability associated with a t-statistic testing the hypothesis of no difference between means. We also calculated 95% Monte-Carlo confidence intervals for consumption rates. The Monte Carlo procedure included drawing a random sample from the *site-specific* distribution for each attribute of sample size 117 and 113, for Knight and Montague respectively. We again calculated the mean values to estimate the new consumption rate and repeated the process 1000 times for each area. Confidence limits were estimated by the 2.5% and 97.5% points in the Monte Carlo distribution of consumption rates.

The consumption rates for sea otters at Knight and Montague Islands in 1996-1998 were contrasted with comparable data from other PWS sites (Garshelis et al. 1986) and from Kodiak Island collected prior to the spill. Means and 95% confidence intervals were estimated for consumption rates at Kodiak largely using published data from these sites as inputs. Calculations were made in the same manner described above for Knight and Montague. Foraging data for Kodiak Island (Doroff & DeGange 1994, AR DeGange, unpublished data) were collected in a

manner similar to those described for PWS. Size distributions of clams at Kodiak Island were based on shell litter collections (Kvitek et al. 1992).

Morphometrics. Age-adjusted body mass and mass to length ratios were compared between sea otters captured from northern Knight, Montague, and Kodiak Islands. Animals at Knight and Montague were captured in 1996, 1997, and 1998 using either tangle nets or diver operated modified Wilson traps (Bodkin et al. 2001). The sex, mass, and body length (from the tip of the nose to tip of the tailbone) of each animal was determined and a tooth (pre molar) was extracted prior to the animal's release. Each tooth was analyzed to estimate the age of the sea otter based on the number of cementum layers (Garshelis 1984, Bodkin et al. 1997). This analysis provides ages accurate to ± one yr on average. The Kodiak data were collected in 1986 and 1987 using methods similar to those described above (Monson 2000b, Monson & DeGange 1995). The Kodiak site was recently occupied by sea otters (within 5 to 15 yr prior to sampling) and had abundant food.

Analyses were conducted for females from 1 to 4 yr of age that did not have dependent pups with them at the time of capture. We restricted the analyses to females because there were too few males captured for the purpose of comparison. Older females were excluded because of possible confounding effects of having a large number of pregnant females among older individuals, and because the effects of a limited food supply were expected to have their greatest impact on younger animals that are generally poorer competitors. Higher starvation- caused mortality in young animals has been suggested for sea otters (Kenyon 1969) and demonstrated for other large mammals (Choquenot 1990, Virgil & Messier 1997). Variation in survival of immature individuals accounts for most of the variation in rates of population increase for marine mammals (Eberhardt & Siniff 1977).

We tested the null hypotheses of no difference between age-adjusted mass and mass to length ratio using an analysis of covariance. Age classes used were 1, 2, 3, and 4 yr. Areas were contrasted using pairwise comparisons of least-square means.

RESULTS

Food availability. The mean energy content of sea otter prey per unit area was nearly twice as high at Montague as at Knight Island (Table 5). This was primarily the result of higher energy per unit area for *Macoma* spp., *Mya truncata*, *Saxidomus gigantea*, *Mytilus trossulus*, and 'other clams' at Montague Island. However, among individual species, only the energy per unit area of *M. trossulus* differed significantly between areas ($p<0.01$), and there was no significant difference between areas for the energy per unit areas summed over all sea otter prey ($p=0.81$).

The relative proportions of total energy contributed by each species differed between areas (Table 5). At Knight Island, the majority of energy available was from *Humilaria kennerleyi* (43%) and *Mya truncata* (21%). At Montague Island, 'other clams', *M. truncata*, and *Macoma* spp. contributed 21, 20, and 16%, respectively.

The prey energy per unit mass of sea otter was 4.8 times higher at Knight Island than Montague Island (Table 6). However, we failed to reject the null hypothesis that energy per unit mass of sea otter was equal within the two areas ($z=1.19$, $p=0.12$). The estimated annual production of prey energy at Knight Island (1.6×10^{10} kj yr^{-1}) was about 26 times higher than that required to support the sea otter population there (6.1×10^{8} kj yr^{-1}). At Montague Island, the

estimate of the mean energy available (2.7×10^{10} kj yr^{-1}) was only about 6 times that required (4.5×10^{9} kj yr^{-1}).

Within sea otter foraging areas, the average clam energy per unit area was substantially higher at Kodiak Island sites sampled by Kvitek et al. (1992) than at either Knight or Montague Islands (Table 7). This was especially true for those Kodiak Island sites where sea otters had only recently become re-established. Frontal areas at Kodiak Island had over 36 times higher densities of clams (in terms of energy per unit area) than Montague Island and over 98 times higher prey energy per unit area than Knight Island. Kodiak sites that were long occupied by sea otters and were considered food limited had approximately three times more kj m^{-2} of clams than Montague, and about seven times more than Knight Island sites. The dominant clam species (in terms of energy per unit area within sea otter foraging sites) at Knight and Montague Islands were *Saxidomus gigantea* and *Mya truncata* respectively. *S. gigantea* dominated at all Kodiak Island sites.

Rate of consumption. Clams comprised the majority of the prey energy consumed by sea otters at both Knight and Montague Islands (Table 8). Sea otter-cracked shell collections indicated that at Knight Island *Saxidomus gigantea* were the most often taken prey and had the majority of prey energy (Table 9). The species composition in the sea otter-cracked shell litter at Montague Island was more varied. Most of the prey energy was supplied by *Mya truncata* and *S. gigantea*. Mean sizes for all species of clams were slightly larger at Knight than at Montague Island.

Sea otters at Knight Island had a slightly higher proportion of successful dives and took prey that were, on average, of higher energy (Table 8). An average of 90.3% of dives was successful in obtaining clams or mussels at Knight Island compared to 88.3% at Montague Island. The average energy provided per prey item was higher at Knight Island for both clams and crabs. At Knight Island, the average clam taken supplied an estimated 51 kj compared to 36 kj for those taken at Montague Island. Crabs taken had 505 kj at Knight Island vs. 274 kj at Montague Island. For all prey items, sea otters at Knight Island also took more individuals per dive. Factors advantageous to a higher rate of consumption at Knight Island were offset to an extent by a higher average dive time at Knight Island. Dive time plus surface time of successful dives were 33% longer, and unsuccessful dives were 28% longer at Knight than at Montague Island. Also, there was a slightly higher proportion of low energy prey (mussels and 'other') taken at Knight Island.

The resulting average consumption rate for sea otters at Knight Island was 2260 kj h^{-1}, about 18.9% higher than at Montague Island (1900 kj h^{-1}, Table 10) and differed significantly between areas ($p=0.001$, 1-tailed randomization test). Using these consumption rates, we estimate that the average size female sea otter at Knight Island fed an average of 9.9 hours per day to obtain energy required for maintenance (1,019 kj kg^{-1} d^{-1}). This is lower than the 11.8 hours needed at Montague Island.

The prey consumption rate for sea otters at Montague Island (1,900 kj h^{-1}) was slightly higher than that observed at nearby Green Island, PWS, prior to the oil spill (1,300 kj h^{-1}, Garshelis et al. 1986) (Table 10). Sea otters had occupied the Green Island site for many years and were considered food limited. The rate for Knight Island was similar to that observed at Nelson Bay, PWS (Garshelis et al. 1986), an area that was occupied by sea otters for only 2 to 3 yr prior to the surveys of consumption and likely not food limited. Both Knight Island

(sampled post-spill) and Nelson Bay sea otters had consumption rates that were higher than at Green and Montague Islands. Consumption rates were much higher at Orca Inlet in 1980-81, and Kuperanof Strait (Kodiak region) sites in 1986-87 than at all other sites. The Orca inlet and Kuperanof sites were surveyed only several years after colonization by sea otters and sea otters there were clearly not food limited. However, sea otters at a long-occupied site at Afognak Island (Kodiak region) had a consumption rate in the same range as animals from Knight and Montague Islands (Table 10).

Morphometrics. The age-adjusted body mass and mass to length ratio of one to four year-old female sea otters (without pups) captured at Knight Island were both significantly higher than for animals from Montague Island in 1996-1998 (Table 11). Body mass was 8.7% higher at Knight and mass: length was 6.3% higher. The difference in mass: length ratio translates to 1.1 kg difference for the average young sea otter (113 cm in length). Body mass and mass: length ratio were significantly higher at Kodiak Island sites that were only recently colonized by sea otters than at either Knight or Montague Islands. Body mass to length ratio at Kodiak was about 6.5% higher than at Knight Island and 13.1% higher than at Montague Island.

DISCUSSION

The availability of food resources for sea otters was the same, if not greater, at northern Knight than Montague Island over the period from 1996-1998. The rate of consumption of food was significantly higher and the condition of young female sea otters was significantly better at northern Knight than at Montague Island. Furthermore, the mean prey energy per unit area did not differ significantly between areas, and the prey energy available per kg of sea otter, while not significantly different between the two areas, averaged about four times higher at Knight Island. Based on the assumption that sea otters were better samplers of their available food supply than we were, and based on the relative lack of precision in estimation of food availability (see discussion below), we suspect that food resources were in fact more available at Knight Island.

Our estimation of food available to sea otters, both in terms of energy of prey per unit area and energy per unit mass of sea otter, were dependent on a number of assumptions. In particular, inaccuracies may have resulted if: 1) densities of prey in unsampled habitats (e.g., at depths greater than 20 m) were different than we assumed, 2) our sampling missed some widely dispersed, high-density patches of prey, 3) summer sampling misrepresented the average yearly energy density of prey, or 4) there were seasonal movements of sea otters (especially winter decreases in the more exposed Montague Island site) that were undetected by our summer sampling. While the estimates of prey per unit area are clearly imprecise and the estimates of the absolute quantity of prey available may be inaccurate, we have no good reason to suspect that there were biases that may have affected the relative measures of food available at our Knight and Montague Island study sites. Therefore, we feel that they provide a reasonable index of the relative abundance of prey at northern Knight and Montague Islands. There is some evidence that sea otters may move from more exposed areas (like our Montague Island site) in winter (Garshelis et al. 1986) and a winter survey of sea otters at Montague Island conducted in March 1998 (JL Bodkin, unpublished data) counted about 44% fewer sea otters than in July 1998. However, low light levels during winter resulted in poor precision and density estimates did not differ significantly between March and July surveys.

Although food was at least as abundant at northern Knight Island than at Montague Island, there was no increase in sea otter population at northern Knight Island between 1993 and 1998, but a significant increase at Montague Island over the same period (Dean et al. 2000). The number of sea otters at Montague Island increased from 335 in 1993 to 623 in 1998, a rate of about 15% per year, but remained almost constant (from 77 in 1993 to 76 in 1998) at northern Knight Island. These data provide evidence that the population of sea otters at northern Knight Island was below its carrying capacity with respect to food resources, and that the lack of growth of the sea otter population at northern Knight Island was due to factors other than the availability of food resources necessary for intrinsic population growth.

There is also demographic evidence that suggests that food was not limiting intrinsic population growth of sea otters at northern Knight Island. The growth of food limited populations is often constrained because of lower juvenile survival (Choquenot 1991). In the several years after the oil spill, survival rates of juvenile sea otters were lower than pre spill rates (Monson et al. 2000a) and lower weanling survival was noted in oil impacted vs unimpacted areas (Ballachey et al. 1994). However, in more recent years (including the years 1996-1998 in which our study was conducted) survival rates for juveniles returned to pre-spill levels (Monson et al 2000a). This is consistent with what would be expected under non-food limiting conditions. Also, birth rates of sea otters did not differ between oiled and unoiled portions of PWS (Johnson & Garshelis 1995, Bodkin et al. 2000). Survival rates of older sea otters decreased with time after the spill (Monson et al. 2000a), but given the better condition of sea otters in northern Knight Island, it is unlikely that the lower survival of older animals was caused by a lack of food.

While there was apparently sufficient food to allow for intrinsic growth of the existing segment of the population at northern Knight Island in 1996-1998, there is some question as to whether there was sufficient food to allow for successful immigration. Food appeared at least equally abundant at northern Knight Island compared to Montague Island, but food resources were still substantially lower at both Knight and Montague Islands than in areas recently reoccupied by sea otters (after decades of absence) where food was clearly not limiting. The relative lack of food at Knight and Montague Islands was probably largely the result of predation by sea otters that occupied these sites for several decades prior to the oil spill (Garshelis et al. 1986, Bodkin et al. 2000), but may have been exacerbated at Knight Island due to impacts of the spill on the prey (reviewed in Peterson 2001). Sufficient food resources are a requisite for successful immigration (Estes et al. 1986), and higher densities of food might be required for successful immigration than are required for growth within resident populations. This may especially be the case because food resources are patchy, and new immigrants may not be as efficient at utilizing food resources as resident adult sea otters or their pups that learn feeding behavior from their mothers. Thus, it is possible that there may have been sufficient food at northern Knight Island for intrinsic growth, but insufficient food to induce potential immigrants to establish residency. However, age-distribution models suggest that there must have been some net immigration to the northern Knight Island area in order to offset losses due to mortality and maintain the current population density (Monson et al 2000a). Therefore, while the relative short supply of food at Knight Island (compared to areas unoccupied by sea otters for decades) may have been sufficient to curtail net immigration, it apparently did not altogether prevent immigration and does not appear to be a primary cause for the lack of sea otter recovery.

While food resources at northern Knight Island appear sufficient to allow for population growth, they do not appear sufficient to allow for maximum potential population growth as observed in some sea otter populations in newly occupied areas. Sea otter populations that inhabit areas unoccupied by sea otters for several decades prior and have unlimited food supplies can increase at the rate of 25% per year (Estes 1990, Bodkin et al. 1999). Food resources at both northern Knight and Montague Islands were much lower than in these newly occupied sites, and were likely insufficient to allow for population growth rates near the maximum. Nonetheless, there was clearly sufficient food at both locations for some sea otter population growth, yet we observed growth at Montague Island, but not at northern Knight Island.

It is also possible that the relatively short supply of food at northern Knight Island (again compared to areas unoccupied by sea otters) may have interacted with other factors (e.g., oil-induced mortality or predation) to constrain sea otter population growth, and therefore sea otter recovery. Higher sea otter mortality were observed in oil-affected areas of Prince William Sound through 1998, perhaps as a result of exposure to oil (Monson et al 2000a, Ballachey et al. 2000). Furthermore, predation by killer whales (Hatfield et al. 1998, Estes et al. 1998, Garshelis & Johnson 1999) and hunters (Bodkin et al. 2001) may have contributed to the lack of recovery. Populations occupying habitats with low potential food supplies are more prone to being regulated at low densities by predation (Messier 1994) or possibly by oil-induced mortality. However, there is little evidence that predation pressure was higher at northern Knight than at Montague Island. Thus, the relative differences in sea otter population growth at northern Knight vs. Montague Island, and the lack of recovery of sea otter populations at northern Knight Island were unlikely the result of higher predation rates there.

Based on the equal if not greater availability of prey energy per otter at northern Knight Island than at Montague Island, and based on the increasing population density of sea otters at Montague Island but not at northern Knight Island, we conclude that factors other than food were primarily responsible for lack of recovery of sea otters in the heavily oiled northern Knight Island portion of PWS following the *Exxon Valdez* oil spill. Evidence presented elsewhere (Ballachey et al. 2000, Monson et al. 2000a, Bodkin et al. 2001) suggests that the recovery was primarily constrained by high rates of mortality and emigration that were linked to continued exposure to oil or persistent sublethal effects of oiling. However, it was also apparent that the *potential* population growth rate for sea otters at both Knight and Montague Islands was food-limited and the relatively short supply of food may have restricted immigration or interacted with other factors (e.g., predation or oil-induced mortality or emigration) to constrain sea otter recovery at northern Knight Island. Nonetheless, our data suggest that impacts of anthropogenic disturbances on large, often food-limited vertebrate predators may persist in spite of the availability of food resources that is sufficient for intrinsic population growth.

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Table 1. Summary of sampling effort for determination of density of sea otter prey at northern Knight Island (KI) and Montague Island (MI). Strata are indicated as +2.5 to +0.5 m = High Intertidal (HI), +0.5 to -0.5 m = Low Intertidal (LI), -0.5 to -5.0 m = Very Shallow Subtidal (VSS), -5.0 to -10.0 m = Shallow Subtidal (SS), -10.0 to -20.0 m = Deep Subtidal (DS). All depths are relative to mean lower-low water.

Prey species	Strata sampled	No. sites sampled per area		Years sampled	No. quadrats or transects sampled per site and stratum	Quadrat or transect size (m)
		KI	MI			
Protothaca staminea	LI	75	75	1996, 1997, 1998	5	0.5 m x 0.5 m x 0.1 m deep
	SS	39	37	1996, 1997	3 to 6 (usually 4)	0.5 m x 0.5 m x 0.3 m deep
	DS	10	10	1998	3 to 4 (usually 4)	0.5 m x 0.5 m x 0.3 m deep
All other clams	LI	45	45	1997, 1998	5	0.5 x 0.5 m x 0.1 m deep
	SS	39	37	1996, 1997	3 to 6 (usually 4)	0.5 x 0.5 m x 0.1 m deep
	DS	10	10	1998	3 to 4 (usually 4)	0.5 x 0.5 m x 0.1 m deep
Telmessus cheiragonus	LI,VSS,SS	60	59	1996, 1997	1	50 x 1 m
Mytilus trossulus	HI	112	107	1996, 1997	10	0.22 x 0.22 m
S*trongylocentrotus droebachiensis*	LI	75	74	1996, 1997, 1998	1	50 m x 0.5 m
	VSS, SS	60	59	1996, 1997	1	50 m x 0.5 m

Table 2. Size to mass and energy to mass relationships for sea otter prey and the source used to quantify these relationships. Sources are as follows: A = our estimate, B = Wacasey & Atkinson 1987; C = Cummins & Wuycheck 1971, D = a mean of 43 bivalve species from Cummins & Wuycheck 1971.

Prey Species	Dry mass (mg) vs. size (mm)	Source	Energy (j mg^{-1} dry mass)	Source
Clinocardium spp.	Mass = 0.000079 x length $^{(2.579)}$	A	18.88	B/C
Diplodonta spp.	Mass = 0.000009 x length $^{(3.186)}$	A	18.85	D
Humilaria kennerleyi	Mass = 0.000018 x length $^{(2.920)}$	A	18.85	D
Macoma spp.	Mass = 0.000006 x length $^{(3.147)}$	A	17.99	B
Mya truncata	Mass = 0.000035 x length $^{(2.903)}$	A	13.90	B
Protothaca staminea	Mass = 0.000098 x length $^{(2.432)}$	A	22.52	A
Saxidomus gigantea	Mass = 0.000100 x length $^{(2.555)}$	A	18.81	A
Other clams	Variable - based on relationships of similar shaped species	A	18.80 to 20.2	A/B/C
Telmessus cheiragonus	Mass = 0.000046 x length $^{(3.354)}$	A	11.94	A
Mytilus trossulus	Mass = 0.000011 x length $^{(2.843)}$	A	17.33	A
Strongylocentrotus droebachiensis	Mass = 0.000650 x test diameter $^{(2.5187)}$	A	3.70	A

Table 3. Benthic areas (km^2) within depth strata in the study areas at northern Knight Island (KI) and Montague Island (MI).

Depth stratum (m relative to mean lower low water)	Area	
	KI	MI
+ 2.8 to + 0.5	0.59	1.92
+ 0.5 to - 0.5	0.39	1.57
-0.5 to -5.0	1.71	7.16
-5.0 to -10.0	2.36	5.88
-10.0 to - 20.0	8.27	10.66
- 20.0 to - 100.0	13.54	46.22
Total	26.86	73.41

Table 4. Assumptions regarding prey densities in unsampled depth strata.

Prey species	Assumption
Protothaca staminea	Density = 0 below 20 m depth and above + 0.5 m
Telmessus cheiragonus and *Strongylocentrotus droebachiensis*	Densities at depths below 10 m are equal to densities in the -5 to - 10 m depth stratum Density = 0 in the + 2.8 to 0.5 m depth stratum
Mytilus trossulus	Density = 0 below the + 0.5 m stratum
All others	Densities at depths below 20 m are equal to densities in the -10 to - 20 m depth stratum Density = 0 in the + 2.8 to 0.5 m depth stratum

Table 5. Mean energy per unit area (kj m^{-2}) for sea otter prey at northern Knight and Montague Islands, 1996-1998.

Taxa	Knight Island Mean	95% CI	Montague Island Mean	95% CI	Z	p
Humilaria kennerleyi	31.66	±67.27	16.76	±39.00	0.38	0.35
Mya truncata	15.82	±49.09	31.47	±97.36	-0.28	0.61
Other Clams	6.73	±26.01	31.09	±57.60	-0.76	0.78
Mytilus trossulus	6.44	±0.83	14.62	±1.48	-9.43	<0.01
Saxidomus gigantea	4.40	±22.88	22.04	±41.68	-0.73	0.77
Protothaca staminea	3.99	±14.13	0.99	±4.04	0.40	0.34
Telmessus cheiragonus	1.62	±3.89	0.34	±1.10	0.33	0.37
Clinocardium spp.	1.89	±4.53	7.66	±23.83	-0.47	0.68
Macoma spp.	1.29	±3.65	23.34	±63.76	-0.68	0.75
Diplodonta spp.	0.32	±1.33	0.96	±5.62	-0.22	0.59
Strongylocentrotus droebachiensis	0.03	±0.23	0.03	±0.23	0.01	0.50
Total	74.20	±91.58	149.30	±144.01	-0.86	0.81

Table 6. Means and 95% confidence intervals of 1996-1998 values for sea otter prey energy per unit area, sea otter abundance, prey energy per unit mass of sea otter, yearly production of sea otter prey, and yearly prey production required to support the average number of sea otters within each study area. Yearly production of prey was calculated as the mean prey energy per unit area x the potential foraging area (i.e., the area less than 100 m depth) x an estimated P:B of 2. The yearly production required to support sea otters was calculated as the number of sea otters x average mass of a sea otter x the energy of prey required by sea otters daily (1,019 kj kg d^{-1}) x 365 d yr^{-1}.

Study Area	Prey energy per unit area (kj km^{-2})	Sea otter Abundance	Potential foraging area (km^2)	Average mass of a sea otter (kg)	Prey energy per unit mass of sea otter (kj kg^{-1})	Yearly production of sea otter prey (kj yr^{-1})	Yearly production of prey required to support sea otter population (kj yr^{-1})	Ratio of prey energy available per year: prey energy required
Knight Island	74×10^6 ($\pm 92 \times 10^6$)	72 ($\pm$ 12)	106	22.85	4.8×10^6 ($\pm 6.0 \times 10^6$)	1.6×10^{10} ($\pm 1.9 \times 10^{10}$)	6.1×10^8 ($\pm 1.1 \times 10^8$)	26.2
Montague Island	149×10^6 ($\pm 144 \times 10^6$)	533 ($\pm$ 202)	89	22.85	1.1×10^6 ($\pm 1.2 \times 10^6$)	2.7×10^{10} ($\pm 2.6 \times 10^{10}$)	4.5×10^9 ($\pm 2.2 \times 10^9$)	6.0

Table 7. Comparisons of clam densities (kj m^{-2}) at sea otter feeding sites at Kodiak, Knight, and Montague Islands. Kodiak Island data are from Kvitek et al. (1992). Data for otter-free sites Kodiak Island are from randomly selected sampling areas.

Region	Areas within region	Year	Sea otter population status	Clam energy per unit area (kj m^{-2})	Dominant clam species
Kodiak	6 otter free sites	1987-88	None	12,958	*Saxidomus gigantea*
Kodiak	6 frontal sites	1986-88	Newly occupied	8,384	*Saxidomus gigantea*
Kodiak	5 intermediate sites	1986-87	Occupied 5 to 15 years	4,008	*Saxidomus gigantea*
Kodiak	2 long occupied sites	1987	Occupied > 25 years	591	*Saxidomus gigantea*
PWS	Montague Island	1996-98	Occupied > 25 years	228	*Mya truncata*
PWS	Knight Island	1996-98	Long occupied -reduced in ‘89	85	*Humilaria kennerleyi*

Table 8. Means (± 1 standard deviation where appropriate) for feeding data for sea otters at northern Knight and Montague Islands, 1996-1997. A total of 117 and 113 sessions were observed at Knight and Montague Islands respectively, with an average of eight dives observed in each session within both areas.

Variable	Units	Knight Island		Montague Island	
Success rate	% of dives in which				
Clam - mussel dives	Prey were captured	90.3		88.3	
Non-clam-mussel dives		80.7		78.8	
Time per dive (dive time + surface time)	Seconds	162	(±66)	121	(±48)
Successful					
Unsuccessful		111	(±43)	87	(±35)
Prey composition	% of successful dives				
Clams		72		80	
Mussels		14		9	
Crabs		3		4	
Other		11		7	
Number of individuals per successful dive	No. inds.				
Clams		2.43	(±0.77)	2.12	(±0.30)
Mussels		11.25	(±4.25)	8.14	(±2.13)
Crabs		1.28	(±0.33)	1.04	(±0.07)
Other		2.90	(±1.66)	2.48	(±2.44)
Energy per prey item	Kj				
Clams		51	(±16.2)	36	(±17.6)
Mussels		5	(±0.8)	5	(±0.8)
Crabs		505	(±50.7)	274	(±27.2)
Other		17	(±1.7)	17	(±1.7)

Table 9. Percentage of clams by species, mean size of each species (± 1 standard deviation), and the percentage of total available prey energy contributed by each prey species in sea otter-cracked shells from sea otter foraging sites at northern Knight (N = 33) and Montague Islands (N = 30). An average of 11 and 20 recently cracked clam shells per site were collected at Knight and Montague respectively.

Clam species	% (by no. inds.)		Mean size (mm)		% (by energy)	
	Knight	Montague	Knight	Montague	Knight	Montague
Saxidomus gigantea	46.2	12.9	61.6 (7.1)	56.8 (9.8)	58.7	23.7
Protothaca staminea	8.4	4.1	49.6 (8.6)	38.2 (8.1)	5.3	2.1
Macoma spp.	11.2	5.6	45.3 (9.7)	42.0 (11.7)	3.3	2.4
Clinocardium spp.	3.6	18.3	42.8 (16.2)	39.5 (5.9)	2.2	8.7
Humilaria kennerleyi	10.9	11.1	47.3 (12.2)	44.3 (5.6)	6.3	8.3
Mya truncata	10.6	33.1	52.6 (10.3)	48.8 (5.0)	9.7	38.2
Serripes groenlandicus	5.3	2.5	64.7 (20.2)	53.9 (13.7)	11.0	4.8
Others	3.8	12.4	62.5 (13.0)	49.5 (4.9)	3.5	11.8

Table 10. Estimates of prey consumption and hours spent feeding by independent adult female sea otters. Data from Green Island, Nelson Bay, and Orca Inlet are from Garshelis et al. (1986). Feeding data from Kodiak are from Doroff & DeGange (1994) and DeGange (unpublished). Shell length and mass to length conversions for Kodiak are from Kvitek et al. (1992) and mass to energy conversions were from Kenyon (1969), Cummins & Wuycheck (1971), and Wacasey & Atkinson (1987). Upper and lower 95% confidence intervals are given in parenthesis.

Region	Area within region	Year	Sea otter population status	Prey consumption rate (kj hr-1)	Hours feeding required per day
PWS	Orca Inlet	1980-81	Occupied < 2 years	6,134	5.0[2]
Kodiak	Kupreanof Strait	1986-87	Occupied < 15 years	5,100 (4230-6230)	4.4[2] (3.6-5.3)
Kodiak	Afognak Island	1986-87	Occupied > 25 years	2,340 (1482-3337)	10.0[2] (6.7-15.1)
PWS	Knight Island	1996-98	Occupied > 25 years-reduced 1989 to 1998	2,260 (1980-2570)	9.9[2] (8.7-11.3)
PWS	Nelson Bay	1980-81	Occupied 2 to 3 years	2,187	8.8[1] (7.7-9.9)
PWS	Montague Island	1996-98	Occupied > 25 years	1,900 (1630-2180)	11.8[2] (10.3-13.7)
PWS	Green Island	1980-81	Occupied > 25 years	1,274	11.3[1] (10.7-11.9)

[1] Based on observations of activity from telemetry.

[2] Estimate based on the number of hours required to obtain the energy needed for maintenance (1,019 kj kg-1day-1) given the measured prey consumption rate. For Montague and Knight, the average sea otter was 22.5 kg. At Orca Inlet, most animals were males and we assumed that the average size was 30 kg.

Table 11. Age adjusted mean body length (cm), mass (kg), and mass to length ratio for 1 to 4 yr old female sea otters from recently colonized Kodiak Island sites, northern Knight Island, and Montague Island. Results of analysis of covariance, and contrasts between sea otters from different areas are given. Means of groups with like letters did not differ significantly at $p<0.05$. Probabilities of equality among areas with respect to age-adjusted means are given. Age effects were significant ($p<0.001$) in all cases.

		Length		Mass		Mass:Length	
Area	N	Mean	Group	Mean	Group	Mean	Group
Kodiak	26	123.63	A	22.46	A	0.181	A
Knight	22	113.88	B	19.43	B	0.170	B
Montague	28	111.09	B	17.88	C	0.160	C
ANCOVA p values for area effect		<0.0001		<0.0001		<0.0001	

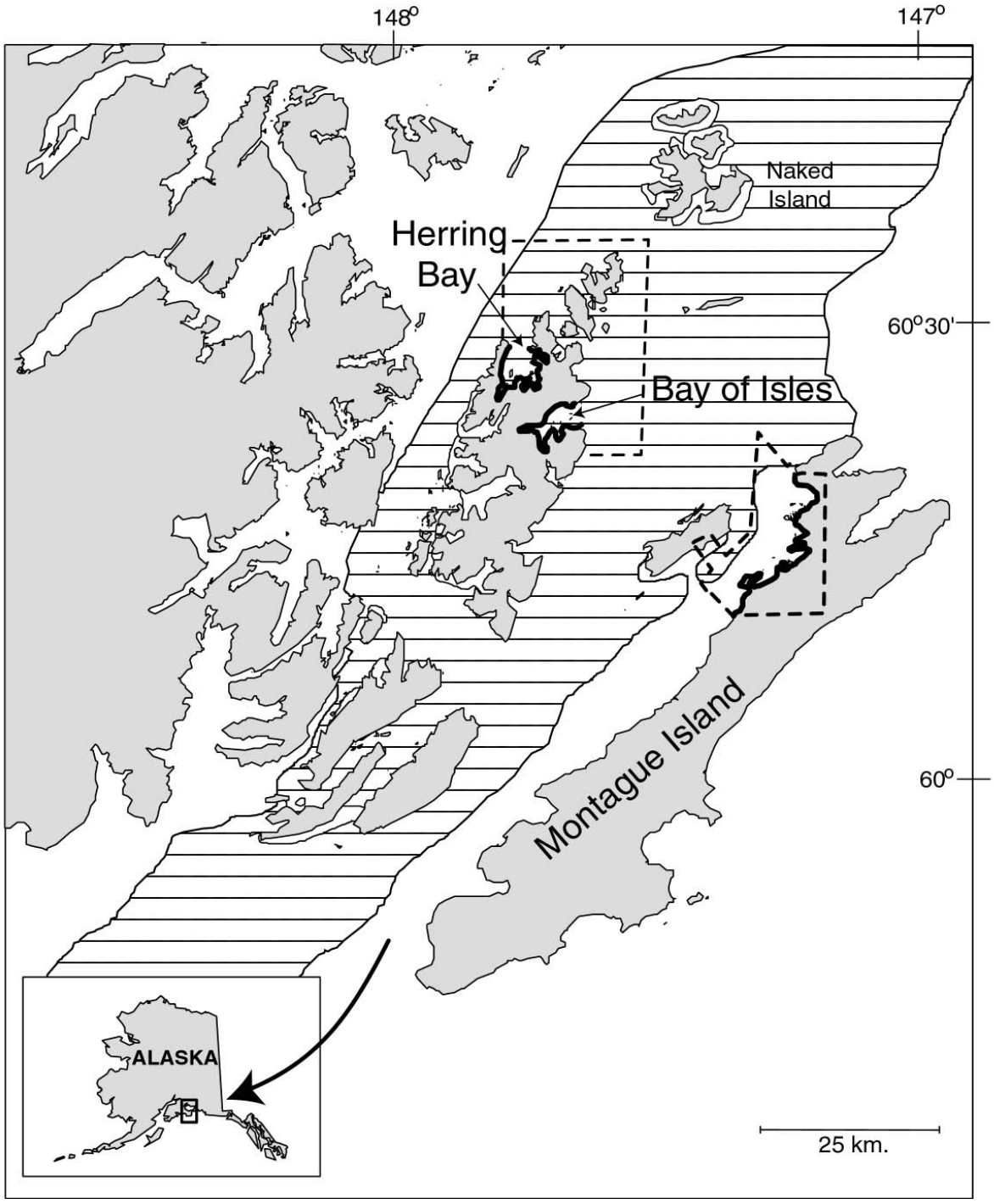

Figure 1. Location of sampling sites for sea otters, and sea otter prey in western Prince William Sound. The cross hatched area indicates the trajectory of oil from the *Exxon Valdez* oil spill based on a hind-cast model (Galt et al. 1991) and shoreline oiling surveys (ADEC 1989, ADNR 1991). Areas where sea otters were surveyed are indicated by a dotted line. Shoreline areas in Herring Bay and Bay of Isles on Knight Island, and Montague Island where prey were sampled in 1996 through 1998 are indicated by thickened lines.

Sea Otter (*Enhydra lutris*) Perspective: Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

Part C. Trophic Linkages among Sea Otters and Bivalve Prey in Prince William Sound, Alaska, in the Aftermath of the *Exxon Valdez* Oil Spill: Implications for Community Models in Sedimentary Habitats

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ABSTRACT

We exploited the *Exxon Valdez* oil spill in Prince William Sound (PWS), Alaska, to evaluate effects of reduced sea otter densities on prey populations in sedimentary habitats. We considered the need for and characteristics of new models for trophic effects of sea otters on coastal marine benthic communities. We viewed evidence for nonlinear or uncertain patterns of prey response to varying sea otter density as particularly significant for new model structure.

We specifically examined responses of densities and size distributions of populations of mussels and clams (several taxonomic and habitat categories), all important sea otter prey in PWS, to reduction in sea otter density caused by the oil spill. We utilized two primary criteria for determining the consistency of prey demographic responses to reduced sea otter densities as predicted by null hypotheses consistent with existing published models. First, prey populations subject to reduced influence by sea otters should be denser and contain proportionately more large individuals than prey populations strongly influenced by sea otter predation. Second, response times of prey demography to reduced otter densities should be similar to response times of prey to increased otter densities, the latter as indicated in existing published models.

Results were disparate with regard to expectation for the six categories of prey evaluated. With few exceptions, density data indicated nonconformance with demographic expectations. In contrast, size data for prey indicated conformance with expectation in about half the categories evaluated. We suggest that lingering effects of the oil spill, nonlinear relationships of sea otters and prey that involve thresholds in otter density, uncertainties in prey recruitment patterns, spatial differences in natural disturbance rate, and differences between areas in effects of competing predators are the main factors possibly accounting for patterns in our data. Recruitment and disturbance effects in particular may include significant stochastic components, especially in a temporal context. We suggest that recovered sea otter populations and their prey do not necessarily exist in long-term stable equilibria, and that development of new models incorporating both trophic thresholds and trophic stochasticity will be important in understanding community-level responses to variable sea otter numbers.

Key words: Clam, community model, *Enhydra lutris*, *Exxon Valdez*, nondeterministic trophic interaction, oil spill, Prince William Sound, sea otter, soft sediment, density threshold in trophic interaction.

INTRODUCTION

The understanding of linkages among top-level predators, primary prey, and associated communities arguably is more complete for sea otters, their prey, and their ecosystems than for any other carnivorous mammal. The history of sea otter populations over the past 260 years has contributed substantially to the current level of knowledge. Sea otters were hunted nearly to extinction during a poorly regulated multinational fur trade from 1741 to 1911 (e.g., Ogden 1941; Barabash-Nikiforov et al. 1947; Kenyon 1969). After receiving protection from Article V of the Treaty for the Preservation and Protection of Fur Seals (37 Stat. 1542) in 1911, many sea otter populations began increasing in numbers and range. By the end of the twentieth century, range-wide sea otter numbers had increased approximately fifty-fold over 1911 numbers (Gorbics et al. 2000), and a number of local populations appeared to be approaching carrying capacity. Much of our understanding of sea otter effects results from analyses of ecosystem behavior as otter numbers have increased rapidly from low densities. We term these results "recovery models."

Recovery models of the influences of sea otters on benthic communities include two components, direct and indirect effects. Direct effects involve three patterns that have been documented repeatedly among locations and habitats, across time, and among prey species e.g., Ebert 1968; Lowry and Pearse 1973; Wild and Ames 1974; Estes and Palmisano 1974; Cooper et al. 1977; Estes et al. 1978; Duggins 1980; Hines and Loughlin 1980; Hines and Pearse 1982; Estes and VanBlaricom 1985; Wendell et al. 1986; VanBlaricom and Estes 1988; Riedman and Estes 1990; Kvitek et al. 1988, 1992; Wendell 1994; Estes and Duggins 1995; Pollard et al. in press). The patterns are a significant reduction in mean density of prey per unit area of substratum, a significant shift in size frequency distribution of prey toward smaller individuals, and a shift in microhabitat distribution of prey in favor of cryptic locations, such as crevices or cracks in rocky substrata, wherein sea otters cannot capture prey efficiently.

The stereotypical direct effects of sea otters, in turn, produce a number of indirect effects that collectively comprise one of the most charismatic and widely recognized of modern ecological paradigms (e.g., VanBlaricom and Estes 1988; Kvitek et al. 1992; Estes and Duggins 1995; Estes et al. 1998). The most commonly cited examples are in kelp forest habitats where direct trophic effects of sea otters on sea urchin populations often produce significant indirect effects. Indirect effects may include proliferation of biomass and diversity of macroalgae, enhanced habitat quality for nearshore marine invertebrates, fishes, seabirds, and marine mammals, enhancement and modification of carbon flow in nearshore food webs, and consequent geological and physical oceanographic changes in the coastal zone (e.g., Estes and Palmisano 1974; Estes et al. 1978; Simenstad et al. 1978; Duggins 1980; Palmisano 1983; VanBlaricom 1984; VanBlaricom and Estes 1988; Duggins 1988; Laur et al. 1988; Duggins et al. 1989; Estes and Duggins 1995). There are, however, substantial published contrary views regarding the ubiquity of recovery models for sea otters and ecosystems (Schiel and Foster 1986; Foster and Schiel 1988). The primary conclusion of the contrarian perspective is that recovery models are over-emphasized and over-generalized in explaining patterns in kelp forests. Criticisms of the models focus primarily on California habitats and may be less applicable in other regions. Although there has been significant work on indirect effects of sea otters in sedimentary (sandy, muddy, or silty) substrata (e.g., Kvitek and Oliver 1988, 1992), less is known in general about sea otter effects on communities of soft substrata.

As sea otter populations approach carrying capacity, fluctuations in otter density become increasingly likely in response to varying food supply and other natural or anthropogenic disturbances. Such fluctuations may include significant reductions in otter density. Whereas recovery models relate are dominated mechanistically by the foraging habits of otters, ecosystem responses to decreasing otter numbers may reflect a larger number of ecological processes, many of them independent of sea otter foraging. Examples include larval recruitment patterns in prey populations and effects of disturbances, such as storm events.

Several recent studies have examined ecosystem responses to perturbations of sea otter populations thought to be recovered from depletion and possibly approaching carrying capacity (Estes et al. 1998; Konar 2000; Appendix SO-07). In each case, ecosystems were observed as otter numbers were locally reduced, either by natural or anthropogenic disturbances or by unknown factors. Collectively, results suggest a range of responses by benthic communities to reductions in density of sea otter populations. In one case (Estes et al. 1998), prey populations responded rapidly to reduced sea otter numbers, and reported patterns at the community level were equal, but opposite to patterns seen previously as sea otter numbers increased. Such responses suggest that linkages of sea otters and communities are both linear and strongly deterministic, and are predictable from recovery models. In two other cases, however (Konar 2000; Appendix SO-07), observed community responses were not consistent with recovery models. The latter results indicate the possibility that connections of sea otters to ecosystems are neither linear nor strongly deterministic. Thus, there may be a need for development of a new category of models that we term “fluctuation models,” improving on recovery models by incorporating more categories of relevant ecological processes.

Our purpose here is to utilize direct effects of the *Exxon Valdez* oil spill (EVOS) of 1989 as a vehicle for approaching the development of fluctuation models for sea otters and bivalve prey in sedimentary ecosystems. The EVOS caused a precipitous reduction of local sea otter densities in parts

of Prince William Sound (PWS), Alaska (Bodkin and Weltz 1990; Bodkin and Udevitz 1994; Garrott et al. 1993; Ballachey et al. 1994; Chapter 3 Part A), although the overall effect of EVOS on the sea otter populations of PWS was uncertain and controversial (Lensink 1990; Garrott et al. 1993; Johnson and Garshelis 1995; Garshelis and Estes 1997; Eberhardt and Garrott 1997). Local depletions of sea otters have persisted in time following EVOS in some areas of PWS, allowing us to study responses of benthic communities of which sea otters are part (Chapter 3 Part A; Chapter 3 Part B).

Available data on foraging patterns of sea otters in PWS indicate that prey are taken primarily from sedimentary habitats, although rocky substrata also are utilized. Bivalve molluscs, especially clams and mussels (*Mytilus trossulus* Gould), generally are the largest dietary component, with crabs, sea urchins, and echiurids also commonly consumed (Calkins 1978; Estes et al. 1981; Johnson 1982; Garshelis and Garshelis 1984; Garshelis et al. 1986; VanBlaricom 1988; Doroff and Bodkin 1994; Chapter 3 Part B). Effects of reduced sea otter numbers on densities of sea urchins and consequent indirect effects on kelp communities of rocky substrata have been considered by Dean et al. (Appendix SO-07) in parallel studies in PWS. We limit our considerations here to bivalve prey in sedimentary habitats.

We ask specifically how populations of six common sea otter prey categories, all bivalves, have responded to the persistent reduction of sea otter density in parts of PWS. We ask if models suggested by our data are linear or nonlinear, deterministic or nondeterministic, and if apparent attributes of fluctuation models are different from known attributes of recovery models. With regard to linearity, we are particularly interested in the possibility that sea otter densities must be reduced below threshold levels before prey populations respond significantly to the reduction in predation rate. The EVOS caused a reduction of sea otter densities in our oiled study areas by at least half (see below). A failure of prey demographic responses to such a reduction is consistent with nonlinear predator-prey interactions, suggesting a threshold reduction of more than 50% in sea otter density before prey populations respond. With regard to determinism, we are particularly interested in the possibility that unpredictable interannual and intersite variability in prey recruitment rates and possibly other factors will influence the capacity of prey populations to respond demographically to reduced rates of mortality from predation. Local recruitment variability in our oiled study areas, over time and compared to unoiled study areas, will introduce uncertainty in the pattern of prey response to reduced sea otter densities.

METHODS

We sampled during summer months in 1996, 1997, and, excepting mussels, 1998. Sampling effort by category is summarized in Table 1.

Sampling Locations and Methods

Study Areas and Sites.—Our study compared abundances and size distributions of sea otter prey populations within a selected oiled vs. a selected nonoiled area. The mobility of sea otters, the diversity of habitats within PWS, and the nonrandom distribution of habitat contamination by EVOS made selection of designated areas, rather than random selection of areas, a necessary approach. Inferential limitations related to our “space for time” study design are, therefore, significant (see also Chapter 3 Part B).

Our oiled study area included two locations on northern Knight Island, Herring Bay, and Bay of Isles that are now well known for the heavy oil accumulations that developed nearshore and onshore as a result of EVOS (Bodkin and Weltz 1990; Galt et al. 1991; Chapter 3 Part B). Our contrasting unoiled area was the northwestern portion of Montague Island. Prey data were collected from approximately Stockdale Harbor southward to "Mooselips Bay" (Chapter 3 Part B). Within each study area (Montague Island or Knight Island), potential sampling sites were chosen by selecting a random starting point, then systematically dividing the shoreline into contiguous, consecutive intervals of 600 m length throughout the rest of the study area. Sampled segments were chosen at regular intervals, beginning at the random starting point. Shoreline segments of 200 m length within selected sites were used for actual sampling.

As a result of heavy oiling, local densities of sea otters declined substantially at northern Knight Island following EVOS (Bodkin and Udevitz 1994; Ballachey et al. 1994; Appendix SO-07; Chapter 3 Part A; Chapter 3 Part B). Available data indicate that sea otter densities at northern Knight Island were less than 50% of pre-EVOS densities during our field work. Sea otter densities at Montague Island did not decline appreciably as a result of EVOS, and oiling levels in our study areas at Montague ranged from none to slight (Galt et al. 1991; Wolfe et al. 1994; O'Clair et al. 1996).

Site locations were determined with Rockwell Precision Lightweight Global Positioning System® (GPS) receivers. Our GPS receivers were equipped with secure differential capability through an agreement with the U.S. Department of Defense allowing locational accuracy of ± 3 m.

Prey Categories.—We focus primarily on six bivalve prey categories: mussels, three taxonomic categories of clams (littleneck clams, *Protothaca staminea* [Conrad]; butter clams, *Saxidomus gigantea* [DeShayes]; bentnose clams *Macoma* spp.), and two habitat-based categories of clams (intertidal and subtidal clams, with all species lumped by category). We found at least 20 clam species in our samples (Fukuyama 2000), with littleneck, butter, and bentnose clams consistently abundant. Our field work included assessments of densities and size distributions of the prey categories. We did not measure patterns of microhabitat distribution of prey species in this study. The significance of our prey categories in the diets of PWS sea otters was evaluated in parallel studies by Bodkin et al. (Chapter 3 Part A), Dean et al. (Chapter 3 Part B), and Fukuyama (2000).

Variables Measured.—Prey density data are represented as individuals per unit of substratum surface area. Prey size distributions are based on maximum shell dimension of individual prey, as measured to the nearest 0.1 mm with Vernier or digital calipers. In the case of clams sampled subtidally, we consider only individuals ≥20 mm in maximum shell dimension. Subtidal sampling methods are considered biased against individuals smaller than this lower limit. In the case of mussels, we consider only individuals 5 mm or more in maximum shell length.

Mussels occur exclusively in intertidal habitats in PWS. Littleneck clams are primarily intertidal, but are found on occasion in subtidal samples. Butter and bentnose clams can be abundant in both intertidal and subtidal habitats. Because intertidal and subtidal sampling for density was done with markedly different methods, we treated intertidal and subtidal density data separately by prey category in all analyses. For evaluations of size distribution we lumped intertidal and subtidal data for littleneck, bentnose, and butter clams. We evaluated the two

multispecies categories in order to achieve greater statistical power in comparisons between oiled and unoiled areas. Contrasts of prey categories between areas are summarized in Table 2.

Sampling Methods: Subtidal Clams.—Subtidal clams were sampled within a randomly chosen subset of the 200-m segments selected for intertidal sampling. Sampling was done directly offshore of intertidal sites. Two depth strata (10 m and 20 m) were sampled at each site for subtidal clam populations. A Venturi suction dredge was used to sample clams ≥20 mm in maximum shell dimension. The dredge hose and nozzle were dropped in the appropriate depth stratum at each site and a 15-m transect tape was laid on the bottom substratum along the depth contour, beginning where the nozzle came to rest on the seafloor. A steel quadrat frame (0.5 x 0.5 m) was placed along the transect tape at a random starting point within the first 3 m of the tape. Three to five replicate quadrats 3 m apart were sampled. All clams excavated by the dredge were collected and placed into mesh bags. All live clams were measured with Vernier calipers to the nearest 0.1 mm and preserved in 10% formalin solution for later identification. Dredging depths depended upon the substratum, but were generally about 0.5 m deep.

Power analysis was performed on preliminary subtidal clam density data collected in 1995. For all clams ≥20 mm lumped across species, power analysis revealed that a sample size of 46 sites would allow the detection of a difference of 50% of the mean at the $\alpha = 0.10$ significance level with power $(1 - \beta) = 0.75$.

Assemblage of Dead Intact Butter Clam Shells.—Unconsolidated sedimentary habitats in PWS often contain patches of empty clamshells, from clams likely killed by an unknown past disturbance event or events. The shells, here termed the “dead intact clam assemblage” (DICA), have been described previously from intertidal habitats in PWS (e.g., Baxter 1971; Estes and VanBlaricom 1985; Kvitek and Oliver 1988). The DICA clams are typically found in life position, both valves present and articulated, with the siphonal aperture oriented upward. In the course of our subtidal sampling for live clams, we found that DICA clams were virtually ubiquitous in the areas we sampled, most often as a layer 30–50 cm below the sediment surface. The DICA was dominated by butter clams. We sampled DICA clams in the same manner as described above for live subtidal clams, and with the same level of sampling effort (Table 1). The value of DICA clam data to the testing of our principal hypotheses is elaborated below in “Discussion.”

Sampling Methods: Intertidal Clams.—Clams were sampled within the 200-m intertidal segments at both Montague Island and Knight Island, as described above. Within each segment, a randomly selected distance (0–150 m) was chosen as the starting point. A transect line was placed at the starting point and extended for 30 m, parallel to the shoreline at the tidal datum. Five 0.25 m^2 samples were collected at randomly selected locations along the line. The sediment in each sample was excavated to a depth of 30 cm. Sediment was first hand sorted to remove larger clams, then washed through a series of screens. The mesh size of the smallest screen was 5 mm x 5 mm. All clams were retained for later identification, counts, and measurements.

Sampling Methods: Mussels.—Mussels were sampled in 500 cm^2 quadrats on transects within 200-m long shore segments. Ten transects were laid 20 m apart within each shore segment. The first transect was placed a random distance between 0 and 20 m from the boundary of the shore segment. The remaining nine transects were laid systematically at 20-m intervals starting from the first. Each transect was laid perpendicular to shore from the upper limit to the lower limit of distribution of mussels. A quadrat was positioned a random distance along each transect. All mussels were removed from within the quadrat, placed in a plastic bag, and frozen within 3–4 hours of collection.

Following cost and power analysis performed on preliminary mussel density data collected in 1995, a sample size of 60 shore segments per study area was chosen. Power analysis revealed that this sample size would allow the detection of a difference of 42 mussels/500 cm^2 (55% of the mean) at the $\alpha = 0.05$ significance level with power $(1 - \beta) = 0.8$. The segments were post-stratified into two strata based on substrate: 1) rocky (including bedrock and boulder areas) and 2) unconsolidated or mixed substrate (including various mixtures of mud, sand, pebbles, cobbles, and shell litter).

Mussel samples were sieved in the laboratory using 4-, 2-, 1-, and 0.5-mm mesh sieves. Small mussels were counted in two size classes (0–2 and 2–5 mm) based on shell length. These data were used for qualitative considerations of recruitment rate (see Discussion), but were not included in quantitative contrasts among area in density, mean size, or size distribution. The shells of all mussels 5 mm or greater in length were measured to the nearest 0.1 mm with a digital caliper connected to a data logger.

Analytical Methods

Data for each prey category are combined among years in most cases. We did not consider year effects in most of our analyses because most sampled taxa are long-lived and grow slowly and because separate analyses (Fukuyama 2000) showed year effects to be generally insignificant for clams. In general, we used student's *t* tests to compare mean densities and mean size distributions and the Kolmogorov-Smirnov (K-S) two-sample test to compare size distributions, with $\alpha = 0.05$. Because of the much larger data set available for mussels and the *a priori* expectation of year effects in mussel data, we utilized analysis of variance (ANOVA) to compare mean density and mean size by stratum and year and between areas for mussels.

Subtidal Clams.—Only densities of clams $\geq$20 mm in size were compared. Several sites were sampled in more than one year. In those few cases, a mean density over the years was used as the single observation of density by site. In all other cases, observation of density by site was the mean number of clams observed in individual plots sampled at each site.

Intertidal Clams.—All collected clams were compared for the three dominant taxa found, *Protothaca staminea*, *Saxidomus gigantea*, and *Macoma* spp. Intertidal sampling methods were considered less biased against small clams than were subtidal sampling methods, so all clams collected from the intertidal were included in the analyses for the multispecies category. Individuals <20 mm shell length were excluded from analyses for individual clam species and for

Macoma spp. because the data were combined with subtidal data that were biased against smaller individuals.

Mussels.—Analysis of variance was used to test for differences between study areas, by stratum and year, in mussel density, and mean size. Transformations (log [y+1], $y^{-0.5}$) were used to stabilize variances. Because the data for mussels ≥40 mm contained many zeros, we used the Mann-Whitney U-test to test for differences in mussel density by stratum between study areas for this size class.

Models and Decision Rules

For each of the six prey categories of interest, we specify a simple model linking density and size distributions with sea otter population status. We then define decision criteria for each category providing the protocols by which we address questions of linearity and determinism in sea otter effects on prey. Because there were few data available on the demography of most taxa of sea otter prey in PWS prior to EVOS, we cannot make confident comparisons of prey density and size in particular locations before and after EVOS. As a substitute, we compare prey demographic data from our two study areas.

We use two criteria to judge if our data are consistent with recovery models for sea otter effects as defined above and to assess nonlinear or nondeterministic tendencies in prey responses to reduced sea otter numbers. The first involves direct contrasts of prey demographic data between study areas. As noted above, in prevailing recovery models, sea otters are known to reduce the mean density and alter the size distribution of preferred prey. Expectations of recovery models regarding density and size are summarized in Figures 1 and 2. We interpret existing recovery models to predict significantly greater measured densities and significantly greater numbers of large individuals at our oiled area as compared to our unoiled area. This follows from the reduction of sea otter density in the oiled area. Results different from stated expectation indicate nonlinear or nondeterministic tendencies in demographic responses of prey, carry important implications for the development of fluctuation models, and require invocation of factors other than sea otter predation for understanding of prey demography.

We assume that, at the time of our study, effects of oiling by EVOS in our oiled study area were limited to the demography of sea otters but not their prey. Studies of mussel populations in PWS suggested that demographic recovery from EVOS effects had occurred by 1995 (Highsmith et al. 1996; Houghton et al. 1996; Coats et al. 1999), supporting our assumption. However, the assumption is untested for other prey categories.

The second criterion involves assessment of time scales of response by prey species to changes in sea otter density. To apply this criterion, we define a concept termed “symmetry.” As sea otters reoccupy a formerly vacant area, primary prey populations will shift to predictable new patterns of density and size distribution over a period T_A (Fig. 3). These changes are the foundation of current recovery models. Although there is no published empirical evidence for bivalves in sedimentary habitats, the simplest interpretation of recovery models leads to predictions that a significant local reduction in otter density would produce a consequent shift in prey density and size distribution. The latter shift would be comparable in magnitude to that associated with reoccupation by sea otters, but opposite in direction. The time required for the

second type of shift in prey demography is defined as T_B. We define the symmetrical case as $T_A \approx T_B$ and the asymmetrical case as $T_A < T_B$. We do not consider a second asymmetrical option ($T_A > T_B$) here. Given the relatively brief duration of T_A in many reported cases, we suggest that the outcome $T_A > T_B$ is implausible in the case of sea otters. To our knowledge, the only published empirical evidence for symmetrical responses is that of Estes et al. (1998) based on data from Aleutian kelp forests.

In some cases, the data needed for a confident estimate of T_A, as defined above, are only marginally adequate. In other cases, the published data suggest somewhat different values of T_A for different locations within the geographic range of sea otters. In cases where data from different locations indicate different estimates, we base the estimate of T_A on data from the locations closest geographically to PWS. In determining T_B in our study, we assume that significant fluctuations in density or size distribution of focal prey populations did not occur between 1989 and 1996. Available data (e.g., Doroff and Bodkin 1994) are consistent with our assumption. It is possible, however, that significant fluctuations could have been overlooked. Estimation of T_A may also be influenced by differences in spatial scales of cited studies used as the basis for estimates. The time scale of community response may be much smaller for small areas of habitat than for regional-scale responses.

***Decision Criteria*.—1. Mussels**. Sea otters are known to forage on mussels in the intertidal zone throughout the North Pacific Rim (VanBlaricom 1988). To our knowledge, the only published data detailing functional interactions of mussel population structure and sea otters are those of VanBlaricom (1987, 1988). VanBlaricom's data, based on work done in PWS from 1978 through 1986, suggested two possible models linking sea otter foraging and mussel population structure. Both are recovery models, as defined above, developed during a period of numerical growth and distributional expansion in the sea otter populations of PWS. In the first, large mussels (≥40 mm maximum shell length) are rare or entirely absent in areas occupied by territorial adult male, breeding female, and dependent juvenile sea otters. In such areas, mean mussel densities were relatively low and mussels often occurred primarily as scattered small patches, frequently associated with cryptic microhabitats. In areas lacking sea otters or occupied by nonbreeding male sea otter aggregations, mussel populations often contained large individuals in dense, multilayered patches. VanBlaricom (1988) proposed that breeding females and dependent juveniles often forage on mussels as a method for fostering competency in prey collection by juveniles, improving the odds that young animals will acquire the skills needed to locate and obtain, independently, more nutritionally rewarding prey, such as infaunal clams. Differences among locations in density, size distribution, and microhabitat use by mussels, thus, reflect differences in frequency of foraging on mussels by breeding females and dependent pups vs. nonbreeding males. The second model takes note of the frequent observation that breeding female and dependent juvenile sea otters often forage preferentially in protected areas of PWS, such as bays or coves. VanBlaricom (1988) suggested that differences in mussel demography between protected coves (breeding areas) and more exposed locations (nonbreeding areas) result from disproportionately high pressure on prey in protected locations during periods of rough weather or seas. Our study areas share similar physiographic characteristics suggesting that, by either of the alternative models, foraging by sea otters in both areas should result in few mussels exceeding 40 mm in length, and in reduced density of mussels compared to unforaged locations.

Both study areas provide some protection from heavy weather and seas. The Montague Island study area is clearly an active breeding area for sea otters (Chapter 3 Part A), and Herring Bay and Bay of Isles likely were prior to EVOS. Thus, the reduction of sea otter numbers at northern Knight Island should result in a significant increase in mussel density per unit area and a significant increase in the density of mussels ≥40 mm in shell length (Figs. 1 and 2).

There are few published data useful in estimating T_A for interactions of sea otters and mussels. VanBlaricom (1988) reported rapid reductions in densities of large mussels in Orca Inlet, PWS, paradoxically associated with a winter influx of male sea otters. The time frame for development of effects of foraging by breeding female and dependent juvenile sea otters on mussels has not been estimated. We assume an estimate of 5 years for T_A given the pattern observed in Orca Inlet and the general time frame of effects of sea otters on easily obtained epifaunal prey observed elsewhere (e.g., Kvitek and Oliver 1988). Because our data were collected 7–9 years after EVOS, we predicted increased densities and size distributions of mussels at northern Knight Island as compared to Montague Island in the symmetrical case.

2. Clams. Clams comprise the bulk of sea otter diet in PWS (Calkins 1978; Estes et al. 1981; Doroff and Bodkin 1994; Fukuyama 2000; Chapter 3 Part B) and are an important component of sea otter diet in much of coastal southern Alaska (e.g., Kvitek et al. 1992; Kvitek and Oliver 1992). Venerid and tellinid clams, including the focal species of our study, typically dominate the bivalve component of sea otter diet in Alaska. Published data indicate that sea otters select as prey the larger clams from the available range of clam sizes, especially in the case of venerids (including butter and littleneck clams). Data from Kvitek et al. (1992) indicate that sea otter predation causes a reduction in density per unit area and a shift to smaller values in the size distributions of subtidal clam populations in the Kodiak Archipelago, Alaska. The modal size of clams taken as prey generally is between 60 and 80 mm (maximum shell length), with a range from 30 to 100 mm. As a consequence, mean clam size at Kodiak is 55 mm or smaller in areas occupied by sea otters and approximately 70 mm in areas without sea otters. Because of typically aggregated distributions in bivalves, it is difficult to generalize about sea otter effects on clam density per unit area. At Kodiak, densities of clam species preferred by sea otters are lower by at least half in areas with sea otters compared to areas without (Kvitek et al. 1992). Kvitek and Oliver (1992) report similar patterns in southeastern Alaska. These patterns form the primary basis for our model of interactions of sea otters and clams (Figs. 1 and 2).

The data from the Kodiak region indicate that effects of sea otter foraging on clam populations are statistically apparent a minimum of 5 years after occupation of an area by sea otters (Kvitek et al. 1992), although demographic responses in some clam populations may require more time (Estes and VanBlaricom 1985; Kvitek et al. 1992). In other locations, especially in California, the effects of sea otters on clam populations are known to become apparent on time scales ranging from <1 year (Wendell et al. 1986) to >10 years (Hines and Loughlin 1980; Kvitek and Oliver 1988). Geographic distances limit applicability of California patterns to PWS. We utilize a value of 5 years for T_A in our model based primarily in patterns from the Kodiak region that are relatively close in space to PWS and involve the same prey taxa as in PWS. We recognize that different values for T_A are plausible. Because our data were collected 7–9 years after EVOS, we view greater mean sizes and greater proportionate numbers

of larger clams at northern Knight Island, as compared to Montague Island, as consistent with recovery models and with a symmetrical response to reduced otter numbers as defined above.

RESULTS

1. Mussels:

Mussel data were stratified by substratum type, size category, and year (Table 2). Mussels were sampled in 1996 and 1997, but not in 1998.

On rocky substrata, there were no significant differences in density between areas for any size class in 1996. Mean density on rocky substrata was significantly higher at northern Knight Island (KI) for mussels $\geq$5 mm in length in 1997. In contrast, mean density was significantly higher at Montague Island (MI) on rocky substrata for mussels $\geq$20 mm in length in 1997. Densities of mussels $\geq$40 mm in length were quite low in both areas and did not differ significantly between areas in either year.

On mixed substrata, differences in density between areas for mussels $\geq$5 and $\geq$40 mm in length were not significant in either year. Densities of mussels $\geq$20 mm in length were significantly greater at Montague Island in 1997, but not in 1996.

Length–frequency data (Fig. 4) indicate greater mean sizes of mussels at Montague Island on both types of substratum in both sampled years (Table 2). Size distributions also were different between areas (Table 2).

Based on our models and decision criteria, we conclude that expectations based on recovery models are not met for mussels. There are significant differences in density in only 3 of 12 possible year-by-stratum contrasts between areas, and two of the three significant differences are opposite to expectation (MI>KI, rather than KI>MI). All 10 possible year-by-substratum contrasts between areas for size were contrary to expectation for mussels. Thus, we conclude that $T_B > T_A$ for mussels in PWS and that the interaction of sea otters and mussels in PWS is asymmetrical.

2. Littleneck Clams:

Density data for littleneck clams were separately analyzed for intertidal and subtidal habitats, but size data were lumped across habitats. Data were lumped across years for density and size. Data were collected in 1996, 1997, and 1998.

There were no significant differences in density of littleneck clams for either habitat category between areas (Table 2). The mean size of clams was significantly greater at Knight Island than at Montague Island (Table 2), but size distributions of clams (Fig. 5) did not differ significantly between areas (Table 2).

Contrasts of density data for littleneck clams failed to meet expectations of recovery models. Contrasts of size data were weakly consistent with expectation, but only with regard to central tendency. The interaction of sea otters with clam density is asymmetrical by our definition. The interaction with size data is unclear.

3. Butter Clams:

Data for butter clams were collected on the same schedule and analyzed in the same manner as for littleneck clams. Results of contrasts between areas for butter clams were the same as for littleneck clams (Table 2), with one exception. Size distributions (Fig. 5) were significantly different between areas (Table 2). Thus, the results for butter clams are divergent. Patterns in density data are not consistent with expectations of recovery models and suggest that the interaction with sea otters is asymmetrical. Results for size data are consistent with expectation and with a symmetrical interaction.

4. Bentnose Clams:

Data for bentnose clams were collected and analyzed in the same manner as for littleneck and butter clams. Densities in intertidal and subtidal habitats and mean sizes were significantly greater at Montague Island than at Knight Island (Table 2). Size distributions (Fig. 5) also were significantly different between areas (Table 2). Thus, data for bentnose clams are entirely inconsistent with the expectations of recovery models for density and size distribution. The data indicate that the interaction of clams with sea otters is asymmetrical.

5. Intertidal Clams:

Intertidal clam data, lumped by species, were collected in 1996, 1997, and 1998. Mean densities of intertidal clams were significantly greater at Montague Island than at Knight Island (Table 2). Length–frequency data for intertidal clams (Fig. 6) indicate a greater mean size at Knight Island, but no significant difference in size distribution between areas (Table 2). Thus, results for intertidal clams also are divergent. Density data are strongly contradictory to the pattern expected from recovery models, but size data are weakly consistent with the models. Likewise, density data are consistent with asymmetrical interactions with sea otters, while size data suggest a more symmetrical interaction.

6. Subtidal Clams:

Subtidal clam data, lumped by species, were collected in 1996, 1997, and 1998. Results of contrasts between areas were generally similar to those for intertidal clams (Table 2), except that the contrast of size distributions (Fig. 7) was significantly different between areas (Table 2). Thus, subtidal clam data are strongly divergent. Density data are opposed to expectations of recovery models and indicate an asymmetrical interaction with sea otters. Size data match model expectations and indicate a symmetrical interaction with sea otters.

7. Comparison of DICA and Live Butter Clams at Knight Island:

The DICA butter clam densities and mean sizes were significantly higher than live butter clam densities at Knight Island, and distributions of sizes of DICA (Fig. 8) and live clams were significantly different (Table 3). The DICA data indicate that habitats at Knight Island have

previously supported subtidal populations of large butter clams at high density. The data are consistent with the argument that clam populations exposed to low levels of predation by sea otters at Knight Island have the capacity to increase in density and mean size. Thus, the contrast of DICA and live clams at Knight Island suggests inconsistency with recovery model predictions and an asymmetrical interaction with sea otters.

DISCUSSION

Our data indicate that bivalve prey of sea otters in PWS did not respond to a significant and extended reduction of sea otter densities in the manner predicted by recovery models as defined above. Our data are the first empirical demonstration of the need for new fluctuation models (as defined above) of trophic interactions of sea otters and prey in unconsolidated sedimentary habitats.

Results of our contrasts of live and DICA butter clams at Knight Island allow us to discount the possible importance of fixed habitat differences between areas for some prey categories. The most plausible hypothesis for the existence of the DICA is a catastrophic, large scale mortality event associated with the great earthquake of 1964, epicentered at Esther Island in PWS, about 60 km north of our study areas. The earthquake is known to have caused tectonic deformation of the sea floor and tsunami-scale water movement and sediment displacement in PWS (e.g., Plafker 1965; Reimnitz and Marshall 1965; Barrett 1966; Baxter 1971). Thus, the DICA assemblage may represent a “snapshot” of subtidal clam populations in 1964, when sea otter numbers in our study areas probably were substantially lower than they were at the time of EVOS. The only pre-earthquake data for the region are aerial survey counts (Lensink 1962), qualitatively suggesting that sea otter numbers in our Montague Island study sites were much lower prior to the 1964 earthquake than they were at the time of our field work. Lensink did not report survey data for Herring Bay or Bay of Isles. Lensink’s survey data are the only scientific surveys of sea otter populations done in PWS prior to the 1964 earthquake (Kenyon 1969).

The DICA butter clams were larger and more abundant than live clams at Knight Island (Table 3), and estimated densities and size distributions of DICA butter clams were quite similar between Knight and Montague Islands (Fukuyama 2000). There are measurable physical differences between areas (Fukuyama 2000), and the differences may be important for some prey species considered here (see below), but patterns in the DICA data are consistent with the capacity of Knight Island habitats to support more abundant live populations of large clams than we observed, at least for one species highly preferred as prey by sea otters.

Our findings indicate differences in response of prey densities and prey size distributions to reduced sea otter densities. For mean prey densities, only 1 of 14 main statistical contrasts between areas produced a result consistent with the predictions of recovery models. The single consistent result, mussels 5 mm or greater in shell length on rocky substrata, held only for one of two sampled years. For mean sizes and size distributions of prey, 6 of 14 main analytical results were consistent with predictions of existing models. Thus, meaningful fluctuation models must include a capacity for predicting apparently divergent responses of density and size to reduced sea otter numbers.

Our divergent results suggest that the processes of density regulation and size regulation may be somewhat independent in fluctuation models. For purposes of understanding our data, we must evaluate factors that regulate recruitment, postmetamorphosis survival, and individual growth in prey populations released from maximum levels of sea otter predation. We suggest that five factors may explain the primary patterns in our data: lingering direct effects of EVOS, thresholds in the relation of sea otter densities and prey demography, infrequent recruitment to prey populations, natural disturbance events, and effects of predators other than sea otters.

Effects of residual contamination of nearshore benthic habitats by the EVOS could account for the apparent failure of prey taxa at northern Knight Island to respond as predicted by recovery models to the reduction in local sea otter density. There is abundant direct evidence of both acute and chronic contamination of sedimentary habitats and ecosystems in PWS by EVOS residues (Paine et al. 1996; Spies et al. 1996; Coats et al. 1999; Jewett et al. 1999; Peterson 2001; Dean and Jewett 2001) and consequent tissue contamination of species consumed by sea otters (e.g., Gilfillan et al. 1995; Dean et al. 1996; Driskell et al. 1996; Highsmith et al. 1996; Houghton et al. 1996; Short and Babcock 1996; Short and Harris 1996; Trowbridge et al. 1996; Roberts et al. 1997; Fukuyama et al. 2000). The bulk of available evidence indicates that direct effects of spilled oil were greatest in intertidal habitats, with effects on subtidal habitats somewhat more equivocal. For example, tissue collection of subtidal clams for hydrocarbon analyses did not show any difference in total alkanes, total aromatics, and unresolved complex mixtures between oiled and unoiled areas (Doroff and Bodkin 1994).

Studies of biochemical markers in sea otters indicate ongoing exposure to hydrocarbons at northern Knight Island (Chapter 2). Because EVOS residues seem the most likely source of exposure and trophic linkages the most likely pathway, the biochemical data from sea otters are indirect evidence of chronic contamination of prey, possibly at levels altering normal processes of individual prey growth and prey population dynamics (see for example Fukuyama et al. 2000 for data on littleneck clams). Exposure of sea otters to EVOS residues could come from nontrophic pathways, and the identity of possible trophic sources remains unknown. However, we suggest that, in general, residual EVOS contamination is a likely mechanism for limiting the capacity of prey populations to respond numerically to reduced sea otter densities. Our conclusions in this regard contradict a central premise of our study design as indicated previously. Thus, in discussing other possible factors affecting responses of prey to reduced sea otter densities, we must regard lingering EVOS effects as possibly interacting with non-EVOS factors to influence prey demographic responses.

One possible form of nonlinear relationship between sea otter density and prey demography involves thresholds in sea otter density. The EVOS event reduced sea otter densities by at least half in our oiled area. Evaluated prey categories may require a larger reduction in sea otter density before significant changes in prey density or size distribution can occur. Our results are consistent with a threshold-based nonlinear relationship of this type. Thus, our results could be entirely deterministic, with the apparent failure of prey categories to respond to reduced otter densities resulting entirely from the nonlinear character of the relationship. Sea otters in PWS seem to have highest preferences for butter clams and for clams in subtidal habitats (Fukuyama 2000). In a nonlinear threshold-based predator-prey relationship, smaller changes in sea otter densities should be required to produce demographic responses by highly preferred prey than by less desirable prey. Our results are consistent with this scenario and strengthen the case for significant nonlinear elements in the predator-prey relationship. However, at present we do not have adequate information to distinguish possible

nonlinear effects from effects of other factors listed below, some of which include substantial stochastic components.

Populations of nearshore benthic invertebrates at temperate and subpolar latitudes often are limited in growth potential by low average annual rates of recruitment (e.g., Quayle and Bourne 1972; Paul and Feder 1976; Estes and VanBlaricom 1985). Chronically low recruitment rates could facilitate the observed absence of a numerical response to reduced sea otter numbers at Knight Island. This factor may be particularly significant for subtidal clams, the mainstay of sea otter diet in PWS. Our data indicate relatively few small individuals among subtidal clams, although the use of the Venturi dredge for sampling may impart a bias against small individuals. A separate analysis of core samples taken at the same times and places as the Venturi dredge samples (Fukuyama 2000) confirms that juveniles of the clam species most preferred by sea otters (venerids and tellinids) were uncommon through the sampling done in 1996 through 1998. However, juvenile venerid and tellinid clams were more abundant at Montague Island than at Knight Island over the time frame of our study (Fukuyama 2000). Small individuals were common in sampled populations of mussels and intertidal clams in both areas, and densities of juvenile mussels at Knight Island were relatively high in both 1996 and 1997. We suggest that low recruitment rates in subtidal clams at Knight Island may contribute to nonconformity with recovery models and asymmetrical predator-prey interactions involving sea otters in PWS.

As in most studies of nearshore ecology in PWS, our field work, done in 1996, 1997, and 1998, was concentrated in the summer months. However, a related study (Gage 1998) included midwinter field work in December 1995. During that period a strong northerly wind event was observed in Herring Bay. The winds and associated low air temperatures produced severe wave action and substantial ice accumulation on exposed rocky intertidal surfaces, particularly those oriented to the north. Such events have the potential to produce significant mortality in intertidal invertebrate populations, including mussels and clams. Other seasonal disturbance events common to PWS include rockslides, avalanches, and floods, all of which can influence nearshore marine habitats, although to our knowledge quantitative verification is lacking. We suspect that physical disturbances such as avalanches and rockslides are common in our study areas, especially at Knight Island, during winter when observational effort is minimal. Effects of such events could on occasion extend to subtidal sedimentary habitats and affect the demography of bivalve populations. The dramatically higher nearshore terrestrial relief of Knight Island, compared to the portions of Montague Island adjacent to our study areas, is consistent with a hypothesis of higher rates of disturbance at Knight Island.

A number of benthic invertebrates are themselves nearshore predators in PWS, and represent potential competitors for sea otters, as well as a mechanism that might facilitate asymmetry in predator-prey interactions involving sea otters. Gage (1998) surveyed predatory invertebrates in our study areas in PWS in 1995 and 1996, assessing spatial and temporal variation in density and diet with an emphasis on subtidal habitats. Of the predatory invertebrates studied, the sea star *Pycnopodia helianthoides* (Brandt) was most abundant and had the greatest overlap in diet with sea otters. Gage reported that diet and density of *Pycnopodia* varied with season, but that there were no trends in diet or density among years or between study areas. *Pycnopodia* diet in PWS is dominated by small gastropod molluscs, including juveniles of some species also consumed by sea otters (Gage 1998). However, the frequency of prey shared with sea otters and the measured rate of prey consumption indicate only a minimal likelihood that *Pycnopodia* is a significant competitor for food with sea

otters, or that predation by *Pycnopodia* facilitates responses of sea otter prey not compliant with recovery models. Thus, we doubt that predation by invertebrates contributed substantially to observed patterns in our data from subtidal habitats.

Predation by other consumers may have influenced patterns of response in mussel populations in our study areas. Carroll and Highsmith (1996) reported effects of predation by the whelk *Nucella lima* (Gmelin) on a mussel population in southern Cook Inlet, Alaska. The mussel population had experienced catastrophic mortality from a cold weather event, resulting in loss of larger size classes in the population. High rates of predation on small mussels by *Nucella* prevented the recovery of predisturbance dominance by large size classes. *Nucella* is common in PWS (VanBlaricom 1987) and could have prevented recovery of larger size classes of mussels at Knight Island following heavy oiling and reduction of sea otter densities by EVOS. O'Clair et al. (Appendix SO-05) found *N. lima* and a similar species, *Nucella lamellosa* (Gmelin), to be more abundant in our oiled study areas at Knight Island than in our unoiled area at Montague Island during the period of our field work. Field estimates of density and laboratory estimates of feeding rate for *Nucella* indicate that the rate of predation by *Nucella* on mussels was 10-fold higher at Knight Island than at Montague Island. Further, mean sizes of mussels on a local scale at Knight Island were significantly negatively correlated with local *Nucella* densities. No such correlation was found in the unoiled site at Montague Island. The data of O'Clair et al. (Appendix SO-05) are consistent with the hypothesis that other predators interfered with recovery of mussels from EVOS at Knight Island, despite reduced densities of sea otters. These data are particularly interesting in the context of data reported by Ebert and Lees (1996), indicating that oiling reduced both growth and survival rates of *N. lamellose* in PWS. We do not have an explanation for the apparent contradictions indicated by the two data sets

Our conclusions are subject to some caveats. Most importantly, we have noted moderate uncertainty in determination of T_A for subtidal and intertidal clams, and substantial uncertainty for mussels. Our general claim of asymmetrical interactions could be tested more definitively with improved data on T_A for clams and mussels in PWS. Our assessments of T_B may also be questioned. Our prey population data were collected in 1996, 1997, and in some cases 1998. There are few intervening data between EVOS in 1989 and the commencement of intensive prey sampling in 1996, in the specific study areas that we sampled. Thus, it is possible that we missed significant increases in prey size distribution or density between 1989 and 1996, obscured by subsequent reductions. In our view such fluctuations are plausible but unlikely. Available published data (e.g., Doroff and Bodkin 1994) are consistent with our position. Thus, there is some risk that we have overestimated T_B for our six focal prey categories. Our discussion is based on the premise that we have estimated both T_A and T_B with reasonable accuracy.

Density and size data for bentnose clams were notable for larger means at Montague Island than at Knight Island. Montague Island clearly supports larger populations of bentnose clams, and we suspect that physical habitat differences between areas (Fukuyama 2000) may be important in this specific case. Bentnose clams are most abundant in silty sediments, and silty sediments are more prevalent at Montague Island. Thus, we suspect that size and density data for bentnose clams may have lesser value in developing fluctuation models than for other prey categories considered here. Moreover, two of us (D. Monson and J. Bodkin) have studied bentnose clam populations in shallow sediments at Glacier Bay, southeastern Alaska, in areas lacking sea otters. Size and density values are comparable to our data from Montague Island despite high sea otter densities at the latter area (Monson and Bodkin, unpublished data).

A primary goal of our project was evaluation of nondeterministic and nonlinear tendencies in the linkage of sea otters to lower trophic levels in sedimentary habitats. Our data indicate that nonlinear patterns cannot be ruled out. Our data also indicate that reduction of sea otter density does not necessarily produce predictable responses in prey demography on a predictable time schedule, in direct contrast to time lines for prey changes resulting from local increases in sea otter numbers. Many of our data for sea otter prey show a lack of compliance with demographic predictions of recovery models (increased size and increased density of prey) and indicate asymmetrical temporal interactions with fluctuating sea otter numbers. These patterns are conceptual surrogates for nonlinear and uncertain trophic linkages. Our design does not allow us to resolve which of the two concepts applies to our study areas. Nevertheless, the patterns indicate the need for significant improvements over recovery models.

Observed patterns in juvenile clam abundance (Fukuyama 2000), sea otter abundance (Chapter 3 Part A; Appendix SO-07), and prey density and size distributions (Table 1 and Figs. 4 through 7) suggest primary attributes of a fluctuation model for our study areas. This qualitative model also provides a possible explanation for the observed divergence in results between size and density data as described above. At Montague Island, recruitment rates of prey are relatively high for reasons unknown, but survival rates of adult clams are relatively low because of a high density of sea otters. The opposing effects of high recruitment and high adult mortality produce dynamic equilibria with relatively dense prey populations dominated by smaller, younger individuals. Should a significant reduction in sea otter density occur at Montague Island, the predicted result would be relatively rapid size distributional and numerical responses in prey populations, contrasting dramatically with our perception of the consequences of EVOS at Knight Island. At Knight Island, dynamic equilibria in prey populations are established by opposing effects of relatively low recruitment rate (with the possible exception of mussels as indicated above) and low adult mortality rate, the latter caused by effects of EVOS on local sea otter densities. Adult prey are fewer in number, but also have lower per capita mortality rates, compared to populations at Montague Island. The result is low density populations with a larger representation of larger individuals. The DICA data for butter clams clearly indicate low adult mortality rates at both locations during the period of years prior to the butter clam mortality event. Such conditions are most likely in PWS if sea otters are either at low densities or absent altogether. If the mortality event occurred in 1964 as we surmise, it follows that sea otters were rare or absent in both study areas in 1964. It is likely that an assemblage demographically comparable to our DICA data can develop in PWS only with an extended period of greatly reduced sea otter densities, although the length of the requisite period should vary by area inversely with local recruitment rate of prey.

We suggest that indications of asymmetrical interactions in our data may result from threshold effects in a nonlinear relationship of sea otters and prey, and from four other factors, three of which are natural processes independent of EVOS. The natural factors (infrequent major recruitments, other predators, and natural disturbances) can interact in a number of ways to extend mean values and variances for TB. We offer one hypothetical scenario for an extended TB in Figure 9, recognizing that the number of possible variants is infinite. In general, the mean value of TB can be represented as a function of the listed factors, with due consideration of stochasticity. In a case with reduction of sea otter densities below the threshold required for demographic responses in prey, the relationship can be stated formally as:

$$T_B = f[M_X, M_Y, M_Z, M_U, \sum\alpha_{i,j}, \sum\beta_{i,j,k}, \chi_{i,j,k,l}, \epsilon_X, \epsilon_Y, \epsilon_Z, \epsilon_U, \sum\epsilon_{i,j}, \sum\epsilon_{i,j,k}, \epsilon_{i,j,k,l}]$$

Where: M_i is the mean contribution of main factors i to T_B;
α, β, and χ are two-, three-, and four-way interaction terms, respectively;
ϵ_i is the error associated with each main factor i;
$\epsilon_{i,j}$, $\epsilon_{i,j,k}$, and $\epsilon_{i,j,k,l}$ are error terms associated with all possible two-, three-, and four-way interaction terms, respectively,for main factors i, j, k, and l;
X, Y, and Z are known, measurable natural main factors, such as recruitment or disturbance interval or effects of alternate predators, that influence T_B; and U is a composite surrogate for unknown factors that influence T_B.

The above applies to sedimentary habitats in PWS under natural conditions without anthropogenic perturbation. In other regions, the same model form should apply, with different terms for main effects. Anthropogenic processes, such as oil spill effects, can also be added as main effects when appropriate.

Based on the above model, we predict that T_B should vary among locations in PWS. For example, recruitment of benthic invertebrates is sensitive to oceanographic factors influencing transport of larvae (e.g., Shepherd and Brown 1993; Hofmann and Powell 1998), and transport processes in semi-enclosed marine systems, such as PWS, are complex and spatially variable. Thus, fluctuation models with T_B as a central element must incorporate spatial effects in parameter estimation. Our present data represent a good example given our arguments above that T_B should be less at Montague Island than at Knight Island.

Available estimates of T_A are based primarily on cases in which sea otters return in large numbers after an absence of decades or more. In such cases, prey populations have developed high densities, with high proportionate representation of large individuals. In these cases, T_A will be determined by the foraging habits of sea otters, and other factors will be unimportant. Given our model for T_A as stated above, our observed result that $T_B > T_A$ for prey densities is to be expected because T_A and T_B are determined by entirely different sets of factors.

We caution that in cases of sea otter populations fluctuating near local carrying capacity, the value T_A may be different than in cases of sea otter recovery after a long absence. In the fluctuation model case, T_A should vary with the period of sea otter population variation and should be influenced by nonlinear characteristics of the trophic linkage of sea otters and prey. For increasingly lengthy periods of significantly reduced sea otter density, T_A should asymptotically approach values observed in recovery model cases as described above. However, if temporary sea otter reductions do not reach thresholds for prey response, prey demography will change little as a result. A subsequent return of sea otter densities to higher levels will, therefore, cause only slight changes in prey over a brief time frame. Thus, T_A will be constrained to small values. Likewise, if a downward turn in sea otter density is sufficiently brief, substantial prey changes are unlikely, particularly if effects M_i in our model for T_B are large and effects ϵ_i are small. Again, the result is a small value for T_A. These arguments suggest an interdependence of T_A and T_B in the fluctuation model case.

We conclude that fluctuation models for trophic interactions of sea otters and primary prey in sedimentary habitats are likely to be quite different from recovery models. There is a clear need for development of fluctuation-phase models in sedimentary habitats. We suggest that

the need for fluctuation models should also be evaluated carefully for other categories of sea otter habitat.

Sea otter populations near local carrying capacity may, nevertheless, be variable emphasizing the need for model development. Perhaps, the most compelling example is the troubling recent widespread decline in sea otter numbers in the Aleutian Archipelago (U.S. Fish and Wildlife Service, unpublished data). This and other cases illustrate that sea otter populations at carrying capacity are not necessarily in stable equilibria with prey populations, nor are they necessarily immune to changes induced by new sources of mortality, or new forms of density-independent population regulation. Effective fluctuation models, therefore, will be needed to accurately quantify trophic linkages of sea otters and coastal benthic communities of the North Pacific Rim and to reliably predict changes in coastal benthic communities induced by changes in sea otter densities.

CONCLUSIONS AND RECOMMENDATIONS

We have presented evidence that existing models of trophic effects of sea otters do not adequately predict responses of prey in soft sedimentary habitats to a precipitous and persistent reduction in sea otter densities. Our data and analyses indicate that a central shortcoming of existing models (recovery models) is the lack of accommodation of ecological uncertainty. Existing models are based primary on contrasts of circumstances where sea otters are absent with circumstances where sea otters have increased in density, rapidly and to a high level. We find that the relatively strong determinism of recovery models is inappropriate for more general models in which sea otter densities may fluctuate downward as well as upward. Thus, new fluctuation models are required, and the new models must effectively accommodate uncertainty in prey recruitment attributes and in stochastic habitat features, such as physical disturbance. Until progress can be made in the lengthy process required to collect data for development of new fluctuation models, it will be necessary to apply existing recovery models with caution. Available information suggests that sea otter numbers may fluctuate both upward and downward in a number of locations. Recovery models will not be useful in predicting prey responses in all such cases. Recovery models must eventually be replaced with more generalized fluctuation models in order to provide predictive power for the general problem of fluctuation in sea otter numbers.

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Table 1. Summary of number of sites sampled for intertidal clams, subtidal clams, and mussels from Montague Island (MI) and Knight Island (KI). ns=not sampled.

Taxa	1996	1997	1998
Intertidal clams			
MI	30	30	15
KI	30	30	15
Subtidal clams			
MI	10	30	5
KI	10	37	5
Mussels			
MI	51	56	ns
KI	57	55	ns

Table 2. Summary of results of analyses for contrasts of prey data between oiled (Montague Island, MI) and unoiled (Knight Island, KI) areas.

Prey category	Variable	Observed mean values	Procedure	Result
Mussels on solid rocky substrata	Density, m^{-2} individuals ≥5 mm	MI: 1,242 (1996), 704 (1997); KI: 1,545 (1996), 2,472 (1997)	ANOVA, area and year effects	No difference in 1996, KI > MI in 1997 ($P < 0.05$)
	Density, m^{-2} individuals ≥20 mm	MI: 188 (1996), 179 (1997); KI: 93 (1996), 101 (1997)	ANOVA, area and year effects	No difference in 1996, MI > KI in 1997 ($P < 0.05$)
	Density, m^{-2} individuals ≥40 mm	MI: 0.4 (1996), 0.4 (1997); KI: 1.8 (1996), 0.6 (1997)	Mann-Whitney U-test, years combined	No difference in either year
	Mean size, mm	MI: 13.1 (1996), 14.0 (1997); KI: 11.8 (1996), 10.0 (1997)	ANOVA, area and year effects	MI > KI ($P < 0.001$), year effects not significant
	Size distribution	See Figure 4	Kolmogorov-Smirnov (K-S) test	Areas different ($P < 0.001$)
Mussels on mixed substrata	Density, m^{-2} individuals ≥5 mm	MI: 636 (1996), 974 (1997); KI: 1,098 (1996), 692 (1997)	ANOVA, area and year effects	No difference
	Density, m^{-2} individuals ≥20 mm	MI: 231 (1996), 340 (1997); KI: 249 (1996), 148 (1997)	ANOVA, area and year effects	No difference in 1996, MI > KI in 1997 ($P < 0.05$)
	Density, m^{-2} individuals ≥40 mm	MI: 3.1 (1996), 11.3 (1997); KI: 5.8 (1996), 2.3 (1997)	Mann-Whitney U-test, years combined	No difference
	Mean size, mm	MI: 17.4 (1996), 18.4 (1997); KI: 15.2 (1996), 15.3 (1997)	ANOVA, area and year effects	MI > KI ($P = 0.002$), year effects not significant
	Size distribution	See Figure 4	K-S test	Areas different ($P < 0.001$)
Littleneck clams	Density, 0.25 m^{-2}, individuals ≥20 mm, intertidal	MI: 0.79 KI: 0.48	Student's *t* test	No difference
	Density, 0.25 m^{-2}, individuals ≥20 mm, subtidal	MI: 0.06 KI: 0.12	Student's *t* test	No difference
	Mean size, mm, individuals ≥20 mm	MI: 26.15 KI: 27.36	Student's *t* test	KI > MI ($P < 0.001$)

Table 2. Continued.

Prey category	Variable	Observed mean values	Procedure	Result
Butter clams	Size distribution, individuals over 20 mm	See Figure 5	K-S test	No difference
	Density, 0.25 m^{-2}, individuals ≥20 mm, intertidal	MI: 0.03 KI: 0.04	Student's *t* test	No difference
	Density, 0.25 m^{-2}, individuals ≥20 mm, subtidal	MI: 0.10 KI: 0.16	Student's *t* test	No difference
	Mean size, mm, individuals ≥20 mm	MI: 34.65 KI: 44.83	Student's *t* test	KI > MI ($P = 0.004$)
	Size distribution, individuals ≥20 mm	See Figure 5	K-S test	Areas different ($P < 0.05$)
Bentnose clams	Density, 0.25 m^{-2}, individuals ≥20 mm, intertidal	MI: 2.45 KI: 0.26	Student's *t* test	MI > KI ($P< 0.001$)
	Density, 0.25 m^{-2}, individuals ≥20 mm, subtidal	MI: 1.30 KI: 0.08	Student's *t* test	MI > KI ($P = 0.006$)
	Mean size, mm individuals ≥20 mm	MI: 30.26 KI: 25.83	Student's *t* test	MI > KI ($P< 0.001$)
	Size distribution, individuals ≥20 mm	See Figure 5	K-S test	Areas different ($P < 0.01$)
Intertidal clams (all species combined)	Density, 0.25 m^{-2}	MI: 3.26 KI: 0.78	Student's *t* test	MI > KI ($P< 0.001$)
	Mean size, mm	MI: 26.04 KI: 26.90	Student's *t* test	KI > MI ($P = 0.003$)
	Size distribution	See Figure 6	K-S test	No difference
Subtidal clams (all species combined)	Density, 0.25 m^{-2}, individuals ≥20 mm	MI: 2.25 KI: 0.69	Student's *t* test	MI > KI ($P = 0.002$)
	Mean size, mm, individuals ≥20 mm	MI: 35.42 KI: 42.87	Student's *t* test	KI > MI ($P< 0.001$)
	Size distribution, individuals ≥20 mm	See Figure 7	K-S test	Areas different ($P < 0.001$)

[a]ANOVA = analysis of variance

Table 3. Summary of results of analyses for contrasts of live butter clam and dead intact butter clam assemblage (DICA; see text) data at Knight Island.

Variable	Observed mean values	Procedure	Result
Density, 0.25 m^{-2}, individuals ≥20 mm	Live: 0.69 DICA: 3.32	Student's *t* test	DICA > Live ($P < 0.001$)
Mean size, mm, individuals ≥20 mm	Live: 51.3 DICA: 73.5	Student's *t* test	DICA > Live ($P < 0.001$)
Size distribution, individuals ≥20 mm	See Figures 7 and 8	K-S test	Distributions different ($P < 0.001$)

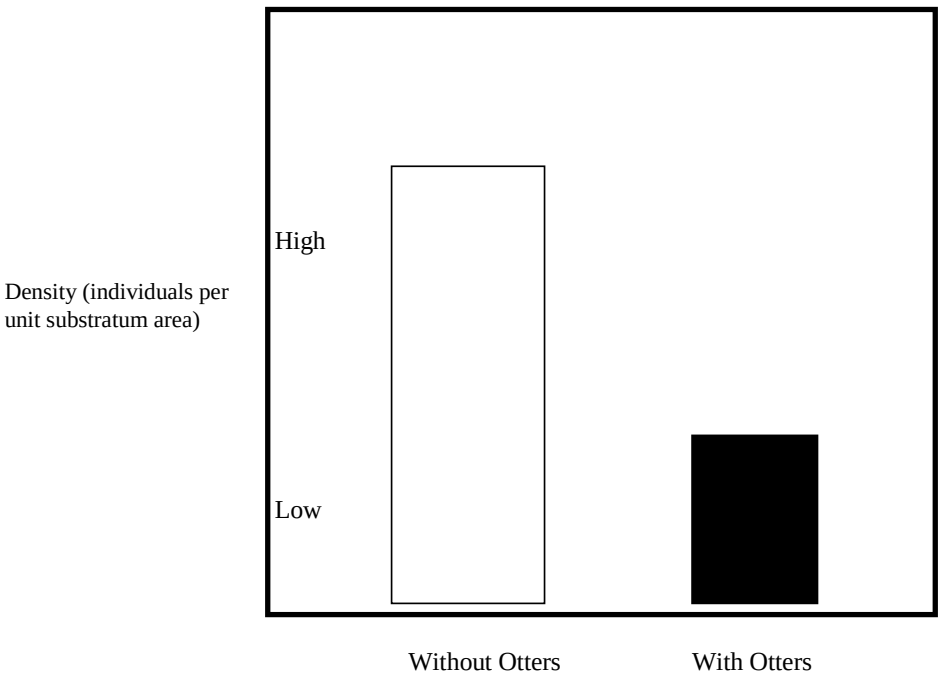

Figure 1. Hypothetical model of densities of prey found in areas with and without sea otters based on recovery models as defined in text.

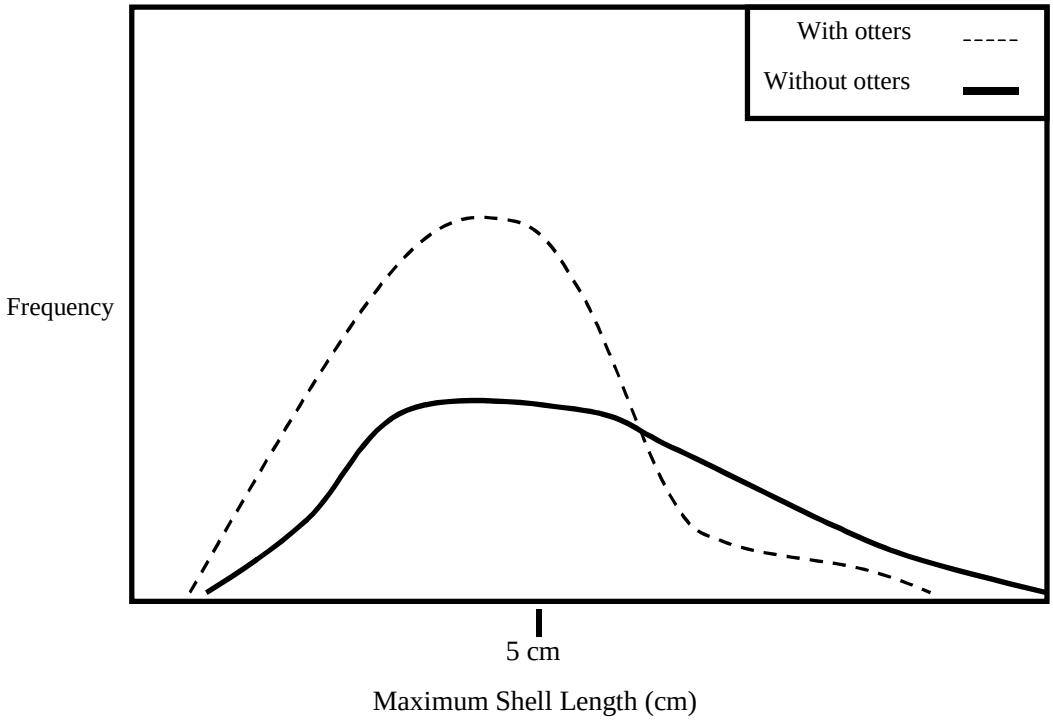

Figure 2. Hypothetical model of sizes of prey found in areas with and without sea otters based on recovery models as defined in text.

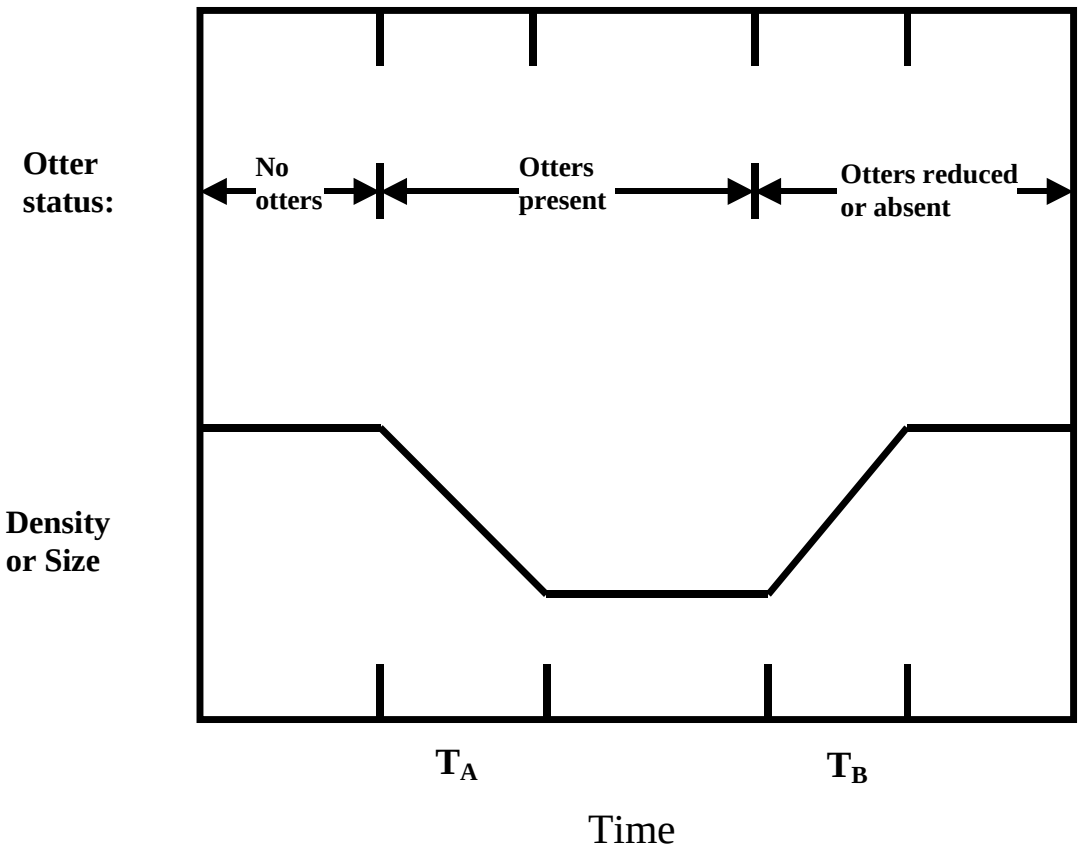

Figure 3. Hypothetical model of the effects of sea otter predation on densities and sizes of prey. T_A = time frame in areas with sea otters immigration; T_B = time frame in areas with loss or reduction of sea otters. The illustrated case shows $T_A = T_B$, the simplest plausible case consistent with recovery models as defined in text.

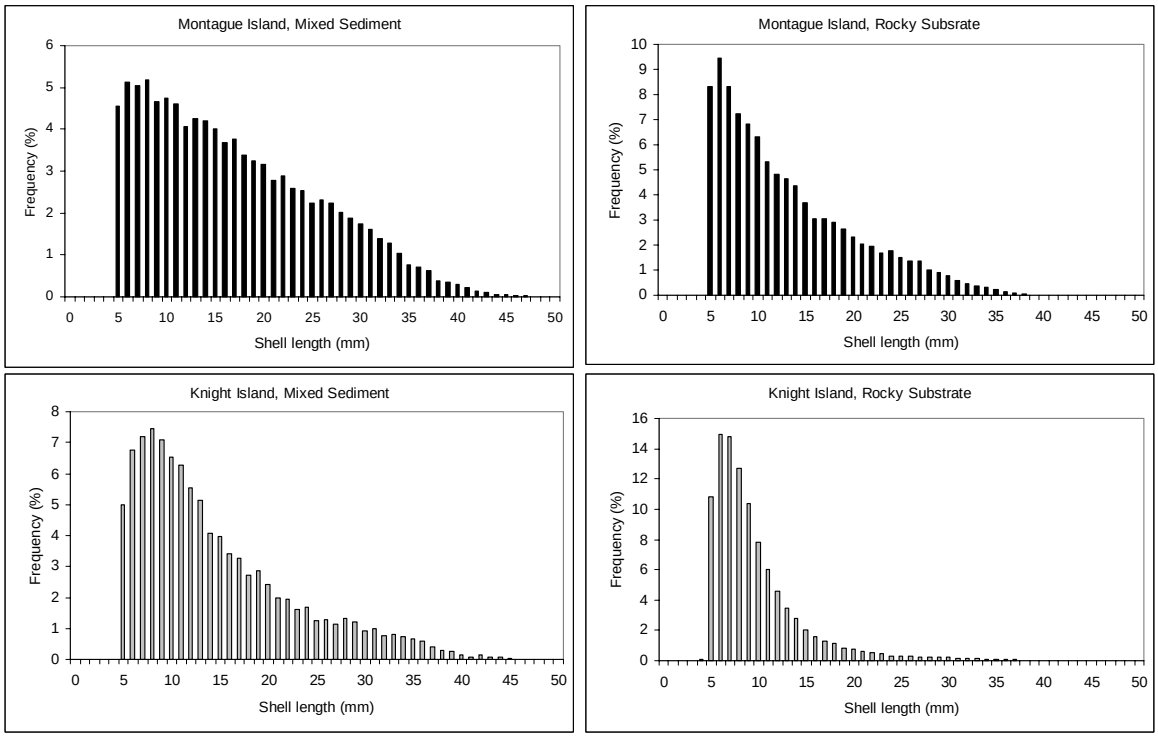

Figure 4. Length frequencies of *Mytilus trossulus* from intertidal sampling, 1996 and 1997 data combined. Data are separated by area and by substratum category.

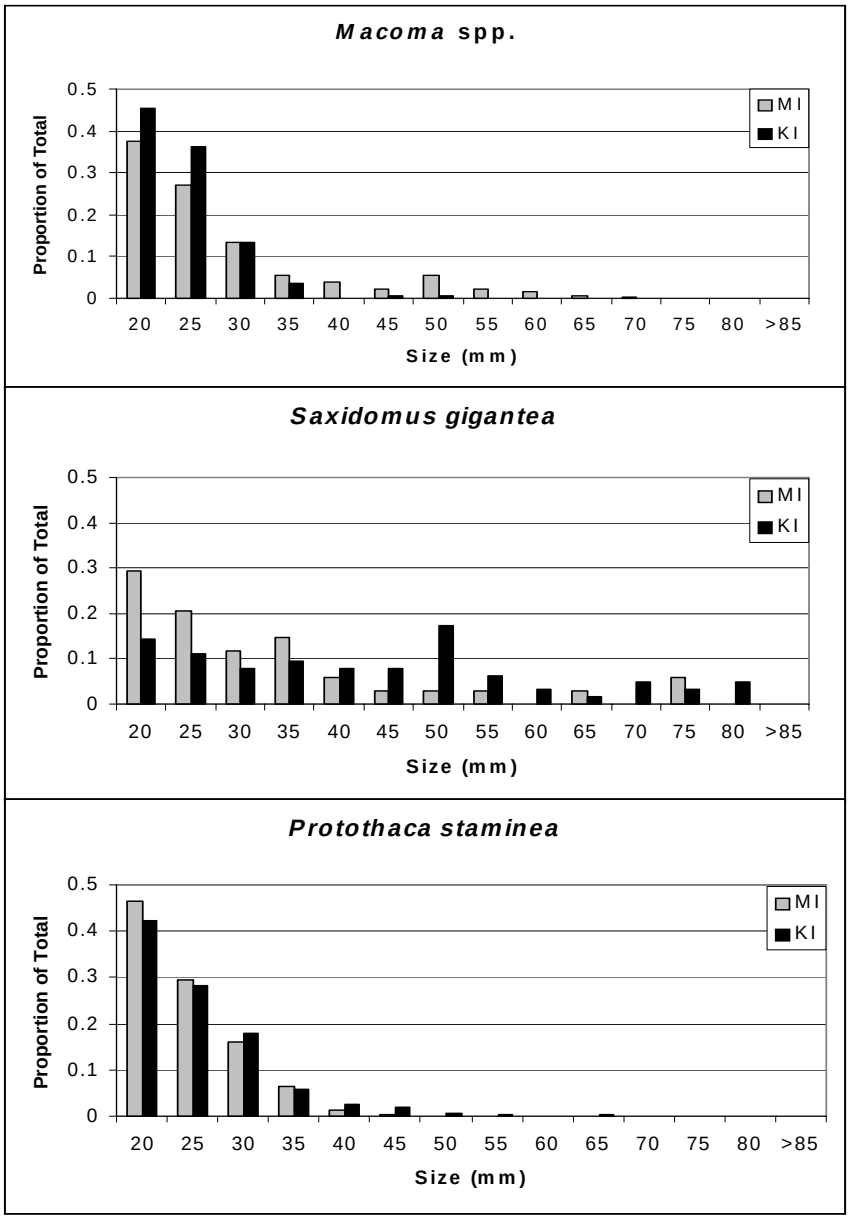

Figure 5. Length frequencies of *Macoma* spp., *Saxidomus gigantea*, and *Protothaca staminea* >20 mm in size from intertidal and subtidal sampling, 1996–1998 combined. MI = Montague Island, KI = Knight Island.

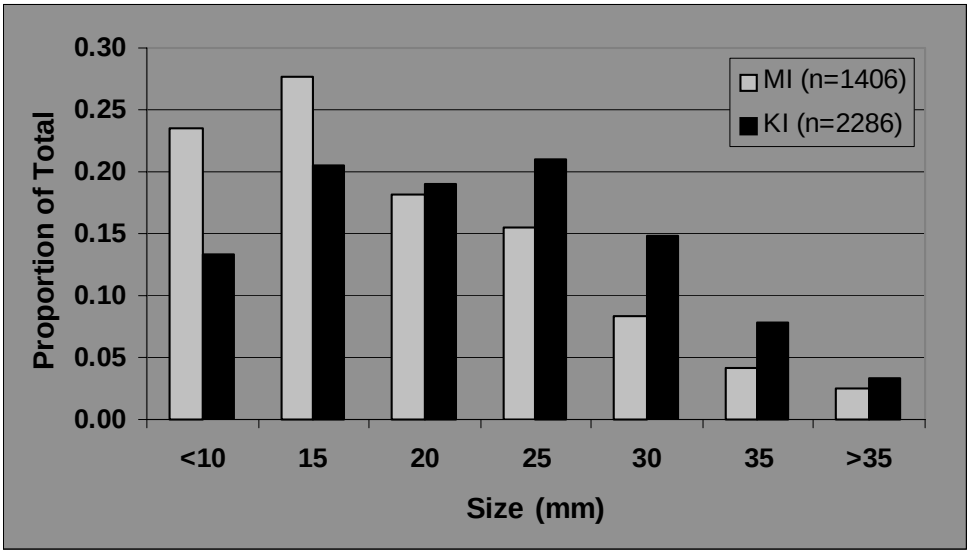

Figure 6. Length frequencies of intertidal clams from 1996 to 1998 at Montague Island (MI) and Knight Island (KI). Data shown include all species and sizes sampled.

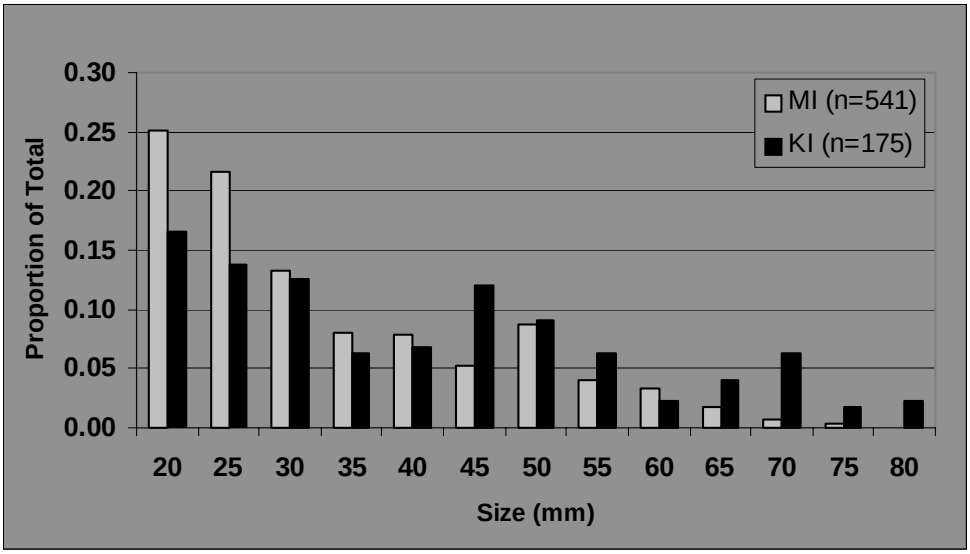

Figure 7. Length frequencies of all sampled species of clams ≥20 mm from subtidal habitats at Montague Island (MI) and Knight Island (KI), 1996–1998 combined.

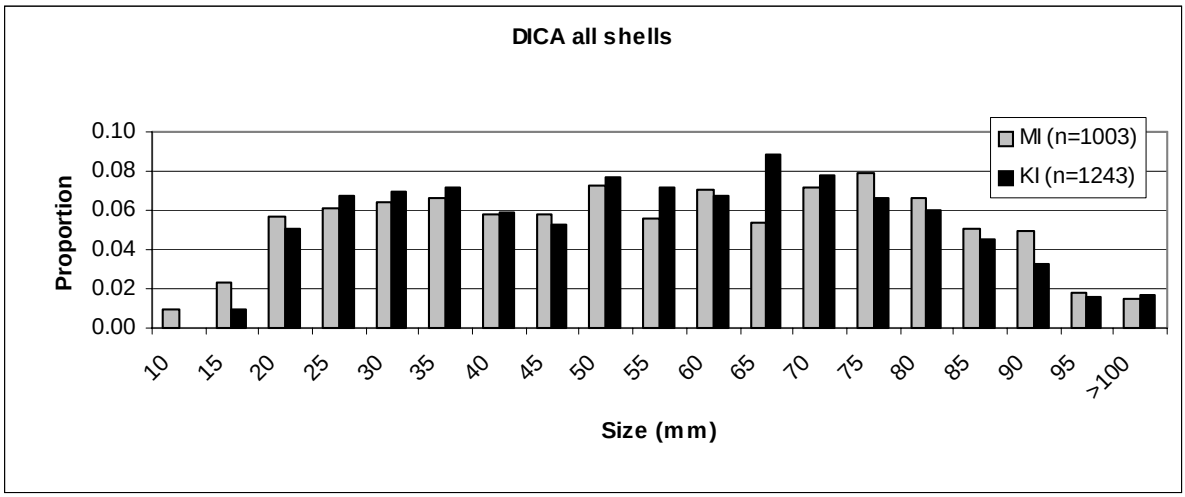

Figure 8. Length frequencies of the dead intact clam assemblage (DICA), as defined in text, at Montague Island (MI) and Knight Island (KI), 1996–1997 combined.

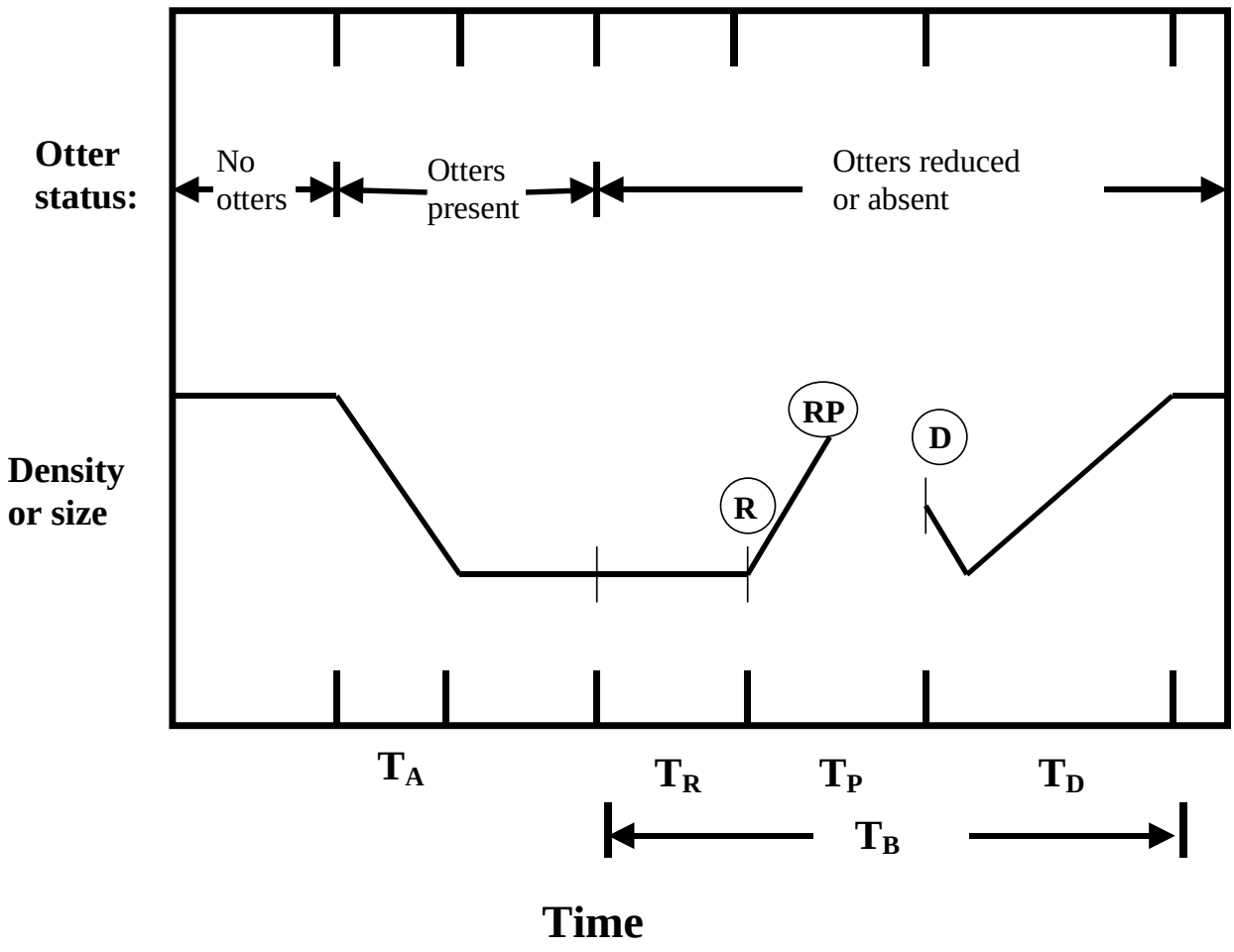

Figure 9. One of many possible scenarios for an asymmetrical interaction, as defined in text, of sea otters *Enhydra lutris* and prey populations. In this case, $T_B > T_A$ and $T_B = T_R + T_P + T_D$ where T_R is the delay in prey response due to infrequent recruitment, T_P is the delay due to effects of a competing predator, and T_D is the delay due to effects of natural disturbance. In the timberline, R represents a prey recruitment event, RP represents a recruitment event for a competing predator, and D a natural disturbance event. There are an infinite number of other possible asymmetrical scenarios. The symmetrical case is illustrated in Figure 3.

Chapter 4. Harlequin Duck (*Histrionicus histrionicus*) Perspective

Harlequin Duck *(Histrionicus histrionicus*) Perspective: Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

Harlequin Duck Population Recovery Following the *Exxon Valdez* Oil Spill: Progress, Process, and Constraints[1]

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ABSTRACT

Following the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, we studied the status of recovery of harlequin duck (*Histrionicus histrionicus*) populations during 1995-1998. We evaluated potential constraints to full recovery, including (1) exposure to residual oil, (2) food limitation, and (3) intrinsic demographic limitations on population growth rates. In this paper, we synthesize the findings from our work and incorporate information from other harlequin duck research and monitoring programs to provide a comprehensive evaluation of the response of this species to the *Exxon Valdez* oil spill. We conclude that harlequin duck populations had not fully recovered by 1998. Furthermore, adverse effects continued as many as 9 years after the oil spill, in contrast to the conventional paradigm that oil spill effects on bird populations are short-lived. These conclusions are based on the findings that (1) elevated cytochrome P450 induction on oiled areas indicated continued exposure to oil in 1998, (2) adult female winter survival was lower on oiled than unoiled areas during 1995-1998, (3) fall population surveys by the Alaska Department of Fish and Game indicated numerical declines in oiled areas during 1995-1997, and (4) densities on oiled areas in 1996 and 1997 were lower than expected using models that accounted for effects of habitat attributes. Based on hypothesized links between oil contamination and demography, we suggest that harlequin duck population recovery was constrained primarily by continued oil exposure. Full population recovery also will

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be delayed by the time necessary for intrinsic population growth to allow return to pre-spill numbers following cessation of residual oil spill effects. Although not all wildlife species were affected by the *Exxon Valdez* oil spill, and some others may have recovered quickly from any effects, harlequin duck life history characteristics and benthic, nearshore feeding habits make them susceptible to both initial and long-term oil spill effects.

Key words: Demography, *Exxon Valdez*, Harlequin duck, *Histrionicus histrionicus*, Marine birds, Oil contamination, Population recovery.

INTRODUCTION

Harlequin ducks (*Histrionicus histrionicus*) spend most of the year in nearshore marine habitats (Robertson & Goudie 1999), where they feed on benthic invertebrates (Goudie & Ankney 1986) in intertidal and shallow subtidal zones. Aspects of harlequin duck ecology make their populations particularly susceptible to perturbations of their wintering environment. Harlequin ducks, like many sea ducks, have a life history in which variable and generally low annual productivity is compensated by relatively high adult survival and long reproductive life spans (Goudie et al. 1994). This type of strategy evolves under conditions of predictable and stable nonbreeding environments, which are required to ensure adult survival (Stearns 1992). Also, harlequin ducks, because of their small body size, are thought to exist near an energetic threshold during winter, with little flexibility for increasing caloric intake or relying on stored reserves (Goudie & Ankney 1986). While this strategy may be tenable under predictable and stable conditions, it does not readily accommodate perturbations that result in either decreases in energy acquisition or increases in metabolic costs. Finally, strong site fidelity, such as that exhibited by wintering harlequin ducks, evolves in predictable and stable habitats (Johnson & Gaines 1990, Robertson & Cooke 1999, Cooke et al. 2000) and does not facilitate movement to undisturbed areas if local habitat quality becomes degraded (Hilden 1965, Cooch et al. 1993).

The release of approximately 42 million liters of crude oil into Prince William Sound (PWS) by the 1989 *Exxon Valdez* oil spill (EVOS) was a significant perturbation to harlequin duck nonbreeding habitat. As much as 40% of the spilled oil was deposited in intertidal and subtidal zones of PWS (Galt et al. 1991, Wolfe et al. 1994) and oil persisted in these areas more than 8 years after the oil was spilled (Hayes & Michel 1999). Vulnerability of harlequin ducks to oil spill effects is exacerbated by their diet of intertidal and shallow subtidal benthic invertebrates (Vermeer 1983, Goudie & Ankney 1986, Gaines & Fitzner 1987, Goudie & Ryan 1991, Patten et al. 2000). Oil constituents can accumulate in bottom sediments and subsequently, in benthic invertebrates (Fukuyama 2000, Peterson 2001). Petroleum hydrocarbons occurred in harlequin duck prey from immediately after the spill through at least 1995 (Babcock et al. 1996, Boehm et al. 1995, Patten et al. 2000, Short & Babcock 1996, Wolfe et al. 1996).

In this paper, we examine effects of the EVOS on harlequin duck populations in PWS and consider potential constraints to full population recovery. The first objective is to review data that provide insight into population injury and recovery status. The second objective is to evaluate mechanisms potentially constraining full recovery, including (1) intrinsic demographic limitations on population growth rates that delay return to prespill numbers despite lack of continuing oil spill effects, (2) health effects of continued oil exposure at levels that have

population consequences, and (3) food limitation due to oil spill-related reductions in prey that either lowers carrying capacity or reduces health and survival of individual ducks.

Data used in this synthesis were gathered from journal publications and *Exxon Valdez* Oil Spill Trustee Council reports, both from our own studies conducted during 1995-1998 and from other research and monitoring programs in PWS after the spill. We also reviewed published studies of harlequin duck ecology conducted throughout their range and their implications for understanding constraints to full recovery from the EVOS.

Prince William Sound is prime nonbreeding habitat for harlequin ducks. It supports about 14,000 birds during winter (Lance et al. 1999), although it is one of the northernmost wintering areas within the species' range (Robertson & Goudie 1999). Some reproduction occurs in streams feeding into PWS (Crowley 1999), but most harlequin ducks that winter in PWS nest outside of the area. Breeding locations have not been determined and could extend throughout the vast breeding range in Alaska and the Yukon Territories (Robertson & Goudie 1999).

INJURY AND RECOVERY STATUS

We reviewed studies conducted after the EVOS that lend insight into harlequin duck population injury and recovery. We categorized these as studies of population status, adult female survival, and body mass variation.

Population Status

Several studies are relevant for evaluating harlequin duck population status, most of which were conducted outside of our own research program. These measured a range of population parameters, including direct mortalities, abundance, numerical trends, densities, age and sex ratios, and habitat use. Generally, we would predict that these population parameters (excluding initial mortalities) would be similar in oiled and unoiled areas once full population recovery had occurred. However, this prediction rests on a suite of assumptions and is subject to a number of problems (Wiens & Parker 1995). For example, lack of comprehensive prespill data for many parameters (e.g., winter densities, habitat use, age and sex ratios) precluded use of a before-after-control-impact (BACI) design, which can be used to distinguish effects of an environmental perturbation from natural spatial and temporal variation. Also, comparisons between affected and control areas, without pre-perturbation data, often assume that natural attributes of the areas are similar (Wiens & Parker 1995); for harlequin duck population parameters sometimes that assumption could be addressed, but not always. Finally, changes in demographic endpoints such as numbers, trends, or densities do not address underlying demographic processes and thus can not indicate mechanisms leading to population change. However, despite these limitations and assumptions, convergence of population densities, trends, and age and sex distributions in oiled and unoiled areas would be consistent with population recovery.

Estimates of direct mortality of birds due to the EVOS were based on recovery of carcasses (Piatt et al. 1990), expanded to account for the proportions of dead birds that were not recovered (Piatt & Ford 1996). The true fraction of all dead birds retrieved after the EVOS is difficult to determine, even with data from experimental carcass drift and recovery studies (Piatt

& Ford 1996). The associated uncertainty has led to controversy about the numbers of birds killed by acute effects of the EVOS (Parrish & Boersma 1995, Piatt & Ford 1996), although Piatt & Ford (1996) convincingly argue that despite uncertainty, incorporation of estimated recovery rates is appropriate and realistic. In the case of the EVOS, even when using a variety of recovery rates, estimates of the magnitude of total bird mortality were similar (Piatt & Ford 1996). Immediately following the EVOS, 212 harlequin duck carcasses were recovered, including 147 in PWS; using a recovery rate of 15% (Piatt & Ford 1996) the estimate of total harlequin mortality due to immediate effects of the EVOS was 1413, with 980 of those in PWS (J. Piatt, pers. comm.). This mortality estimate represents roughly 7% of the harlequin ducks wintering throughout PWS, and a much higher proportion of those wintering in oiled areas of PWS. Sea ducks were quite vulnerable to immediate effects of the oil spill; numbers of oiled sea duck carcasses recovered in PWS exceeded those of any other taxa (Piatt et al. 1990). Mortality estimates from carcasses retrieved just after the spill indicate immediate population injury, but do not address any subsequent, longer-term effects of the EVOS.

Patten et al. (2000) conducted damage assessment studies immediately following the EVOS, focusing on contaminant exposure and abundance. They found hydrocarbon metabolites in 74% of live harlequin ducks collected from oiled areas in 1989 and 1990, consistent with exposure to oil and implying potential for injurious effects. Also, numbers of adults and broods were lower in oiled areas of PWS than in unoiled areas (Patten et al. 2000); however, this study did not account for potential geographic variation from natural causes, which may contribute to or explain observed differences. For example, lower numbers of broods in oiled areas do not necessarily indicate that harlequin productivity was affected by the EVOS because (1) most of the wintering population migrates outside of PWS to breed, (2) within PWS, breeding habitats used by harlequin ducks (Crowley 1994) are found primarily in eastern, unoiled areas (Rosenberg & Petrula 1998), and (3) prespill records of broods in oiled areas could have been flightless birds during wing molt that were misclassified (Rosenberg & Petrula 1998). However, no data have been collected to explicitly examine reproductive effort of harlequin duck subpopulations from oiled areas, so we can not eliminate the possibility that the EVOS had deleterious effects on harlequin duck reproduction.

The U. S. Fish and Wildlife Service has conducted marine bird surveys during summer (July) and winter (March) in PWS since 1989 (Lance et al. 1999). While these were not explicitly designed to estimate harlequin duck numbers or population trends, they do provide a long-term assessment of population status. Pre-spill survey data exist for PWS from summers 1984 and 1985, which have been used for comparison to post-spill data (Irons et al. 2000). Unfortunately, pre-spill survey data for PWS in winter, the period of high and stable harlequin duck numbers, are not adequate for before-after comparisons, although post-spill data can be used to compare winter trends between oiled and unoiled areas (Lance et al. 2001). Also, from 1995 to 1997, the Alaska Department of Fish and Game conducted surveys designed specifically to assess harlequin duck population status (Rosenberg & Petrula 1998). They surveyed during spring (May and June) and fall (late July to September) and measured numbers, pair status (paired versus unpaired), sex ratios, age composition, and molt chronology in oiled and unoiled areas (over more than 250 km shoreline in each area). These surveys have more power for estimating abundance and trends than U. S. Fish and Wildlife Service surveys (Rosenberg & Petrula 1998),

and their fall data provide the best estimates of population trends for nonbreeding populations during the course of our research (1995 - 1998).

Using a BACI design, Irons et al. (2000) found that harlequin duck densities were lower than expected in oiled areas of PWS during summers of 1990 and 1991, based on comparison to observed changes in reference areas. This effect was not evident in subsequent years. Lance et al. (2001) reported stable densities of harlequin ducks in oiled areas of PWS during summer, and increasing densities during winter from 1989 through 1998; the increasing trend during winter could be interpreted as evidence of recovery of winter numbers. However, trends in oiled areas did not differ from those in unoiled (during summer or winter). Lance et al. (1999) interpreted this result as evidence of lack of recovery, under the premise that an EVOS-injured population should have a higher growth rate than reference populations for convergence and thus recovery to be occurring. Alaska Department of Fish and Game surveys (Rosenberg & Petrula 1998) indicated that fall numbers significantly declined on oiled areas from 1995 through 1997, whereas numbers were stable on unoiled areas, consistent with a hypothesis that continued, negative effects of the EVOS were occurring during the time of their survey. Measures of other population attributes (age ratios, sex ratios, and phenology) did not differ between oiled and unoiled areas (Rosenberg & Petrula 1998). Results of U. S. Fish and Wildlife Service and Alaska Department of Fish and Game support the general conclusions that harlequin duck populations were reduced in the years immediately after the spill, that populations were not increasing more quickly on oiled areas through at least 1998, and that the most powerful monitoring study indicated declines in wintering numbers on oiled areas through 1997, consistent with continuing negative effects of the EVOS.

Exxon Corporation sponsored studies to assess effects of the EVOS on marine birds (Wiens et al. 1996, Day et al. 1997, Murphy et al. 1997). These studies relied on data collected following the EVOS (1989 through 1991) in 10 bays in western PWS across a range of oil contamination levels. While designed to examine all marine birds, these studies also drew conclusions relevant to assessment of harlequin duck population status. Authors of these studies concluded that oil spill effects were short-lived for most bird species based on their response parameters of species richness (Wiens et al. 1996), habitat use (Day et al. 1997), and summer abundance relative to prespill data (Murphy et al. 1997). In the studies that present results for harlequin ducks explicitly, Day et al. (1997) concluded that harlequin duck densities showed negative relationships with oiling intensity during 1989 and 1990, but not in 1991, and Murphy et al. (1997) concluded that summer abundance in western PWS did not differ from prespill numbers. Generally, these studies imply initial population injury and recovery within 2 years; these results are contrasted with U. S. Fish and Wildlife and Alaska Department of Fish and Game studies in our Discussion.

As part of our research, we examined correlates of harlequin duck densities within oiled (Bay of Isles and Herring Bay on Knight Island) and unoiled (Montague Island) study areas (Fig. 1). We formally evaluated variation in duck densities in relation to habitat characteristics including substrate, exposure to wind and waves, distance to stream mouths and offshore reefs, intertidal slope, prey biomass, and history of contamination by the EVOS (Esler et al. 2000a). Habitats within PWS are diverse, making it necessary to segregate effects of oil contamination from other naturally varying environmental factors (Wiens & Parker 1995). Persisting lower densities than expected on oiled areas (after accounting for other factors) could result from either

failure of the population to recover from the immediate impact or from ongoing, longer-term negative effects of the EVOS; in either case, this result would be consistent with a lack of full population recovery. During 1995 to 1997, we surveyed densities of wintering harlequin ducks and measured habitat attributes at 216 shoreline segments (113 on oiled Knight Island and 103 on unoiled Montague Island; Fig. 1). We used general linear models to evaluate variation in harlequin duck densities in relation to habitat attributes and history of oiling, using information-theoretic methods for model selection (Burnham & Anderson 1998). We found (Esler et al. 2000a) that harlequin duck densities during winter were related to several habitat attributes, including substrate type, distance to offshore reefs, distance to stream mouths, and exposure to wind and wave action (Table 1). After accounting for these habitat relationships and their interactions with area, oiling history was negatively related to harlequin duck densities (Table 1). These data are consistent with a hypothesis of lack of complete population recovery from the EVOS.

Adult Female Survival during Winter

Within our research program, we used radio telemetry to measure adult female survival during winter (Esler et al. 2000b), because (1) population dynamics of species with a life history like harlequin ducks are particularly sensitive to adult female survival (Goudie et al. 1994, Schmutz et al. 1997); and (2), as described above, harlequin duck populations are likely sensitive to perturbations on wintering areas, which could result in reductions in survival. As an assessment of recovery status, we would predict similar harlequin duck winter survival between oiled and unoiled areas in the absence of continuing EVOS effects. We also would predict survival rates that result in stable or increasing numbers on oiled areas if there were no lingering effects of the EVOS.

During autumns of 1995 through 1997 we captured adult females during wing molt throughout the oil spill zone and on nearby Montague Island (Fig. 1) and surgically implanted conventional radio transmitters. Radio signals (n = 294 over the 3 winters) were monitored by air approximately weekly from October through March. We used an information-theoretic approach to data analysis (Burnham & Anderson 1998, White & Burnham 1999), in which we contrasted the fit of our data to 11 models with various combinations of season and area (history of oil contamination) parameters.

The data strongly supported the inference that survival was lower in oiled areas than unoiled areas (Esler et al. 2000b). The top 3 models, i.e., those with the best fit to the data, all included a difference in survival between areas. Further, models without an area term had very little support, emphasizing the importance of including a term for an area effect. Winter survival rates from the best-fitting model were 78.0% (SE = 3.3%) on oiled areas and 83.7% (SE = 2.9%) on unoiled areas, due primarily to a divergence between areas during mid-winter (Fig. 2). We also determined that survival differences between oiled and unoiled areas were more likely related to history of oil contamination than intrinsic differences (such as habitat, disease, climate, social influences, or predator densities), based on a closer evaluation of survival rates of birds from oiled Green Island, which is very close to Montague Island (Fig. 1) and is similar in most attributes. Survival of Green Island birds was much more similar to that of all oiled area birds combined than to Montague Island birds. Finally, we incorporated survival estimates into a pre-

existing harlequin duck population model (Robertson 1997), holding all other parameters constant, to evaluate the effect of differences in survival on population dynamics. The estimate of annual population change (λ) was 0.9464 for oiled areas (i.e., annual population declines of about 5.4%). For unoiled areas, $\lambda = 1.0054$, suggesting an approximately stable population. These estimates were consistent with the Alaska Department of Fish and Game (Rosenberg & Petrula 1998) fall survey results, showing declining numbers from 1995-1997 on oiled areas and stable numbers on unoiled areas.

Variation in Body Mass and Composition

Body mass and body composition (relative amounts of lipid and lean mass) often are used as indicators of individual and population health under the assumption that fitness increases with increases in mass and energy reserves. This assumption is likely untrue in a number of situations (King & Murphy 1985), i.e., optimal body mass may not be the maximum, particularly for birds. However, in our situation, in which we were comparing populations of harlequin ducks experiencing similar extrinsic environmental conditions with the exception of oiling history (and thus presumably similar body mass optima), differences in body mass between areas could reflect continuing effects of the EVOS. Thus, we would predict that EVOS effects related to changes in prey abundance or sublethal effects of oil exposure could result in lower body mass and smaller lipid reserves on oiled areas than unoiled.

We compared body mass between oiled and unoiled areas during wing molt (late summer and early fall) 1995-97 and winter 1997-98 as part of our research program, using general linear models to determine factors explaining variation in harlequin duck body mass and to evaluate any area differences after accounting for other explanatory variables. We used separate models for wing molt and winter and, within each season, separate models for each sex. To select the model from which we drew inference, we used Mallow's C_p values to contrast all possible combinations of explanatory variables (Burnham & Anderson 1998). Explanatory variables in wing molt models included year, age, ninth primary length (as a measure of stage of molt), and area. For winter, explanatory variables included age, season (December versus March and April), and area. We also compared estimated lipid and lean (lipid-free) masses of female harlequin ducks captured during wing molt based on condition indices created from a sample of collected harlequin duck females collected during wing molt for which composition was measured using laboratory analysis.

During wing molt, variation in female harlequin duck body mass was related to stage of wing molt, age, and year. After accounting for effects of these variables, females averaged 9.6 g lighter on oiled areas than unoiled (Table 2). This represents 1.6% of average body mass of molting females on unoiled Montague Island. Similarly, estimated body lipid and lean averaged 2.5 g and 7.0 g lower, respectively, in oiled areas than unoiled areas. Like females, male body mass was related to stage of wing molt. After accounting for molt stage, average body mass differed by area, although unlike females, male body mass averaged 13.4 g higher in oiled areas than unoiled, a 2.0% increase over average body mass on unoiled areas.

During winter, female body mass varied with season (mid versus late winter) and age; however, no area effect was detected (Table 2). Body mass of males also varied seasonally

during winter and averaged 21.6 g lower in oiled areas than unoiled areas, which corresponds to 3.3% of average body mass on unoiled areas.

Most of the body mass and composition data were consistent with a hypothesis of no continuing effects of the EVOS. Area differences during wing molt were small and were in different directions for males and females; the high statistical power due to the large sample size of captured birds allowed statistically significant detection of small differences of little biological meaning. The 21.6 g body mass difference between areas for male harlequin ducks during winter suggests potential residual EVOS effects; however, because the effect is relatively small and because females captured during the same time on the same areas did not show a similar effect, this does not constitute strong evidence of an EVOS effect.

We also tested whether body mass of wintering harlequin ducks was related to induction of cytochrome P4501A (Trust et al. 1998), an indicator of exposure to oil (see below). For birds captured during March and April 1998 on both oiled and unoiled areas, we used a regression approach to measure the effect of cytochrome P4501A on body mass after accounting for body mass variation due to sex. We found that sex-corrected body mass was negatively related (-0.11 ± 0.05; g/pmol/min/mg ± SE) to EROD activity (Fig. 3). These data suggest physiological consequences of oil exposure, with potential demographic consequences. Survival of some wintering ducks has been demonstrated to vary with body mass (Conroy et al. 1989, Longcore et al. 1991, Bergan & Smith 1993), indicating a mechanism linking contaminant exposure and reductions in survival.

INTRINSIC LIMITATIONS ON POPULATION GROWTH RATES

Full recovery of Harlequin Duck populations could be delayed by the time needed for intrinsic population growth to replace birds removed by initial or early oil spill effects. In other words, even if negative effects related to the EVOS (mortality and emigration) had ended, the time required for demographic effects (recruitment and immigration) to rebuild populations to prespill conditions could be considered a constraint to full recovery. In this section, we review data on harlequin duck demography and population structure that lend insight into this possible mechanism constraining recovery.

Population models, based on demographic data collected from throughout the range of the harlequin duck (Goudie et al. 1994, Robertson 1997), provide an indication of population growth potential. Goudie et al. (1994) concluded that the potential growth rate of harlequin duck populations is low relative to most other ducks because of their life history strategy favoring high survival and long lifespans over high annual productivity. Other waterfowl with similar life histories also have low population growth rates (Schmutz et al. 1997). These data suggest that full recovery of harlequin duck populations could be delayed by the relatively long time frames needed for recruitment to replace birds removed as a result of EVOS effects, even if those effects were no longer operating.

Local wintering aggregations could constitute demographically independent subpopulations if site fidelity is high and dispersal among areas low (Cooke et al. 2000). We reviewed published studies addressing harlequin duck site fidelity and movements in coastal British Columbia; these studies consistently indicated high molt and winter site fidelity and low dispersal (Breault & Savard 1999, Cooke et al. 2000, Robertson et al. 1999, Robertson et al.

2000). Also, Regehr et al. (2001) reported evidence that juvenile harlequin ducks accompany their mothers to wintering areas, which further indicates that local wintering groups represent aggregations that are largely independent.

We also examined data collected during our own studies to assess molt site fidelity based on recapture locations. We conducted captures of flightless (due to wing molt) birds along discrete, non-overlapping stretches of shoreline that were 1 to 3 km in length during autumns of 1995 through 1997. These captures occurred throughout the oil spill zone and along Montague Island. For individuals that we captured in more than 1 year, we summarized recaptures in relation to the distance from the original capture. Of 151 harlequin ducks recaptured during wing molt, 135 (89.5%) were in the same shoreline segment as their original capture, 10 (6.6%) were in an immediately adjacent shoreline segment (i.e., a segment within 1 km of the original capture segment), and 6 (4.0%) had moved to a molting area > 1 km from their original capture location. Also, of the birds recaptured at a different shoreline segment, none was > 20 km from its original capture location. Larger scale movements may have occurred, but we would have detected them if they were common, given that we sampled broadly and intensively throughout western PWS. These data, and the results from other studies, indicate that groups of wintering harlequin ducks are largely demographically independent and that local subpopulation recovery would have to occur largely by recruitment rather than by immigration. Without positive inputs by immigration, local population recovery from the EVOS is more likely to be constrained.

Lanctot et al. (1999) used genetic data to evaluate whether harlequin duck aggregations within the EVOS zone were demographically isolated. DNA was obtained from blood samples of molting harlequin ducks from oiled and unoiled areas of PWS, the Kodiak Archipelago, and the Alaska Peninsula; PWS, Kodiak, and Alaska Peninsula are separated at the scale of hundreds of kilometers. Under this approach, significant differences in nuclear DNA allele frequencies or mtDNA haplotype frequencies among areas would be strong evidence that aggregations are demographically independent (e.g., Slatkin 1995) and, thus, that intrinsic limitations on population growth rates could constrain population recovery. However, Lanctot et al. (1999) found that molting aggregations in PWS, Kodiak Archipelago and the Alaska Peninsula did not have different allele or haplotype frequencies. Lack of genetic differentiation does not necessarily imply demographic panmixia; genetic panmixia also could occur from historical gene flow or from low levels of immigration (Wright 1931) that have little effect on local demography.

CONTINUED EXPOSURE TO OIL

Exposure to oil has been documented to have a suite of deleterious toxic (Leighton 1993) and energetic (Jenssen 1994) consequences for birds. To determine if harlequin ducks in PWS were still being exposed to residual oil, we (Trust et al. 2000) measured induction of cytochrome P4501A (CYP1A) in harlequin ducks captured during March and April 1998 in both oiled and unoiled areas. CYP1A is induced upon exposure to polycyclic aromatic hydrocarbon (PAH) constituents of crude oil and has proven to be a sensitive and fairly specific indicator of oil exposure (e.g., Woodin et al. 1997). In addition to oil-derived PAHs, certain polychlorinated biphenyl (PCB) congeners can induce cytochrome P450 systems (Rattner et al. 1994). Therefore, we also measured congener-specific PCB concentrations in plasma from harlequin ducks

wintering in PWS to contrast with CYP1A enzyme activity. Evidence of exposure to oil would not necessarily imply that exposure had adverse physiological or demographic consequences. However, evidence of exposure would be consistent with potential for these deleterious consequences, and could be interpreted in light of other available data as a possible mechanism constraining full population recovery.

Liver 7-ethoxyresorufin-O-deethylase (EROD) activity (± SE), an indicator of CYP1A induction, of wintering harlequin ducks was higher in oiled areas (204.6 ± 20.3 pmol/min/mg protein; $n = 19$) than on unoiled Montague Island (70.7 ± 21.5 pmol/min/mg protein; $n = 18$; $P < 0.001$; Fig. 4; Trust et al., 2000). This is strong evidence of continued exposure to *Exxon Valdez* oil, given that background PAH concentrations in intertidal sediments and mussel tissues were negligible in PWS immediately prior to the EVOS (Short & Babcock 1996). Area differences in CYP1A induction could not be explained by differences in PCB exposure (Trust et al. 2000); congener-specific PCB concentrations were low and did not differ between areas. These data suggest that continued oil exposure could be limiting population recovery if there were physiological and population consequences of this exposure.

FOOD LIMITATION

Food limitation could constrain population recovery if the EVOS resulted in reduction in abundance of harlequin duck prey. This could occur from either direct effects (e.g., acute toxicity or habitat destruction during cleanup activities) or indirect effects (e.g., changes in food web structure; Peterson 2001). In turn, prey reductions could lead to increased intra-specific competition or reduced health of individuals, either of which could have population-level consequences.

During winter, the diet of harlequin ducks consists of a broad array of benthic marine invertebrates, especially amphipods, limpets, other snails, chitons, and mussels (Vermeer 1983, Goudie & Ankney 1986, Gaines & Fitzner 1987, Goudie & Ryan 1991, Patten et al. 2000). Goudie & Ankney (1986) hypothesized that harlequin ducks are trophic generalists because they must feed continuously to meet metabolic needs during winter; high-energy prey (e.g., amphipods) are consumed when encountered, but lower-quality prey are consumed when high-energy prey are not available.

Effects of the EVOS on populations of several important harlequin duck prey were evaluated by sampling at multiple pairs of oiled and unoiled sites in intertidal and nearshore subtidal habitats shortly after the spill (Highsmith et al. 1996, Jewett et al. 1999). Numerically dominant taxa within several important harlequin duck prey groups (limpets, other snails, mussels, and amphipods) were reduced in density by the oil spill. At oiled sites, numbers of *Mytilus trossulus* (mussels), *Tectura persona* (limpets), and *Littorina sitkana* (littorine snails), were reduced in the years following the EVOS (Highsmith et al. 1996). Similarly, several numerically dominant amphipod taxa were reduced at oiled sites in the nearshore subtidal zone (Jewett et al. 1999). Many of these differences in mean abundance at oiled and reference sites were no longer evident in 1993 (Hooten & Highsmith 1996, Houghton et al. 1996), suggesting that recovery of the intertidal and nearshore subtidal community was well underway. However, the last reported values suggest that there continued to be fewer individuals of some important prey at selected oiled sites at least through 1993 in the intertidal (Hooten & Highsmith 1996,

Houghton et al. 1996) and through 1995 in the subtidal (Jewett et al. 1999). Reduced prey densities at oiled sites can be largely attributed to the direct toxic effects of oil and impacts associated with cleanup procedures (Boehm et al. 1995, Wolfe et al. 1996, Houghton et al. 1996, Jewett et al. 1999). These results are consistent with food limitation of harlequin duck population recovery, at least within the few years immediately following the EVOS.

We estimated availability of harlequin duck prey items (Esler et al. 2000a) on oiled Knight Island (Bay of Isles and Herring Bay) and unoiled Montague Island study areas (Fig. 1) in summer 1997. Although prey availability may vary seasonally, we assumed that relative differences between study areas in summer would also reflect relative winter prey abundance because these invertebrates, as a rule, do not experience multiple generations within the year. To sample intertidal blue mussels (*Mytilus trossulus*), we removed all mussels from within 10 500-cm^2 quadrats placed in the mussel zone of each site. Ash-free dry mass of each mussel 5-25 mm in length (the size range appropriate for consumption by ducks) was estimated based on predictive equations of biomass by length. Samples of other invertebrate prey (limpets, chitons, lacunid snails, littorine snails, other snails, amphipods, and other crustaceans) were obtained at 6 intertidal and shallow subtidal locations within each prey sampling site. All epifauna were removed from a 0.25-m^2 quadrat at each location. Ash-free dry weights of each prey item < 25 mm in length were determined using a muffle furnace. We compared two metrics of food availability between areas: food biomass density and total food biomass relative to harlequin duck abundance. Food biomass density was defined as average g ash-free dry weight per 100 m^2; we used t-tests to compare food biomass densities between areas. Total food biomass was estimated as density expanded to the area of potential foraging for each sampling site (a 200 m shoreline segment by the width of the intertidal zone). Average total food biomass across sampling sites was divided by average number of harlequin ducks per sampling site to generate the metric describing food availability per duck; variance was calculated for a ratio of two independent estimates (Seber 1973) and 2-tailed *Z* scores were calculated to compare areas (Snedecor & Cochran 1980). Biomass density and total food biomass comparisons were conducted for all food items combined and also with mussels excluded because mussel abundance was much higher than for other prey species, yet they constitute a relatively minor part of the diet. Higher food densities or more food per duck on oiled than unoiled areas would imply that food limitation does not constrain recovery. Similar densities or quantities of food per duck between areas would be somewhat equivocal. Higher food densities or more food per duck on unoiled areas than oiled would be consistent with food limitation to population recovery. In 1997, food biomass densities were similar between oiled Knight Island and unoiled Montague Island study areas (Esler et al. 2000a) (Table 3). Also, on a per duck basis, total food biomass was similar between areas (Table 3). These data were highly variable, but generally consistent with a hypothesis of no food limitation to population recovery.

Food variables also were incorporated into habitat association models to determine whether harlequin duck densities were related to food biomass density or total food biomass (Esler et al. 2000a). Strong relationships between food density or abundance and duck densities would suggest that harlequin ducks may be susceptible to food limitation. Biomass density and total biomass of harlequin duck prey items did not explain additional variation in harlequin duck densities beyond effects of habitat and history of oil contamination (Esler et al. 2000a). However, when data for mussels were excluded, prey biomass density was slightly, positively related to

harlequin duck density, although this was strongly influenced by a single observation, without which there was no relationship.

Finally, body mass (see above) should provide strong evidence for the potential for food limitation. We would expect body mass to be lower in oiled than unoiled areas if food were limiting recovery, although other factors also could cause body mass differences. Body mass (see above) did not differ dramatically between areas, suggesting that food limitation was not occurring.

DISCUSSION

Injury and Recovery Status

Our harlequin duck studies, and this review, were focused on effects of the EVOS at the level of the population. We agree with Underwood & Peterson (1988), who described population-level evaluation as a link between the physiological mechanisms that affect individuals, subsequent effects on demography, and potential impacts on community and ecosystem processes. In this light, we summarize the findings of our review in terms of level of biological organization in Table 4 and discuss below the implications for population injury and recovery.

The evidence from post-spill harlequin duck research and monitoring supports the conclusions that: (1) harlequin duck populations were reduced by the EVOS, (2) these populations had not fully recovered by 1998, and (3) continued, direct effects of the EVOS were still occurring as much as 9 years after the EVOS. Key data (Table 4) leading to these conclusions include evidence of acute harlequin duck mortality immediately following the spill (J. Piatt, pers. comm.), continued exposure of harlequin ducks to oil through at least 1998 (Trust et al. 2000), differing fall population trends in oiled and unoiled areas from 1995 to 1997 (Rosenberg & Petrula 1998), lower densities than expected on oiled areas during winters 1995-96 and 1996-97 (Esler et al. 2000a), and differences in adult female survival between oiled and unoiled areas during winters 1995-96 through 1997-98 (Esler et al. 2000b). These results are internally consistent, i.e., predictions from each study are confirmed in the others. Differences in adult female survival offer a likely mechanism for divergence in population trends between areas. Under conditions of low survival, population declines, and high site fidelity, densities would be expected to be depressed in the oiled area, which we observed. The adult female survival analysis is particularly important for our interpretation; it demonstrates continued EVOS effects and describes the demographic mechanism that would lead to persistent population declines. Missing from these studies (Table 4) is a clear link between exposure and subsequent variation in survival; changes in physiology and metabolism presumably result from oil exposure (Leighton 1993, Jenssen 1994), although such responses are difficult to detect under field conditions. Laboratory experiments addressing these mechanisms would be exceedingly useful. The negative relationship between CYP1A induction and body mass suggests that such a mechanism may have been operating. Also, there are no data that address changes in community structure or ecosystem processes that may have resulted from lack of recovery of harlequin duck populations (Table 4); hence, it is impossible to evaluate indirect effects on other system components or processes.

Not all studies of harlequin duck population status and trends give consistent conclusions. U. S. Fish and Wildlife marine bird survey data (Lance et al. 1999) suggest increasing numbers on oiled areas during winter through 1998, consistent with ongoing population recovery, although these surveys are statistically less powerful than those of the Alaska Department of Fish and Game (Rosenberg & Petrula 1998) that described declining numbers on oiled areas. However, lack of differences in population trends between oiled and unoiled areas based on the U.S. Fish and Wildlife Service surveys was interpreted as evidence of lack of recovery (Lance et al. 1999).

Wiens et al. (1996) reported rapid recovery of bird communities following the EVOS based on measures of species richness and diversity. These parameters are derived from measures of presence or absence of a species within the study areas. For understanding recovery of populations, occurrence in oiled habitats is an incomplete measure. For example, occurrence of harlequin ducks in oiled areas likely reflects high site fidelity (Cooke et al. 2000) despite deleterious changes in habitat quality (Hilden 1965, Cooch et al. 1993) and declines in abundance. Occurrence in an area does not indicate a recovered population; populations could, in fact, be declining or a demographic "sink" (Pulliam 1988). We agree with Paine et al. (1996) that measures of population demographic processes are more powerful measures of injury and recovery than occurrence or abundance.

The habitat use studies of Day et al. (1997) indicated no EVOS effects on harlequin ducks during winter 1989-1991, in contrast to our findings of lower densities on oiled than unoiled areas (Esler et al. 2000a). This inconsistency may be a consequence of accumulating deleterious effects of the spill that extended beyond the study period of Day et al. (1997) and through at least our study period (Rosenberg & Petrula 1998, Esler et al. 2000, Trust et al. 2000). Thus, density differences may have been larger and more detectable during our study in 1995-1997 than in 1989-1991. Also, we collected harlequin duck abundance and habitat data at the scale that harlequin ducks use wintering sites (i.e., hundreds of meters reflecting specific shoreline segments; Robertson et al. 1999, Cooke et al. 2000) rather than at the scale of entire bays used by Day et al. (1997); our approach presumably results in greater resolution and power to determine habitat affiliations and evaluate oil spill effects.

Because most harlequin ducks wintering in PWS breed elsewhere, results of pre- and postspill comparisons of summer abundance by Murphy et al. (1997) have limited relevance for understanding dynamics of wintering populations, which we consider to be the core, demographically-distinct population segments. Also, although Murphy et al. (1997) had high power for detecting a 50% postspill population decline, they did not report power for detecting smaller but biologically meaningful reductions (e.g., 10%). In fact, they estimated 13.5%, 6.4%, and 11.9% reductions in harlequin duck numbers from prespill counts to 1989, 1990, and 1991, respectively, although these were not statistically significant. In contrast, Irons et al. (2000) detected significant negative effects for summer harlequin duck numbers in 1990 and 1991.

Body mass comparisons between oiled and unoiled areas did not indicate a mechanism for the observed lack of recovery. However, differences in body mass that are demographically relevant may be difficult to detect in wild populations, as significant mass declines may precede death by only a short period, particularly for animals naturally existing near a metabolic threshold (Goudie & Ankney 1986). For example, body mass declines in captive, oiled mallards faced with other environmental stressors were detectable only within 2 weeks of death (Holmes

et al. 1979). Because dead animals are not available to sample, detecting population-level differences in body mass or condition of field-captured ducks may be unlikely. The subtle but significant body mass decline with increasing CYP1A induction (Fig. 3) suggests that oil exposure may have been directly linked to changes in physiology of individuals.

Our conclusion of lack of full population recovery is supported by those data sets and approaches that are most powerful for assessing population status. Below we consider the potential mechanisms involved in lack of full population recovery.

Intrinsic Limitations on Population Growth Rates

Aggregations of harlequin ducks on wintering areas constitute demographically independent subpopulations from a population structure standpoint (Cooke et al. 2000). Winter site fidelity of harlequin ducks is high (Robertson 1997, Cooke et al. 2000) and pair formation occurs on the wintering areas (Gowans et al. 1997, Robertson et al. 1998). Because dispersal from wintering areas is limited, recovery of groups of birds in oiled areas must occur primarily through recruitment specific to that group (i.e., immigration from other areas does not contribute much to population change). Thus, factors that affect wintering aggregations are influencing subpopulations that are largely distinct demographic units, suggesting that harlequin ducks may be susceptible to constraints to population recovery due to intrinsic demographic limits to population growth rates.

However, demographic limitations on population growth rate can not be invoked as the primary constraint to harlequin duck population recovery until lingering effects of the EVOS on survival are gone and the population in the oil spill zone can achieve positive growth. This was not the case through 1998 and the time frame for cessation of EVOS effects is unknown. However, once freed from other constraints to recovery (see below), recovery of populations then will be limited by the time necessary for intrinsic rates of increase to operate (Goudie et al. 1994). Because it is not clear what naturally regulates harlequin duck populations, nor the life stage where regulation or limitation occurs, it is difficult to predict recovery times of an injured winter harlequin duck subpopulation.

Results from genetic studies offer some good news for harlequin duck populations. Levels of dispersal, either historical or contemporary, have resulted in subpopulations within the oil spill zone that are not genetically distinct (Lanctot et al. 1999), i.e., the EVOS does not threaten a unique genetic resource. Also, these results may reflect low levels of juvenile dispersal that we were unable to detect; if this is the case, population recovery could be enhanced by some immigration.

Continued Exposure to Oil

A growing body of evidence indicates that PAHs from residual *Exxon Valdez* oil were responsible for the observed CYP1A induction in oiled areas of PWS in sea ducks (Trust et al. 2000) and several other vertebrates (Marty et al. 1997, Woodin et al. 1997, Bodkin et al. 2002, Jewett et al. 2002). However, exposure does not necessarily indicate effects on individuals or populations (Underwood & Peterson 1988). A critical question is whether oil exposure could cause physiological challenges that affect demographic properties which, in turn, have population-level consequences.

As described above, our data on adult female winter survival offer a likely explanation for continued injury to harlequin duck populations and, hence, lack of recovery. Although the survival differences between oiled and unoiled areas may appear small, harlequin duck population dynamics are particularly sensitive to changes in adult female survival (Goudie et al. 1994) because their life history is oriented toward long reproductive life spans (Stearns 1992). Oil exposure (Leighton 1993, Jenssen 1994) could negatively affect harlequin duck health and subsequent survival. Continued oil exposure was the most likely mechanism constraining full population recovery through at least 1998.

Most lab studies have shown that mallards (*Anas platyrhynchos*) do not suffer acute toxic effects of oil ingestion until very high doses. These studies have been used to infer that harlequin ducks also should not suffer deleterious physiological responses to residual *Exxon Valdez* oil (Stubblefield et al. 1995, Boehm et al. 1996). However, these lab studies have been conducted under relatively benign conditions. Other lab studies have found that, with addition of other stressors such as cold temperatures, ducks that ingested oil suffered higher mortality than unoiled birds (Holmes et al. 1978, 1979). This is a more appropriate analog for wild harlequin ducks, which exist under winter conditions with cold temperatures and limited foraging time and, hence, little flexibility for accommodating additive stresses (Goudie & Ankney 1986).

The divergence of survival probabilities between oiled and unoiled areas during midwinter (Fig. 2) is consistent with the hypothesis that effects of oil are exacerbated by other stressors. Midwinter is presumably the most stressful period for harlequin ducks under natural conditions. Harlequin ducks feed by sight and during midwinter, when day length is shortest, they spend most of their day time foraging (Fischer 1998, Goudie & Ankney 1986). PWS is one of the farthest north wintering areas for harlequin ducks (Robertson & Goudie 1999); thus, daylight available for foraging is particularly limited. Thus, we suggest that observed differences in winter survival and populations trends are linked to observed differences in contaminant exposure.

Oil exposure could occur through consumption of contaminated prey. In the marine environment, oil constituents can accumulate in bottom sediments and benthic, filter-feeding invertebrates (Fukuyama et al. 2000, Peterson 2001). Studies have documented hydrocarbons in harlequin duck prey from immediately post-spill through 1995 (Babcock et al. 1996, Boehm et al. 1995, Patten et al. 2000, Short & Babcock 1996, Wolfe et al. 1996). Also, contamination could occur through external contact with residual oil; surface sheening was observed in nearshore areas of PWS during the same period as our studies (Hayes & Michel 1999), suggesting that this also could be a potential route of exposure. Metabolic consequences of external oiling are well documented (Jenssen 1994) and could result in increased mortality.

Food Limitation

Available evidence suggests that food availability or quality is not limiting harlequin duck population recovery. Recovery of most duck prey, lack of a strong relationship between harlequin duck densities and food biomass density or abundance, similar food biomass density and abundance per duck between areas, and similar body masses between areas all support this conclusion.

Interpretation of food data is hampered by a lack of understanding of harlequin duck foraging strategies and the role of winter food abundance, density, or quality in harlequin duck population regulation or limitation. Furthermore, we have no data to test causal, mechanistic relationships between winter food supply and carrying capacity. Thus, body mass data provide perhaps the strongest evidence against food limitation. Because harlequin duck body masses across seasons, sexes, and ages did not show a consistent difference between oiled and unoiled areas, we conclude that food is unlikely to be a primary constraint to recovery of populations from oiled areas.

Conclusions and Recommendations

We conclude that, as of 1998, harlequin duck population recovery had not occurred, continued oil exposure may be the primary mechanism constraining recovery, and lack of full recovery likely will be further delayed after deleterious EVOS effects are gone due to intrinsic demographic limits to population growth rates. Our findings are concordant with studies of other nearshore vertebrates. For example, sea otters (*Enhydra lutris*) had elevated CYP1A (B. Ballachey, unpubl. data), increased mortality in oiled areas through at least 1998 (Monson et al. 2000), and lack of return to pre-spill numbers in the most heavily oiled areas of PWS (Bodkin et al. 2002). Like harlequin ducks, food limitation did not appear to limit sea otter population recovery (Dean et al. 2000, Dean et al. 2002). However, sea otters and harlequin ducks both rely on benthic invertebrate prey that can accumulate hydrocarbons, which may explain the parallel findings.

Response of bird populations to the EVOS varied considerably. Populations of some bird species apparently were not reduced by the EVOS, or recovered quickly (Bowman et al. 1995, Wiens et al. 1996, Bowman et al. 1997). Black oystercatcher (*Haematopus bachmani*) breeding was depressed in 1989-90 but nearly recovered by 1991 (Andres 1997). Direct effects of oil exposure and indirect effects of prey reduced by the EVOS were indicated as constraints to full recovery of injured pigeon guillemot (*Cepphus columba*) populations in Prince William Sound for at least a decade (Golet et al. 2002). Harlequin duck populations have an unfortunate combination of characteristics that make them particularly vulnerable to effects of the oil spill during nonbreeding parts of the annual cycle. These characteristics include a life history requiring high adult survival, occurrence in nearshore habitats that are strongly affected by oil spills and which may hold residual oil for years, adaptation to stable and predictable marine environments, high site fidelity, and a diet of benthic invertebrates. The traits of harlequin ducks that make them (and other wildlife species sharing these traits) vulnerable to catastrophic oil spill effects also render them susceptible to effects of chronic, low-level pollution. Sensitive species like harlequin ducks, sea otters, and pigeon guillemots appear to suffer deleterious effects of oil pollution at lower levels and for longer time periods than other species. The duration of the population level effects far exceeds the few years that have conventionally been assumed to represent recovery times for wildlife populations injured by oil pollution.

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Table 1. Results of general linear model analyses to evaluate relationships of Harlequin Duck densities (square-root transformed) in Prince William Sound, Alaska, during winters 1995-1997, with habitat attributes and history of oil contamination by the 1989 *Exxon Valdez* oil spill. The parameter estimates (± SE) are from the best-fitting model, based on comparisons of all possible combinations of habitat attribute variables, habitat by area interactions, and an area (history of oil contamination) term. From Esler et al. (2000a).

Response variable	R^2	Explanatory variable	Parameter estimate
Ducks per 400 m	0.45	Intercept	1.17 ± 0.12
		Reef 200-500 m[a]	0.51 ± 0.15
		Stream 0-200 m[a]	0.34 ± 0.14
		Full exposure to wind/waves[a]	0.45 ± 0.12
		Mixed substrate[a]	0.32 ± 0.14
		Mixed substrate × Area[b]	-0.48 ± 0.18
		Area[b]	-0.69 ± 0.12

[a]Parameter estimate value is the difference in duck density in relation to all other levels of the explanatory variable.

[b]Reference value for area is unoiled Montague Island; parameter estimates are interpreted as effects on oiled Knight Island.

Table 2. Effect sizes (g ± SE) of body mass and composition comparisons between oiled and unoiled areas of Prince William Sound, Alaska. These are parameter estimates for an area term from multiple regression analyses, and represent differences in body mass or composition between areas after accounting for the other explanatory variables in the best-fitting model. The reference value for the area term was unoiled areas, and hence the parameter estimates represent the difference in body mass or composition on oiled areas.

Sex	Season	
Response variable	Wing molt	Winter
Female		
Body mass	-9.6 (± 2.6)	0.00 (± 0.0)[a]
Lipid mass	-2.5 (± 0.7)	-----[b]
Lean mass	-7.0 (± 2.0)	-----[b]
Male		
Body mass	13.4 (± 4.5)	-21.5 (± 8.7)

[a]The area term was not included in the best-fitting model.
[b]Lipid and lean mass were not estimated for females during winter.

Table 3. Average (± SE) biomass density and total biomass of harlequin duck prey (amphipods, chitons, limpets, other snails, and mussels < 25mm) at oiled and unoiled study areas within Prince William Sound, Alaska, 1997.

Parameter	Montague Island (Unoiled)	Knight Island (Oiled)	*P*
Biomass density (g AFDW[a]/100 m^2)	2030.8 (± 555.2)	1964.1 (± 638.9)	0.94 (*t* = 0.08)
Total biomass (kg AFDW/duck)	51.8 (± 16.4)	100.5 (± 52.0)	0.81 (*Z* = 0.24)
Biomass density w/o mussels (g AFDW/100 m^2)	45.9 (± 10.1)	42.8 (± 7.3)	0.80 (*t* = 0.251)
Total biomass w/o mussels (kg AFDW/duck)	3.9 (± 1.2)	3.2 (± 1.5)	0.94 (*Z* = 0.08)

[a]Ash free dry weight.

Table 4. Summary of findings from studies relevant for understanding harlequin duck population injury, status of population recovery, and constraints to full recovery in Prince William Sound, Alaska following the 1989 *Exxon Valdez* oil spill. Findings are arranged by level of biological organization and are interpreted in terms of population processes in the text.

Level of effect Parameter	Findings	Citations
Individual		
Oil Exposure	Hydrocarbon metabolites in 1989-90	Patten et al. 2000
	Elevated CYP1A through 1998 on oiled areas	Trust et al. 2000
Mass Variation	No consistent, strong population-level differences between areas	This document
	Negative relationship between mass and CYP1A induction in late winter	This document
Population Demography		
Initial Mortalities	Approximately 1000	Piatt et al. 1990; J. Piatt, pers. comm.
Adult Female Survival	Lower in oiled areas than unoiled areas	Esler et al. 2000b
Dispersal	Generally low in the species; molt site fidelity high in PWS	Breault and Savard 1999, Cooke et al. 2000, Robertson et al. 1999; This document
Reproduction/Recruitment	Similar age ratios between areas; most breeding outside PWS	Rosenberg and Petrula 1998
Population Status		
Abundance/Densities	Lower densities on oiled areas during summers 1990 and 1991	Irons et al. 2000

Level of effect Parameter	Findings	Citations
	Summer abundance 1989-91 unchanged from pre-spill	Murphy et al. 1997
	Stable summer and increasing winter densities in oiled areas 1989-98	Lance et al. 2001
	Similar summer and winter trends in density 1989-98	Lance et al. 2001
	Numerical declines during fall 1995-97 on oiled areas, stable on unoiled	Rosenberg and Petrula 1998
	Densities lower than expected on oiled areas, winter 1995-97	Esler et al. 2000a
	Densities related to oiling intensity 1989-90, not 1991	Day et al. 1997
	Population model predicts declines on oiled areas, stable on unoiled	Esler et al. 2000b

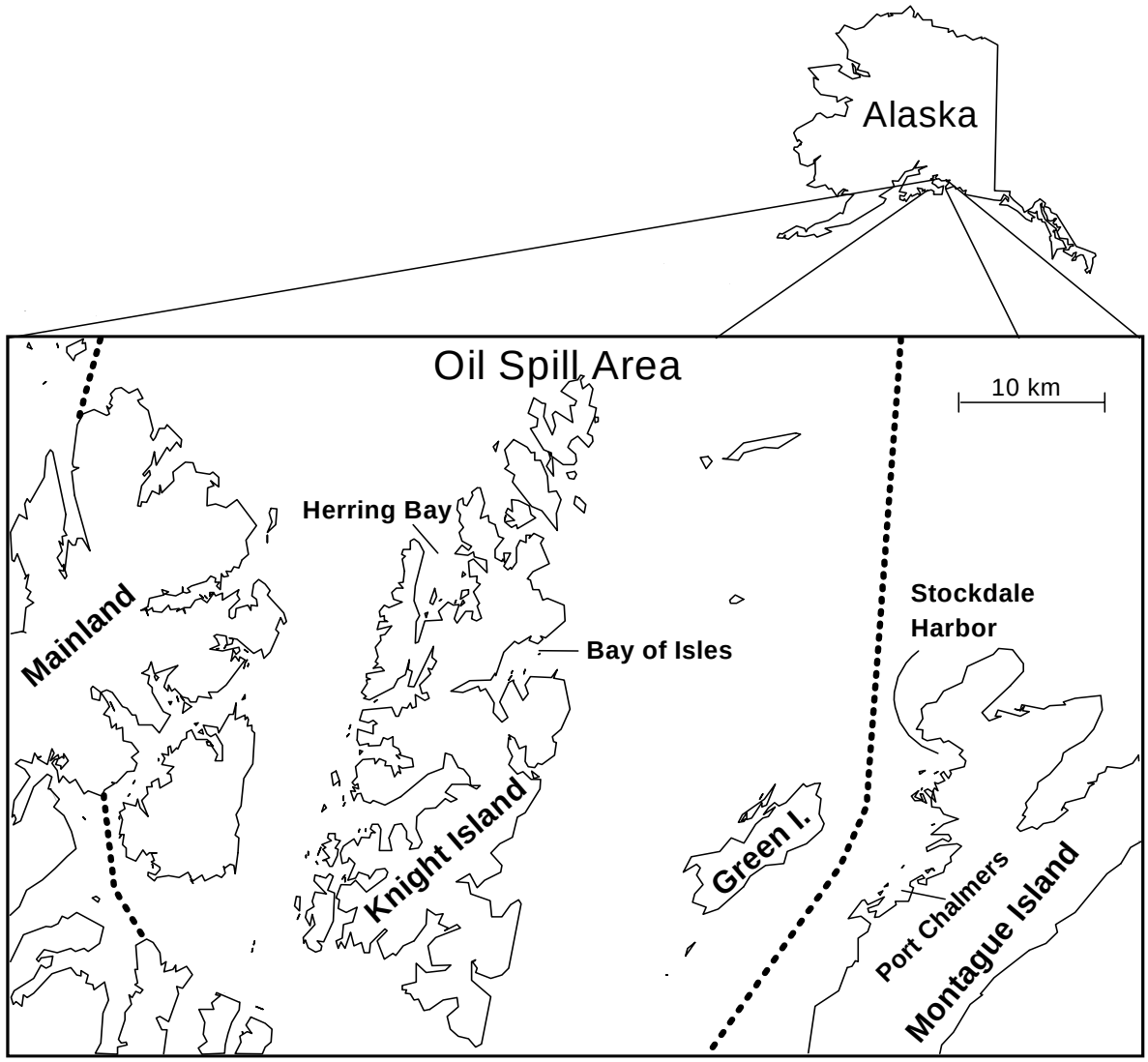

Figure 1. Study areas for the authors' harlequin duck studies in Prince William Sound, Alaska, 1995-1998. Dashed lines indicate the bounds of the area contaminated by the 1989 *Exxon Valdez* oil spill.

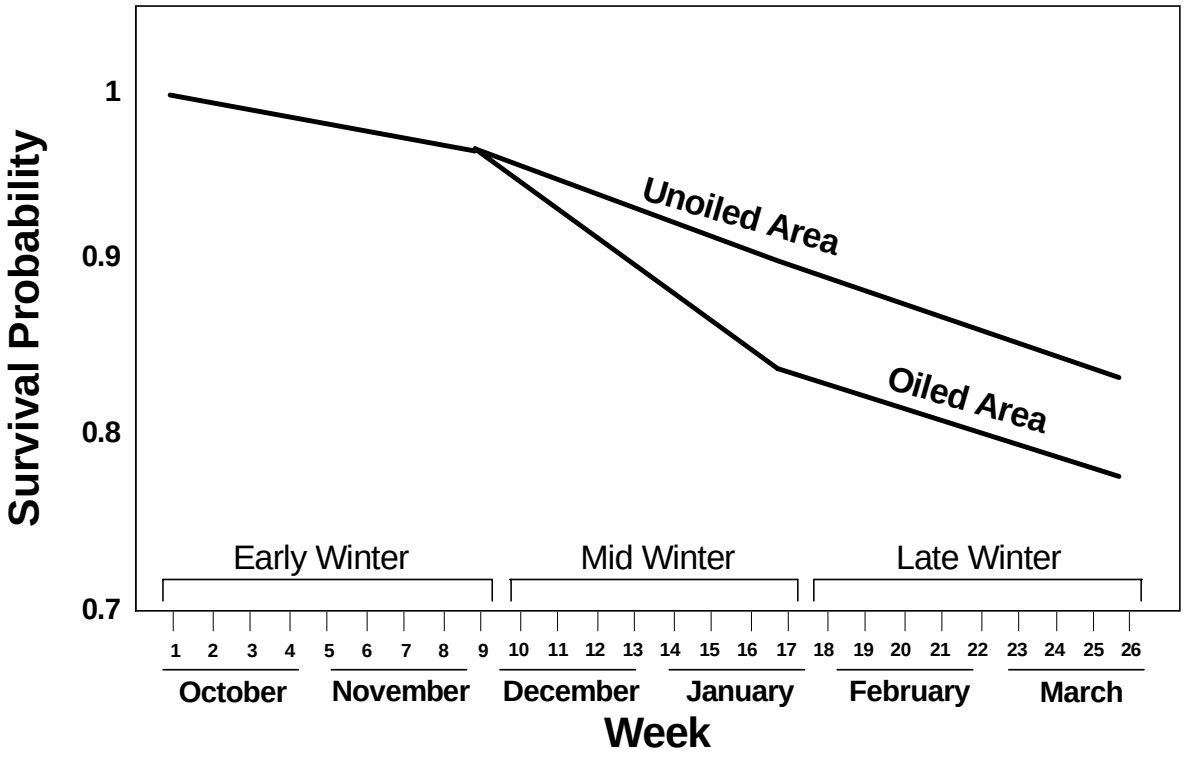

Figure 2. Winter survival probabilities for adult female harlequin ducks in Prince William Sound, Alaska based on radio telemetry data combined over winters 1995-1996 through 1997-1998 (Esler et al. 2000b).

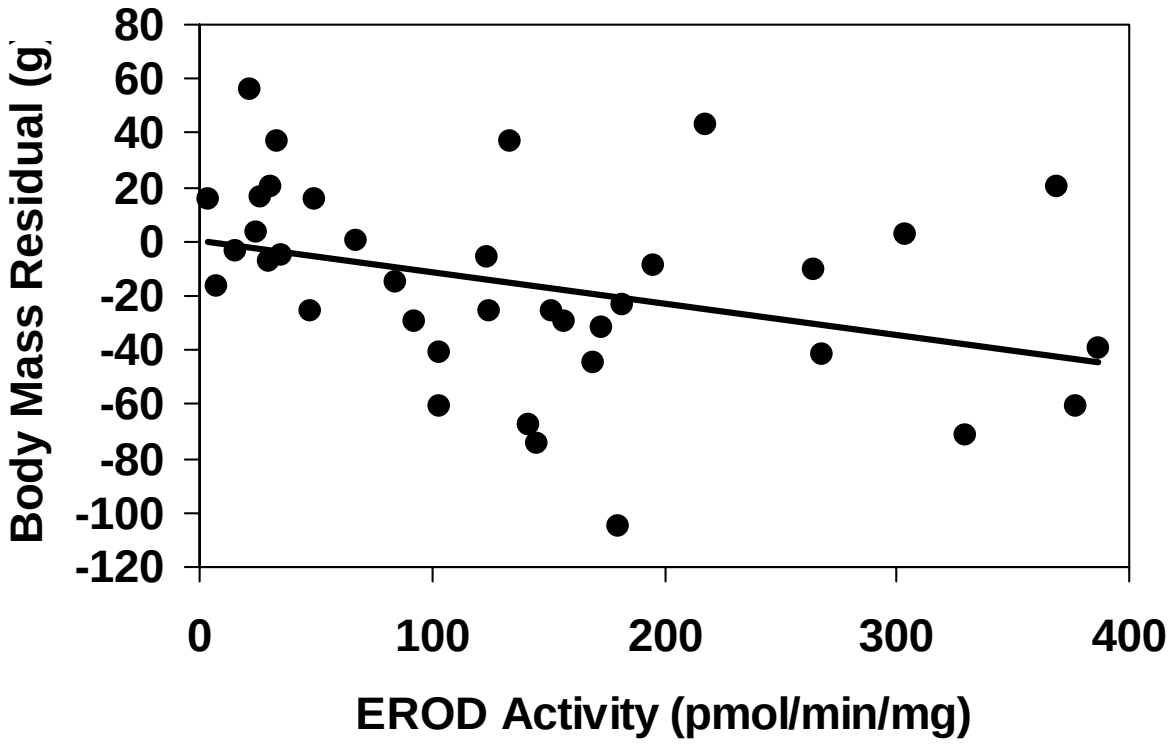

Figure 3. Relationship of body mass residuals (i.e., accounting for mass differences between sexes) with cytochrome P4501A induction, as measured by liver EROD activity, an indicator of exposure to PAHs.

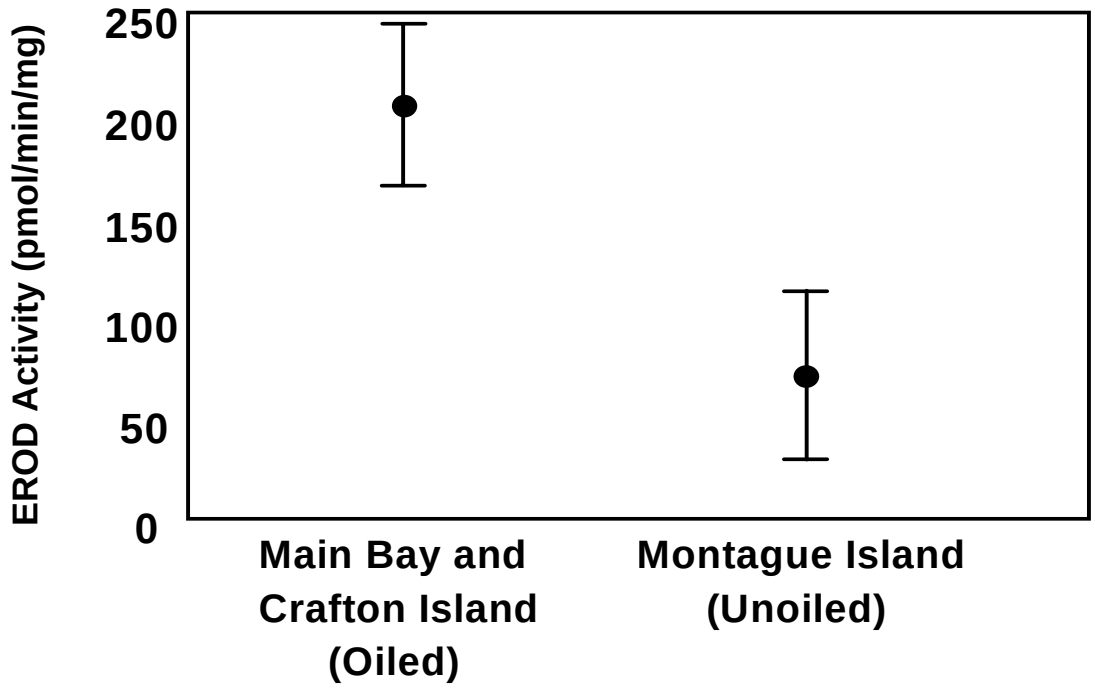

Figure 4. Comparisons of average (± 95% confidence intervals) liver EROD activity, as a measure of cytochrome P4501A induction, of harlequin ducks captured from oiled and unoiled areas of Prince William Sound, Alaska in March and April 1998 (Trust et al. 2000).

Chapter 5. River Otter (*Lontra canadensis*) Perspective

Effects of the *Exxon Valdez* Oil Spill on River Otters:

Injury and Recovery of a Sentinel Species[1]

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ABSTRACT

Integration of individual-based and population-level studies is essential to understanding effects of pollution on populations and ecosystems. Here we provide an example of such integration from our exploration of effects of the *Exxon Valdez* oil spill (*EVOS*) on river otters (*Lontra canadensis*) inhabiting the terrestrial-marine interface in Prince William Sound, Alaska, USA. Our research was divided into 2 phases: an early phase (1989-92) immediately following the oil spill; and a late phase (1996-99), which focused on potential chronic effects of oil contamination in the Sound. We used a variety of measurements that considered the physiological status and health of individual river otters, as well as aspects of their ecology, behavior, and demography. We then conducted meta-analysis to explore interactions between individual-based and population-level data in demonstrating injury and subsequent recovery of otters from ill effects of *EVOS*. During both phases of our studies, we first conducted intensive research at 2 study sites (oiled and "nonoiled"), and then expanded our investigations throughout similar areas of Prince William Sound. Nonetheless, our data are best interpreted as differences between heavily oiled areas and lightly oiled sites because later information indicated that our reference sites were lightly oiled. In the later phase, we were part of a broader ecosystem-based project (Nearshore

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Vertebrate Predators) designed to assess the long term effects of *EVOS* on a suite of key organisms, and to determine whether those species had recovered from that catastrophic accident.

We used radiotelemetry to locate carcasses of animals that died from natural causes, and documented that searching beaches immediately following the spill was not a reliable method for locating dead river otters. Our early research (1989-92) demonstrated that river otters living in oiled areas had lower body mass ($P < 0.04$) and elevated biomarkers ($P < 0.05$) in their blood (e.g., haptoglobin [Hp], interleukin-6 immunoreactive [IL-6 *ir*], aspartate aminotransferase [AST]) than otters inhabiting "nonoiled" areas. Likewise, otters from oiled areas had higher levels of fecal porphyrins ($P < 0.001$), ate a less-diverse diet ($P < 0.001$), had larger home ranges ($P < 0.05$), and selected habitats differently ($P < 0.01$) than otters living in areas that were not heavily oiled. A mark-recapture analysis based on radiotracers in otter feces during 1990 indicated no difference ($P > 0.10$) between density of otters in Herring Bay (oiled) or Esther Passage ("nonoiled"), but no prespill data were available. Likewise, by 1992, biomarkers (Hp, IL-6 *ir*, AST) did not differ ($P > 0.05$) between oiled and "nonoiled" areas.

During the later phase of research, hydrocarbons on the pelage of river otters and the elevation of endothelial *P*450-1A, a biomarker sensitive to hydrocarbon exposure, indicated that river otters were exposed to oil still present in Prince William Sound. Nonetheless, body mass of otters continued to increase on oiled areas over time ($P < 0.05$), and eventually did not differ from otters living in "nonoiled" sites ($P > 0.05$). All blood biomarkers (Hp, IL-6 *ir*, AST) were markedly reduced from the early phase of our research, and no longer differed ($P > 0.10$) between oiled and "nonoiled" sites. We used Principal Component Analysis (PCA) to determine that few differences existed in an array of blood characteristics for otters inhabiting oiled and "nonoiled" sites, and those differences that did exist likely were related to diet. Corpoporphyrin III, a key biomarker in heme synthesis, was reduced ($P = 0.008$) from post-spill collections made in 1990 in the oiled area, and no longer differed ($P > 0.05$) between oiled and "nonoiled" areas in 1996. We used stable isotope analysis to investigate differences in diet of river otters inhabiting oiled and "nonoiled" areas in 1996-97. When we controlled for otters inhabiting extensive freshwater habitats (which did not occur in our early studies), no differences in diet or the trophic level of otters were identified ($P > 0.20$) for otters living in oiled versus "nonoiled" sites. Similarly, density of marine fishes ($\geq$8 cm in total length) on underwater transects did not differ ($P = 0.97$) between oiled and "nonoiled" areas, although an area by year interaction occurred ($P = 0.01$). Habitat selection by otters also was altered from the early phase; river otters on both study areas selected vegetated slopes that were not steep, and selected sites with more understory (brush) and greater exposure; selection for those characteristics was more pronounced in the oiled area. Otters on both sites avoided (use < availability) gravel and small rocks. Although selected variables differed between oiled and "nonoiled" sites ($P < 0.001$), the direction of selection did not differ between areas. Moreover, tidal slope did not enter any of the models, in contrast to our early studies, indicating that differences in selection were not related to avoidance of oiled shores. Home-range size declined ($P < 0.05$) for otters living in oiled areas, and no longer differed ($P > 0.7$) from animals inhabiting "nonoiled" sites. We enumerated populations from oiled and "nonoiled" areas using a combination of live-captured individuals and DNA fingerprinting using microsatellite from otter feces at latrines. We also performed a conventional reconstruction based on age structure to calculate population size in 1997. Those methods indicated that most animals in the population were recruited following the oil spill and both

methods characterized slowly (λ = 1.03-1.06) growing or stable population in the oiled area. Age structure of river otters in the Sound differed neither between oiled and "nonoiled" areas ($P > 0.36$), nor from a harvested population of river otters in Maine ($P > 0.49$). Finally, survivorship of river otters did not differ ($P > 0.2$) between oiled and "nonoiled" areas of Prince William Sound and was high compared with data on other otter populations in North America. Our data indicate that although river otters continued to be exposed to low levels of crude oil, effects of that exposure were no longer sufficient to cause obvious injury. We cautiously conclude that river otters have recovered from the more pernicious effects of *EVOS*.

Based on our experiences in this research, we provide theoretical considerations for use of biomarkers in wildlife studies and describe statistical approaches, including principal component analysis blood variables, which may assist researchers with interpreting complicated results of multiple variables and data sets. Likewise, we describe how dose-response curves should be used in understanding population-level responses to pollutants. We hope that this monograph will provide valuable insights for other wildlife biologists on the process of integration of toxicological data with that of ecological data useful for studying effects of pollution on wildlife populations and their habitats.

Key words: Alaska, biomarkers, body mass, demography, diet, *Exxon Valdez* oil spill, habitat selection, home range, hydrocarbons, *Lontra canadensis*, pollution, river otter.

INTRODUCTION

Studies investigating factors attributable to declines in wildlife populations have resulted in a distinct dichotomy in research emphases. Wildlife and conservation biologists have concentrated on ecological phenomena such as habitat destruction and fragmentation, and reduced genetic variability (Harris 1984, Zacharias and Roff 2000, Eldridge et al. 1999). In contrast, toxicologists and veterinarians have focused on species or individual responses to environmental pollutants using biomarkers (Wilson and Hunt 1975, Broman et al. 1992, Huggett et al. 1992, Vanden Heuvel, and Davis 1999). Variation in individual responses to environmental pollution, however, can have profound effects on population dynamics and stability (Lomnicki, 1988). Integrating both approaches, therefore, is essential to understanding processes connecting individual-based and population-level studies of pollution. For example, sublethal and chronic effects of contaminants can result in decreased reproductive success or survivorship, both of which can lead to a decline in population densities and reductions in genetic variability within populations (Peterson 2001, Weis et al. 2001).

Devastating effects of pollution, especially in aquatic systems, have been the focus of research for many years (Wilson and Hunt 1975, Broman et al. 1992, Huggett et al. 1992, Beckman et al. 1997). Few studies, however, have addressed potential effects of pollution in one system on processes occurring in another through changes in population dynamics of animals (Newman 1998). For instance, aquatic and terrestrial systems are intricately connected through activities of vertebrate predators in riparian zones (Naiman 1988, Ben-David et al. 1998*a*, Hilderbrand et al. 1999, Helfield and Naiman, 2001). Similarly, fertilization of beach-fringe vegetation with marine-derived nitrogen by birds and mammals connects marine and terrestrial systems (Anderson and Polis 1998, Ben-David et al. 1998*a*, 1998*b*; Hilderbrand et al. 1999,

Hobson et al. 1999). Thus, a decline in numbers of semi-aquatic mammals may alter interactions between ecosystems. Likewise, recovery of those animals can have profound importance for ecosystem structure and function in both aquatic and terrestrial habitats. Pollution holds the potential to force those ecosystem processes. Accordingly, integrating individual-based and population-level responses of semi aquatic mammals provides a model for understanding effects of pollution in one system on another.

River Otters as a Sentinel Species

Semi-aquatic mammals, especially mustelids, are ideal sentinel species. A sentinel species is sensitive to pollutants, and useful for measuring or indexing levels of environmental contamination (Prichard et al. 1997, Newman 1998). Species such as mink (*Mustela vison*), European otter (*Lutra lutra*), river otter (*Lontra canadensis*), and giant otter (*Pteronura brasiliensis*) have been the subject of numerous toxicological studies (Table 1). For example, European otters were extirpated from much of their historic distribution in Europe - that decline was strongly linked to environmental contamination (Baker et al. 1981; Kruuk 1995; Gutleb et al. 1998).

Populations of river otters inhabit freshwater systems throughout most of North America (Lariviére and Walton 1998), but also occur in marine environments. Although legally not considered a marine mammal, the distribution of river otters extends along the Pacific Coast from north of the Arctic Circle to central California, and down the Atlantic seaboard from the coast of Labrador to the Gulf of Mexico (Hall 1981, Lariviére and Walton 1998). This wide distribution, with river otters feeding near the apex of the trophic pyramid (Larsen 1984, Stenson et al. 1984, Bowyer et al. 1994, Ben-David et al. 1998*b*), makes these predators a good sentinel species (Duffy et al. 1996, Hecker et al. 1997; Table 1). Indeed, populations of river otters in North America were reduced throughout much of their range in the eastern and midwestern United States by the early 1900s because of pollution and urbanization (Lariviere and Walton 1998). Diminished levels of pollution coupled with reintroductions have expanded distributions of river otters in recent years (Erickson and McCullough 1987; Serfass et al. 1993, 1998; Lariviere and Walton 1998; Johnson and Berkley 1999; Raesly 2001).

River otters are relatively long-lived ($\leq$13 years in the wild; Docktor et al. 1987), reproduce at an early age (some females as yearlings but most as 2-year olds; Hamilton and Eadie 1964, Docktor et al. 1987), and neither migrate nor hibernate (Melquist and Hornocker 1983, Bowyer et al. 1995). Consequently, this mustelid may be exposed year-round to localized sources of pollution and offers opportunities to study acute and chronic effects of toxins, including biomagnification of heavy metals (Duffy et al. 1998, 1999*a*; Ben-David et al. 2001*a*), and accumulation of petroleum hydrocarbons (Ben-David et al. 2000). Further, river otters have home ranges that are sufficiently large (10-45 km of shoreline; Bowyer et al. 1995; Blundell et al. 2000, 2001) to integrate effects of pollution along coastlines. Vagility of this semi-aquatic mammal makes the river otter well suited for studying effects of pollution at the scale of the nearshore ecosystem.

Finally, river otters transport nutrients between marine and terrestrial systems (Ben-David et al. 1998*b*). This mainly piscivorous predator acquires nutrients by foraging in the nearshore environment (Bowyer et al. 1994) and then deposits them at latrine sites that are located along

the coast (Ben-David et al. 1998*b*). Plants growing on latrine sites used by river otters incorporate marine-derived nitrogen from otter feces, urine, and anal-gland secretions. This fertilization may have a substantial effect on community composition of the beach-fringe forest (Ben-David et al. 1998*b*). Consequently, variation in otter numbers or distribution caused by marine pollution holds the potential to uncouple or perturb marine-terrestrial relations. Thus, river otters may be a keystone species (Mills et al. 1993, Estes 1996, Simberloff 1998) for the land-margin ecosystem.

The *Exxon Valdez* Oil Spill

On 24 March 1989, the super tanker *Exxon Valdez* ran aground on Bligh Reef just beyond Valdez Arm in Prince William Sound, Alaska, USA. The accident spilled 39,000 metric tons of North Slope crude oil, which ultimately spread across 3,500 km of shoreline in western Prince William Sound, as well as contaminating portions of the Kodiak Island Archipelago, and Kenai and Alaska peninsulas in southwestern Alaska. Crude oil initially was concentrated in the nearshore environment (O'Clair et al. 1996), a pristine system known for its rich and abundant marine life (Dean et al. 2000*a*). In 1996, microbial analyses indicated that oil in sediments along contaminated shorelines in Prince William Sound still occurred in much higher concentrations than in "nonoiled" areas (Braddock et al. 1996). Oil buried in sediments, which may be resuspended during storms and tidal action, is not subject to degradation by marine organisms and therefore remains in a form that is toxic to many vertebrates (Braddock et al. 1996). Hence, effects from the *Exxon Valdez* oil spill (*EVOS*) may be long term and chronic. Short et al. (1996) documented a continuous decline in oil concentrations in the Sound, indicating that buried oil became less available for biological transport to marine organisms as time progressed following the catastrophe.

Numerous marine organisms were injured as a result of *EVOS* (Collier et al. 1996, Laur and Haldorson 1996, Loughlin et al. 1996, Piatt and Ford 1996, Ballachey and Kloecker 1997), including coastal river otters (Duffy et al. 1993, 1994*a*). The degree of injury and level of recovery of these species and the marine ecosystem has been the topic of international concern since 1989.

Hypotheses and Research Design

Herein, we provide a comprehensive study of effects of *EVOS* on river otters. We combine individual-based and population-level approaches to gain a better understanding of effects of *EVOS* on this sentinel and keystone species. We also provide an integrative model useful for future studies on effects of pollution on wildlife populations and their habitats.

Remaining crude oil buried in sediments of Prince William Sound offered the opportunity to follow effects of hydrocarbon pollution on the nearshore environment through time. We hypothesized that exposure to petroleum hydrocarbons would adversely influence individuals and, consequently, populations of river otters, and that the negative effect would diminish as oil levels were reduced over time.

Our studies were initiated immediately following the spill (1989-92; i.e., early phase) to test for deleterious consequences of *EVOS* on river otters (Duffy et al. 1993, 1994*a*, 1994*b*, 1996;

Bowyer et al. 1994, 1995; Testa et al. 1994; Blajeski et al. 1996). We later resumed our efforts to investigate chronic effects of the spill (1996-99; i.e., late phase) as well as to document the status of recovery of river otters as a part of the Nearshore Vertebrate Predators (NVP) project. That ecosystem-based project combined studies of river otters with those of sea otters (*Enhydra lutris*), pigeon guillemots (*Cepphus columba*), and harlequin ducks (*Histrionicus histrionicus*) to test for lingering effects of the oil spill in Prince William Sound (Dean et al. 2000*a*, *b*; Esler et al. 2000; Monson et al. 2000; Seiser et al. 2000; Dean and Jewett 2002; Bodkin et al. 2002; Dean et al. 2002; Esler et al. 2002; Golet et al. 2002; Peterson and Holland-Bartles 2002). The central question posed by the NVP study was this: had recovery occurred, and if not, was that lack of recovery a function of direct toxicological effects of oiling, indirect effects on habitat, food or its acquisition, or lingering demographic consequences from the spill (Fig. 1)? This logical approach for understanding consequences of oil fouling on a sensitive marine vertebrate has been adopted in another fragile ecosystem (Wikelski et al. 2002). Nonetheless, those potential effects of the oil spill may not be mutually exclusive; accordingly, we tested hypotheses relative to each, as well as potential interactions between direct exposure, habitat degradation, food limitation, and demographics using a weight-of-evidence approach.

Sparse data on river otters prior to the spill necessitated a comparative design, contrasting the status of otters inhabiting oiled and "nonoiled" areas of Prince William Sound. Studying effects of oil pollution on river otters, however, was challenging because of the natural history of this semi-aquatic mustelid (Lariviére and Walton 1998). The secretive nature of river otters makes them extremely difficult to observe and, therefore, estimation of population densities and gathering behavioral data are problematical. Moreover, otters are difficult to capture and especially to recapture (Serfass et al. 1993, 1996; Duffy et al. 1994*b*; Blundell et al. 1999), necessitating the use of some indirect methodologies.

Our methodologies included: 1) biomarker responses as a measure of physiological stress (morphometrics, blood and tissue parameters), 2) assessing diet and prey availability, 3) evaluating use of landscape (habitat selection and home range), and 4) studying demographics (age structure, population estimates, and survival). This design provided an integrative assessment of both injury and recovery when the weight of evidence from data pertaining to toxicological damage and resulting ecological phenomena were considered simultaneously.

Most data we collected shortly following the oil spill already have been published in a series of journal articles, although we have not compiled them previously into a cohesive document that examined overall consequences of *EVOS* on river otters. We have incorporated much of those early data in combination with information collected in the late phase of our study to test hypotheses related to the injury and potential recovery of river otters in Prince William Sound.

Acknowledgments.-This long-term study was initiated immediately following *EVOS* in 1989 when competing proposals from C. C. Schwartz (Alaska Department of Fish and Game - ADF&G) and R. T. Bowyer (University of Alaska Fairbanks - UAF) were combined into a collaborative effort. J. B. Faro soon replaced Schwartz as the principal investigator (PI) for ADF&G, and Bowyer was joined by L. K. Duffy (UAF) and J. W. Testa (UAF) as PIs in 1990. Laboratory procedures at UAF were performed by A. Porchet throughout the course of this study. Veterinarians W. Taylor and G. Grady assisted with surgical procedures in the early phase of our study (1989-92), and J. B. Blake and T. O'Hara offered important advice throughout our

project. B. Ballachey and A. Rebar provided thoughtful discussions on blood chemistry of otters. We are indebted to students from UAF, who helped with fieldwork in the early phase of our research, including J. Kristopeit, N. Chelgren, K. Rock, E. Rock, M. Strauss, K. Wilson, S. Olsen, and K. Olsen. We also thank ADF&G biologists J. Lewis, R. Nowlin, D. McAllister, and S. Patton, and ADF&G technicians B. Porter, C. Hastings, S. Bowen, and K. Koener for their assistance and hard work. Likewise, we thank ADF&G volunteers K. Dowd and B. Weiss for their help. J. B. Browning identified prey remains in feces of otters. We thank D. Albert and M. Charpeniter for assistance with statistics and Geographic Information Systems (GIS) analyses, respectively. M. Ben-David (UAF) joined the project as a volunteer in 1991, later conducted research as a postdoctoral associate, and became a PI in the later phase of our research. She also conducted a companion experiment on effects of oil on captive river otters at the Alaska Sealife Center in Seward, Alaska, USA.

Findings from our earlier studies led to the listing of river otters as an injured resource by the *EVOS* Trustees Council in 1993; unfortunately, funding for our early research was cancelled in that same year. D. D. Roby (UAF) was largely responsible for reinitiating research in 1995, and helping to formulate ideas and write proposals that included river otters; A. D. McGuire (UAF) replaced him in 1997. G. M. Blundell (UAF) assumed responsibility for field investigations of the river otter component of the NVP project in 1996, as part of her Ph.D. research. T. A. Dean and S. C. Jewett joined the NVP project as PIs for the subtidal fish component in 1995. In the later phase of our research, we are indebted to the people of Chenega Village and the Chenega Native Corporation for permission to conduct research on their land. Radiotelemetry transmitters (implants and trap transmitters) were supplied by ADF&G, and ADF&G biologists H. Golden and D. Rosenburg provided logistical support and assistance in the field. We thank R. Colona, P. Sumner, L. Sevin, and N. Kinler for consultations regarding trapping techniques for river otters. We are indebted to students from UAF and elsewhere for assistance in the field in the later phase of our research, including L. Faro, P. Berry, S. Andersson, and C. Durham. We also thank P. Groves, E. A. Rexstad, O. A. Ormseth, J. B. Faro, H. Kruuk, J. Balke, and C. Taylor for assistance in the field, and M. Hoberg and D. Jung for assistance with collection and analysis of data on fishes. H. Kruuk provided thoughtful discussions that markedly improved our study. J. G. Kie assisted in reviewing the manuscript. We are indebted to R. Stowell and M. Stowell for logistical support in the field, and to numerous employees and volunteers with Migratory Bird Management of the U.S. Fish and Wildlife Service, and ADF&G for assistance in establishing field camps. We thank J. Decreeft (Northwind Aviation), our radiotelemetry pilot, J. A. K. Maier for GIS analyses, and E. A. Rexstad and E. Debevec for statistical advice. M. K. Hecker analyzed oil on the pelage of otters, and A. Blajeski and C. Taylor conducted research on fecal porphyrins. All analyses of endothelial *P*450-1A were performed by B. Woodin in the laboratory of J. J. Stegeman at Woods Hole Oceanographic Institute. We also thank N. Haubenstock and C. Restrepo for analyses of stable isotopes, and P. Groves, and D. Ziel for analyses of DNA microsatellites. We thank the crew of the *M/V Babkin* for providing a logistical platform as well as enthusiastic assistance with our fieldwork in 1998. S. Jewett, J. Philips and M. Hoberg provided training for research diving and cold-water diving certification. We thank L. Holland-Bartels, the chief scientist of the NVP project, and the other PIs on that project, including B. Ballachey, M. A. Bishop, J. Bodkin, D. Esler, L. McDonald, C. O'Clair, A. Rebar, S. Snyder, and G. VanBlaricom for their help.

EVOS Trustees Council, ADF&G, and the Institute of Arctic Biology and the Alaska Cooperative Fish and Wildlife Research Unit at UAF provided funding. All methods used in this research were approved by the Institutional Animal Care and Use Committee at UAF; trapping permits were issued by ADF&G and all procedures adhere to guidelines for animal care and use adopted by the American Society of Mammalogists (Animal Care and Use Committee 1998).

STUDY AREA

Prince William Sound is located in southcentral Alaska (Fig. 2). The area possesses a cool maritime climate and receives approximately 2,200 mm of annual precipitation and accumulates >1,000 mm of snowpack; snow often persists along shorelines until late April or early May. Dense, old-growth forest characterized by western hemlock (*Tsuga heterophylla*) and Sitka spruce (*Picea sitchensis*) with a well-developed understory of *Vaccinium, Menziesia*, and *Rubus* is typical at lower elevations. Muskegs occasionally are interspersed with old-growth forest. Alder (*Alnus* sp.) occurs on disturbed sites near the boundary of terrestrial vegetation and the intertidal zone. Alpine tundra occurs at elevations >300 m.

In both phases of our study (early phase, 1989-92; late phase, 1996-99) we used 2 approaches: intensive studies in 1 oiled and 1 "nonoiled" area, and Sound-wide sampling of multiple oiled and "nonoiled" sites. Intensive studies conducted in 1 location for each treatment (e.g., oiled and "nonoiled") allowed us to collect more types of data over a long period. That approach, however, created the potential for bias relative to site-specific phenomena. To overcome that problem, we employed Sound-wide surveys that provided replicate data from several sites for each treatment.

Our intensive sites of study from 1989 to 1991 were Herring Bay and surrounding areas (90 km of shoreline) on northern Knight Island (60° 30'N, 147° 40'W) as the oiled area, and Esther Passage (82 km of shoreline) between the mainland and Esther Island (60° 53'N, 147° 55'W) as our "nonoiled" site. Study areas were composed of continuous coastline and the circumference of all islands adjacent to that shore. The distance between those 2 areas is approximately 60 km. In 1991-92, Sound-wide sampling included the following oiled areas: 1) Herring Bay and Bay of Isles (60° 23'N, 147° 40'W) on Knight Island, 2) Eleanor Island (60° 32'N, 147° 37'W), 3) Naked Island (60° 40'N, 147° 25'W); and "nonoiled" areas: 1) Esther Passage, 2) Eaglek Bay (60° 52'N, 147° 45'W), 3) Unakwik Inlet (60° 55'N, 147° 30'W), and 4) Olsen Bay and surrounding areas (60° 44' N, 146° 10'W).

Because of the need to coordinate our research with other components of the NVP study, we selected a new reference site in 1996, while the oiled site remained the same throughout. We did not observe obvious signs of oil contamination in areas we considered our reference sites. Nonetheless, a LANDSAT Thematic Mapper (TM) image taken by the Geophysical Institute at UAF about 2 weeks after *EVOS*, but published years later (Stringer et al. 1992), clearly showed light oiling along the shorelines of our original reference area (Esther Passage). Consequently, we selected a new reference site for the later phase of our research that, based on field observations and LANDSAT imagery, was similar in degree of oiling to our original reference site. In retrospect, we believe our data are best interpreted as differences between heavily oiled areas and lightly oiled sites. For simplicity, however, we refer to those areas as oiled and "nonoiled" throughout the monograph.

Our intensive sites in the late phase of the study (1996-99) were (oiled) Herring Bay and surrounding areas (45 km of shoreline) and ("nonoiled") Jackpot, Ewan, Paddy bays (55 km of shoreline) along Dangerous Passage (60° 20'N, 148° 10'W) hereafter collectively referred to as Jackpot Bay. Study sites in 1996-97 were located approximately 30 km apart. In 1998, Sound-wide sampling included the following oiled areas: 1) Herring Bay, 2) Eleanor Island, 3) Naked Island; and "nonoiled" areas: 1) Esther Passage, 2) Wells Bay (60° 55'N, 147° 20'W), and 3) Unakwik Inlet.

METHODS

Because most data we collected in the early phase of our research (1989-92) already have been published with detailed description of methods, we refer to those initial publications for methods not included herein. Here we only provide a short account of those methods to assist the reader in interpreting the associated results.

Live-capture of River Otters

In the early phase of our research from 1989 to 1992, river otters were captured with Hancock traps (Northcott and Slade 1976). All traps were placed in blind sets (i.e., no bait or lure) on trails at latrine sites and monitored by means of trap transmitters (Telonics®, Mesa, Ariz., USA) that signaled when a trap had sprung (Duffy et al. 1993). River otters were anesthetized in the trap with a hand injection of ketamine hydrochloride (22 mg/kg). Otters then were transported to a 15-m boat that served as a logistical center.

River otters were captured (Blundell et al. 1999) from May through July 1996-97, and from mid-April through May 1998, with No. 11 Sleepy Creek® double-jaw leg-hold traps (Sterling Fur and Tool Co., Sterling, Ohio, USA). Those traps have a center-mounted swivel located under the trap pan instead of in the customary location on 1 of the springs. We inserted additional swivels into the chain approximately every 35 cm and at the anchor point for the trap; chain length varied according to the topography of the site. Those precautions allowed the trap to swivel on the chain as the captured otter rolled, thereby avoiding serious injury to the animal (Blundell et al. 1999). Vegetation that could become tangled in the chain and prevent it from swiveling was removed from within the range of the trap. Blundell et al. (1999) provide further information concerning trapping techniques. In addition to leg-hold traps, we also used Hancock traps to capture otters in 1996 (Duffy et al. 1993, Blundell et al. 1999). As with our early capture efforts, all traps were placed in blind sets on trails at latrine sites and monitored by means of trap transmitters. River otters captured in leg hold traps were anesthetized with Telazol® (9 mg/kg; A. H. Robins, Richmond, Virginia, USA) administered by Telinject® (Saugus, California, USA) darts with a blowgun. Otters were processed at the capture site during the later phase of our studies, and were placed in holding boxes to recover from anesthesia. Once sufficiently recovered, river otters were able to release themselves (usually within 1-2 hr) by investigating the recovery box and pushing open a hinged door. We used leg-hold rather than Hancock traps later in our project because they were smaller and easier to transport and set, and because leg-hold traps caused less serious injury to the teeth of river otters than did Hancock traps (Blundell et al. 1999).

In the later phase of our research, we inserted a passive integrated transponder (PIT) tag under the skin between the scapulae of each new otter captured to provide a method of permanent identification. All otters captured since 1996 were electronically scanned for PIT tags to determine whether they were recaptures.

Legal harvest of river otters was closed by the Alaska Department of Fish and Game on our study areas during both early and late phases of the research. That closure prevented sport harvest of otters but not subsistence-related use. In the early phase, there was no harvest of otters in our study areas. In the later phase, however, subsistence harvest occurred in the western Sound although no otters were taken from our study sites. As a precaution to prevent the loss of our study animals, and with the cooperation of the native corporations of Prince William Sound, in particular Chenega Native Corporation, we obtained a federal subsistence closure for harvest of river otters in western Prince William Sound in 1997-98. That federal closure was the first granted for research purposes.

Surgical Procedures

Throughout our research, after morphometric measurements and biosamples were collected, a subset of captured otters that were deemed to be in good health were implanted with radiotelemetry transmitters (Telonics®; Rock et al. 1994, Testa et al. 1994, Bowyer et al. 1995, Blundell et al. 2000). We determined health status based on pelage quality, body mass, body temperature, and the absence of severe injury or signs of illness (e.g., respiratory distress, diarrhea, or mucosal discharges). No adverse effects on reproduction have been reported for river otters implanted with intraperitoneal transmitters (Reid et al. 1986). In 1990-91, otters were implanted with transmitters in both Herring Bay and Esther Passage. In 1996, only otters captured in Jackpot Bay were implanted, whereas in 1997 otters in both Herring and Jackpot bays were implanted with transmitters. In 1998, we implanted otters captured in Herring Bay, but did not implant otters in Jackpot Bay during that year (Appendix A).

In the later phase of our research, the abdomen of each otter was palpated for potential nonfunctional transmitters that may have been implanted previously (either from 1990-92 studies in Herring Bay, or a failed transmitter from more recent studies in both areas). Only 1 previously telemetered individual was captured; that otter was released without removing or re-implanting a transmitter.

Shortly before surgery, we examined the otter to ascertain depth of anesthesia and proper analgesia. If a second dosage of anesthetic was required in 1990-91, Telazol® (11 mg/kg) was administered. In 1996-98, additional anesthesia was achieved with a combination of ketamine hydrochloride (100 mg/ml, Ketaset®, Aveco Co., Fort Dodge, Iowa, USA) at a dosage of 10 mg/kg, and midazolam hydrochloride (5 mg/ml, Versed®, Hoffman-LaRoche, Nutley, New Jersey, USA) at a dosage of 0.25 mg/kg (Spelman et al. 1993) mixed in the same syringe and administered intramuscularly. All surgeries employed sterile techniques. The site was shaved and surgically scrubbed with Nolvasan soap, alcohol, and a final iodine preparation was applied. An incision was made on the right side, posterior to the last rib to introduce a hermetically sealed radiotransmitter (IMP/400/L — Telonics®) into the peritoneal cavity. The transmitter signaled a mortality mode if the otter remained motionless for 9 hrs. Each muscle layer was closed separately with absorbable suture material (00 Vicryl® ProVet, Seattle, Wa., USA) with simple

interrupted sutures. The skin was closed with simple interrupted sutures in the early studies, but with a continuous subcuticular suture line in the later years to prevent the otters from accessing the sutures. As a final precaution, the skin incision was sealed with surgical glue. In 1990-91, otters were allowed to recover from anesthesia on the boat and released 5-13 hr after surgery near their site of capture, whereas in 1996-98 individuals were allowed to recover in a box at the capture site and released themselves once they recovered sufficiently (1-2 hr after surgery).

Morphometrics

Field Methods. – Data on morphometrics, collected from anesthetized river otters, included body mass (nearest 0.1 kg); body length, tail length, and total length (nearest 1 mm); total skull length and width of zygomatic arch (nearest 1 mm); length and diameter of canine tooth, distance between canines (nearest 0.01 mm); length from hock to toe, and interdigital spread of the right hind foot (nearest 0. 1 mm). Sex was distinguished by the relative position of urogenital openings and palpation of bacula (Stephenson 1977, Chilelli et al. 1996). Females were examined for evidence of estrus and lactation. For males, bacula lengths and testicle widths were measured to the nearest 0.1 mm. Age of otters (pup, young adult, adult, and old adult) was estimated based on body size, tooth wear and staining, and in 1997 the first upper premolar was extracted for age determination with cementum annuli (Matson Laboratory, Milltown, Montana, USA).

Data Analysis. – We tested for differences in body mass between oiled and "nonoiled" areas. We performed analysis of covariance (ANCOVA) to control for effects of age and sex in river otters with body mass as the dependent variable, area as the factor, and age, sex, and total length as covariates (Zar 1999). We tested changes in adjusted mean body mass of otters living in oiled areas through time with the Spearman rank correlation (Conover 1980).

Biosampling in the Field

Rationale. – We judged that killing a sufficient number of river otters to test for differences in concentrations of hydrocarbons in tissues, and any pathological expression of that exposure, between otters from oiled and "nonoiled" areas might extirpate subpopulations or cause genetic bottlenecks; otters occurred at low densities even in "nonoiled" areas (Testa et al. 1994). Thus, we recognized the need to develop a suite of biomarkers to assess exposure to and damage from hydrocarbons in living vertebrates (Duffy et al. 1996). In early studies, our analyses included a suite of blood-serum parameters as well as haptoglobin (Hp) and interleukin-6 immunoreactive (IL-6 *ir*). Hp and IL-6 *ir* indicate increased liver activity from synthesizing acute phase proteins in response to trauma, toxicological damage, or infection (Duffy et al. 1993, 1994*a*, 1994*b*). Additionally, we assayed porphyrins extracted from fecal samples (Blajeski et al. 1996), which are tetrapyrrolic pigments involved in biosynthesis of the heme molecule. Chemically induced changes in patterns of porphyrins have been observed in several avian species following exposure to aromatic hydrocarbons (Miranda et al. 1987). Our analyses on river otters were the start of a broader biomarker program that became central to the NVP project

beginning in 1996. We employed the previous suite of blood-serum parameters (Appendix B), a complete blood count (CBC; Appendix C), and 2 additional tests to document exposure to hydrocarbons, using newly developed methodologies. We tested for the presence of hydrocarbons on the pelage of river otters with a gas chromatograph-mass spectrometer (GC-MS), and included the biomarker *P*450-1A in our analyses. Cytochrome *P*450s are a group of enzymes that metabolize a wide variety of xenobiotic compounds. *P*450-1A is induced by planar aromatic or chlorinated hydrocarbons, and thus its presence serves as a nonspecific bioindicator of hydrocarbon exposure (Woodin et al. 1997; Ben-David et al. 2001*c*).

Documenting Exposure to Oil. – Extensive searches of oil-contaminated shorelines were conducted in Prince William Sound immediately following *EVOS*. Carcasses recovered from 1989-90, which were suitable for toxicological analysis, were submitted to an *EVOS*-approved laboratory for evaluation of metabolites of hydrocarbons in different tissues and bile.

To establish the presence of hydrocarbons on pelage of river otters in the latter phase (1996-97), swabs were collected from fur with a 5 × 5-cm gauze swab saturated with isopropanol for GC-MS assay. Areas of pelage we suspected to be contaminated with petroleum were swabbed for 15 sec, as well as the ventral aspect of the neck, the abdomen, a swath along each side, and 1 over the length of the back. Swabs were handled with gloved hands only. Once pelage had been sampled, the swab was completely enclosed in aluminum foil and frozen (-8° C) in a portable propane freezer for later analysis in the laboratory at UAF.

Collection of Blood. – We drew blood from the jugular vein of each otter with care taken to keep samples sterile. A portion of the sample was preserved with EDTA (purple top Vacutainer®) for complete blood counts (CBC). The remaining blood (approximately 10 ml) was collected in a red top Vacutainer® and allowed to clot; serum was removed (within 8 hr) following centrifugation at 3,000 rpm for 10 min, and frozen (-8° C) for subsequent analyses of serum chemistry. Three blood smears were made for each river otter at the time the blood was drawn. In the early phase of our studies (1989-92), blood samples collected for CBC analyses were unusable because logistics resulted in long delays between blood collection and processing in the laboratory. Therefore, from 1996 to 1998, we transported blood samples via airplane to the nearest laboratory facility (Quest Laboratories, Anchorage, Alas., USA) for analyses.

Timing of Sampling. – Because many of the biomarkers we evaluated are nonspecific responses to stress or injury, we attempted to control for the influence of sexual activity on induction of biomarkers. Most captures of otters occurred during the height of the mating season (May), along with some captures before and after that period. To control for potential bias relative to differences in capture chronology, we reversed the sampling order of our study sites between years. In 1990 and 1992, otters were captured in the “nonoiled” area during the mating season, whereas in 1991 otters were captured in the oiled area in May. We captured otters in 1996 in the oiled area during the peak of the mating season, and in 1997 we captured otters in the “nonoiled” area in May. In 1998, all otters were captured within a 6-week period immediately prior to and during mating season, alternating oiled and “nonoiled” sites for $\leq$5 calendar nights of trapping in each area. Thus, all otters had a similar biological status with respect to mating season and area (oiled or “nonoiled”) in 1998.

Skin Biopsy and Collection of Fur. – From 1996 to 1998, a 3-mm disposable skin-biopsy punch was used to obtain a tissue sample from each river otter for analysis of endothelial *P*450-1A. Prior to collecting the sample, we clipped hair on the medial surface of the triceps on the left front limb, and a surgical scrub was performed. The tissue specimen was preserved in 10% neutral-buffered formalin immediately after collection. Fur samples (under fur and guard hair) were collected in 1996-98 for diet analysis with stable isotope ratios (Ben-David et al. 1998*b*).

Collection of Feces. – Feces of river otters, deposited during winter prior to the oil spill were collected immediately post-spill (1989), providing rare data on diets of river otters in Prince William Sound prior to *EVOS*. We collected additional feces for analyses of otter diets in summer 1989-90 (Bowyer et al. 1994). For those analyses, the latrine site was considered the sampling unit and all feces collected on a sampling occasion were stored in 1 bag. Feces collected in 1990 and 1996 and used for porphyrin analyses (Blajeski et al. 1996, Taylor et al. 2000*a*) were stored individually. All feces were frozen (-70° C) until analysis.

Laboratory Procedures for Biomarker Assays

GC-MS Assays. – Swab samples collected to detect the presence of hydrocarbons on pelage of river otters were extracted into isopropanol, and that extract was analyzed by gas chromatography with mass spectrometry detection (GC-MS). Mass-spectral data were acquired with selected ions for each of the hydrocarbons (phenanthrene, chrysene, petocosane, and hexacosane). The GC-MS was calibrated by injection of a standard at 6 concentrations reaching 5 mg/g (ppm). Sample concentrations for each hydrocarbon were calculated from the area under the curve generated by the mass spectrometer (Duffy et al. 1999*b*).

Profiles of Whole Blood and Serum Chemistry. – Serum-chemistry profiles (Appendix B) were assayed with an Olympus 7000 analyzer (Olympus, Melville, New York, USA) and complete blood counts (Appendix C) were performed with a Stack-S whole-blood analyzer (Coulter, Miami, Florida, USA). Samples were analyzed at Quest Laboratories (Anchorage, Alaska, USA).

Haptoglobin. – Haptoglobins (Hp) are serum-plasma proteins that bind free hemoglobin (Hb). In the laboratory at UAF, a standard Hp assay, which used electrophoresis to separate the Hp-Hb complex from free Hb, was used to quantify the complex with densitometry (Duffy et al. 1994*a, b*). Results were expressed as mg Hb-bound/100 ml serum.

Interleukin 6 immunoreactive. – Levels of IL-6 *ir* were determined at UAF with an immunochemical assay. Replicates of each sample were added to a microtiter plate coated with a monoclonal antibody for IL-6. After washing away any unbound proteins, an enzyme-linked polyclonal antibody for IL-6 was added to the wells and incubated to allow for binding. After a final wash, a substrate solution was added to the wells. Following color development, sample concentrations were determined from a standard curve (Duffy et al. 1994*a, b*).

Cytochrome P450-1A. – The induction of cytochrome *P*450-1A (CYP1A) in endothelial tissues of river otters was evaluated by immunohistochemistry. Tissue samples were analyzed at the Woods Hole Oceanographic Institute (Woods Hole, Massachusetts, USA), in the laboratory of J. J. Stegeman. To assay *P*450-1A activity, tissue samples were prepared, embedded, sectioned, stained, and scored for staining intensity by the same technician using procedures described in Woodin et al. (1997) and Ben-David et al. (2001*c*). The intensity score was multiplied by the occurrence of staining and reported as a staining index. A higher number for the index indicates a greater response in the individual to exposure to petroleum hydrocarbons (Ben-David et al. 2001*c*).

Fecal Porphyrins. – The protocol used for extraction of fecal porphyrins was modified from Lockwood et al. (1985) for the earlier studies, and from Bowers et al. (1992) for the later period. Fecal extractions were measured with a Perkins-Elmer diode-array spectrophotometer. The relative concentration of total porphyrins was measured against a standard porphyrin kit (Porphyrin Products, Logan, Utah, USA). The concentration of total porphyrins in each sample was calculated from the equation:

$$\text{Total Porphyrins (nmole/g dry feces)} = TD \times (6/stdTD) \times 20 \text{ ml}/(DW \times VU),$$

where: TD = trough depth of sample, measured from baselines; 6/stdTD = trough depth of standard kit (6 nmole); DW = dry weight of sample initially used for extraction; VU = volume of sample used for diode-array analysis.

In addition, we used high-performance liquid chromatography (HPLC) to determine porphyrin profiles. The gradient-solvent system for the HPLC was modified from Lim and Peters (1984) in the earlier period of study, and from Kennedy and James (1993) in the later study. Concentration of porphyrins in each fecal sample was calculated from a calibration curve (Taylor et al. 2000*b*, 2001).

Data Analyses. – To establish differences in levels of hydrocarbons extracted from otter pelage (GC-MS), we used a *t*-test for unequal variances (Zar 1999) to examine differences between areas. To determine differences in levels of biomarkers between oiled and "nonoiled" areas we used several different tests such as multiple response permutation procedures (MRPP; Slauson et al. 1991), Mann-Whitney tests, as well as analysis of variance (ANOVA) when appropriate (Zar 1999). We report the test used for each analysis in results. For data collected in 1991, we used logistic regression (Agresti 1990), with 26 blood values, sex, length, body mass, and age class of otters as potential dependent variables, and area as the factor, with oiled coded 0 and "nonoiled" coded 1. We repeated that analysis for data collected in 1996-98, with the model derived from the earlier analysis.

We expanded our analyses for data collected in 1996-98 to further explore the status of recovery. We first reduced the dimensionality of the data set (i.e., 28 blood characteristics) with principal components analysis (PCA). PCA is widely used to investigate complex data sets by accounting for relations between variables and condensing the information portrayed by multiple variables into single components (McGarigal et al. 2000). PCA has been used effectively in many wildlife studies and provided insights that were not possible from other multivariate and

univariate examinations of data. This methodology is used most often in studies of morphology (Bowyer et al. 2001), but Nudds (1983) delineated the niche relationships among guilds of waterfowl (Anatidae), and Kie and Bowyer (1999) evaluated the dietary niche of white-tailed deer (*Odocoileus virginianus*) using PCA.

We used the correlation rather than covariance matrix to correct for different scales of measurement among blood variables (Johnson and Wichern, 1982). We then compared 95% confidence ellipses for otters living in oiled versus "nonoiled" areas for the first 3 principal components (PCs), separated by year. By using this approach, we were able to control for the different sampling designs between years as well as to document changes through time between oiled and "nonoiled" areas. For each PC, we used those variables (blood parameters) with strong positive or negative loadings of eigen vectors to interpret the physiological significance of that PC; variables with loadings near zero contributed little to separation on a particular axis (Johnson and Wichern 1982). We followed PCA with multivariate analysis of variance (MANOVA) on untransformed data for those variables with influential loadings as dependent variables, and area and year as main effects. If MANOVA is performed on the loadings, PCA must be developed from the variance-covariance matrix, which yields biased results when original data vary markedly in scale (a common occurrence in blood values). Therefore we used a correlation matrix for PCA followed by MANOVA on original data.

If oil persisted in the environment and otters were chronically exposed, we predicted a negative relation between haptoglobin or *P*450-1A and adjusted body mass of otters (from ANCOVA). Therefore, we investigated those relations using linear regression (Zar 1999) of our data collected from 1996 to 1998.

In the initial porphyrin analyses (Blajeski et al. 1996), we used the 2-sample *t*-test to compare total porphyrins in feces of river otter (Zar 1999). A 2-sample z-test for proportions (Remington and Schork 1970) was used to compare selected porphyrins detected by HPLC. In later analyses (Taylor et al. 2000*a*), we used 2-way ANOVA on ranked data, with year and area as main effects, and coproporphyrin III as the dependent variable.

Diet Analyses

Prey Remains in Feces. – Fecal samples containing primarily skeletal remains of fish and invertebrates, fish otoliths and scales, avian feathers, and mammalian hair were collected from latrine sites in our study areas in 1989-90. Samples were washed to remove soft material, air dried, and analyzed to identify prey remains. Those remains were compared with reference specimens and identified, when possible, to the species level. Keys to otoliths, scales, feathers, and mammal hair also were used for identification (Bowyer et al. 1983, 1994).

Stable Isotope Analysis. – We used stable isotope ratios to index diets of otters (Ben David et al. l997*a*, 1997*b*, 1998*b*). The specific combination of values of $\delta^{13}C$ and $\delta^{15}N$ result from the dietary interaction of species or individuals (Hobson 1999). Applying this technique to tissues such as blood and hair allows repeated sampling of known individuals throughout the year (Ben-David et al. l997*a*, 1997*b*) and evaluation of changes in diet of the same individuals under differing circumstances. Stable isotope ratios of intertidal, pelagic, and freshwater fishes differ from each other (Ben-David et al. 1998b; Blundell et al. 2002). Similarly, stable isotope

ratios of marine invertebrates differ from those of intertidal fishes (Ben-David et al. 1997*b*, 1998*b*). Although this technique may not identify individual species of prey, a difference in isotopic signatures in otter hair would reflect consumption of different foods (i.e., intertidal, pelagic, and freshwater fishes, and invertebrates - Ben-David et al., 1998*b*, Ben-David and Schell 2001).

Hair samples collected from river otters in 1996-98 were dried at 60-70° C for 48 hr. Subsequently, a subsample (1-1.5 mg) was weighed in a miniature tin cup (4 × 6 mm) for later combustion. We used a Europa C/N continuous flow mass-spectrometer (at UAF) to obtain the stable isotope ratios. Each sample was analyzed in duplicate and results were accepted only if the variance between the duplicates did not exceed that of the peptone standard ($\delta^{13}C_{std}$ = -15.8, $\delta^{15}N_{std}$ = 7.0, CV = 0.1; Ben-David et al. 1997*a*, *b*).

Data Analysis. – For analyses of prey remains in feces, we calculated species richness and diversity using the Shannon-Wiener index (Ricklefs 1973) for oiled and "nonoiled" areas. We used MANOVA, weighted by number of feces collected at each latrine site to evaluate effects of area and year on abundance of species in diets of river otters (Bowyer et al. 1994). We employed the McNemar test for significance of change to evaluate temporal differences in species richness in otter diets (Bowyer et al. 1994).

To establish differences in diets of otters in 1996-97, we first determined that data for stable isotopes were normally distributed. We then used MANOVA with $\delta^{13}C$ and $\delta^{15}N$ as dependent variables, and area, year, and sex as independent variables to test for differences in diet between Jackpot and Herring bays. We used ANOVA with $\delta^{13}C$ as the dependent variable, and year and area as factors to examine interannual variation in diet for data collected from 1996 to 1998. In that analysis, we used only $\delta^{13}C$ because that parameter more clearly distinguished between prey of river otters in Prince William Sound (Blundell et al. *in press*).

Prey Availability

Field Methods. – Demersal (nearshore) fishes were sampled at 30 latrine sites used by otters in both Herring and Jackpot bays in July 1996-97, as well as at 30 random sites at each location in both years by a team of certified research divers (American Academy of Underwater Sciences, University of Alaska Scientific Diving Program). The same scientist (S. C. Jewett) trained all personnel in fish identification prior to data collection, and data collection followed a strict protocol developed by NVP principal investigators.

Demersal fishes were counted by 2 SCUBA divers at each site along 2 transects oriented perpendicular to shore. Transects extended 30 m, or in instances where the tidal zone was steep, until a depth of 15 m was reached. The 2 transects at each site were separated by 20 m, and originated 10 m to either side of the center of a particular site. Fishes in the water column were enumerated over a 2 m-wide swath by the first diver. Demersal fishes were counted along a 1 m-wide swath on each transect by the second diver while gently moving aside algae and other vegetation. All fishes counted were classified into 3 size classes (<8 cm, 8-15 cm, >15 cm total length) and were assigned to 8 categories: the peciformes-ronquils (Bathymasteridae), pricklebacks (Stichaeidae), and gunnels (Pholididae); as well as families in other orders, including cod (Gadidae), rockfish (Scorpaenidae), sculpin (Cottidae), greenling

(Hexagrammidae), and other. Fishes <8 cm were not considered because otters seldom consume fishes that small (Kruuk 1995).

Data Analysis. – To determine differences in densities of total fishes (≥8 cm in length) between areas and years, we employed a 2-way fixed-effects ANOVA (Zar 1999) on log transformed data (e.g., log density + 0.1) with areas (oiled vs. "nonoiled") and site (latrine vs. random) as main effects. Gadidae, which constitute <11% of the diet of river otters (Bowyer et al. 1994), were excluded from analyses because their behavior resulted in overestimation of their numbers; those fish tended to follow divers. Fish densities recorded at latrine and random sites were compared for Jackpot and Herring bays in 1996-97. Sample sizes were too small to permit additional analysis with families of fishes as dependent variables.

Habitat Selection

Field Methods. – Habitat selection by river otters and other mustelids can be determined by comparing habitat features at latrines and random sites, including terrestrial and marine features (Dubuc et al. 1990, Bowyer et al., 1995, Ben-David et al. 1996, Swimley and Serfass, 1998, Macdonald and Strachan 1999). Active latrine sites of river otter were identified by the presence of fresh feces and well-established trails that were free from recent leaf litter. Each active site was characterized with respect to topography and composition of terrestrial and intertidal substrates to represent both aquatic habitats used for foraging, and terrestrial sites used for denning and social interactions (Bowyer et al. 1995, Ben-David et al. 1998*b*). Vegetation and intertidal substrates were assessed for a 10-m arc with its pivotal point at the mean high-tide line. We estimated relative cover of vegetation visually, with a Likert scale that ranged from 0 to 4 (Bowyer et al. 1995). We used the same method to categorize intertidal substrates. We measured vegetated slopes and tidal slopes to the nearest 5°, from the mean high-tide line to a point 10 m distant, landward and seaward, respectively, with a hand-held compass. Aspect of the site was recorded in 8 compass directions, and exposure to wave action was ranked into 3 broad categories from protected to exposed.

In 1996-97, shorelines in Herring (45 km) and Jackpot (55 km) bays were surveyed in a similar fashion to that of the early phase. Emphasis was placed on identifying active sites (>10 fresh feces) in the oiled area that were not established by river otters in 1990-91. Only new latrine sites were evaluated in Herring Bay in an effort to avoid replicating our earlier surveys. Sites that were inactive or abandoned in the later phase of the study were not included in the analysis of habitat selection for that period.

Data Analyses. – We employed step-wise logistic regression (Agresti 1990) to develop models best separating otter latrine sites (coded 1) and random sites (coded 0) for each of our study areas (oiled and "nonoiled"). Additionally, we developed a similar model for the pooled data set. We controlled for multicollinearity by eliminating 1 of any pair of variables with an absolute value of $r = 0.35$. We ensured that data did not depart from a logistic-regression model with the Hosmer-Lemeshow goodness-of-fit test. Variables that entered models were tested with a use (latrine and random sites) by area (Herring Bay and Esther Passage) MANOVA to determine whether selection differed between areas (Bowyer et al. 1995). Those analyses were

conducted with data collected in both phases of our research. In the second phase, however, we collected a subset of variables: those that distinguished between random and latrine in the early analyses. Those variables were tested with MANOVA to determine if selection still differed between oiled and "nonoiled" areas. We also developed new logistic-regression models to evaluate habitat selection in the later phase of our research.

Radiotelemetry Tracking

Field Methods. – Radiotelemetry tracking was accomplished mostly from a boat traveling along shorelines in 1990-91, as well as in 1996, because little funding was available for aerial telemetry. To minimize a possible bias from otter activities, we randomized starting times for our surveys and collected data across the full 24-hr period during summer. In the early studies, triangulations relied solely upon compass bearings, whereas in later studies a Global Positioning System (GPS) aided in triangulation. All otter positions were plotted on USGS maps (1:63360 scale).

In the early studies, otters were radiotracked from the air once per month if individuals were not located on the study areas. All river otters were radiotracked in 1997-99 with aerial telemetry, although some supplemental radiotracking of otters in Herring Bay was conducted from a boat in 1997. Aerial tracking occurred about every 4 days from mid-April to mid-June; thereafter, tracking was conducted weekly until September. Tracking was attempted every 2-3 weeks during winter, depending upon weather. Once a telemetered otter was located from the air, GPS data were obtained for each otter by flying the plane directly over the location and recording UTM coordinates. Additionally, point locations for each otter were plotted on USGS maps (1:63360 scale). Accuracy of telemetry locations (± 30 m) was confirmed in both phases of the research by relocating transmitters placed at sites unknown to those operating receivers (Testa et al. 1994, Bowyer et al. 1995, Blundell et al. 2000).

Data Analyses. – We compared home-range size for otters inhabiting oiled and "nonoiled" areas as a method of assessing damage or recovery. In the early studies, we established the total length of shoreline used by otters with a method developed by Bowyer et al. (1995). In that conservative method (Sauer et al. 1999), locations of otters were considered extreme values, and eliminated from the calculation of shoreline length, if an otter was located >1 km from the nearest location of that same otter. In our later studies, we calculated home-range size using a nonparametric kernel-density estimator, which defined a utilization distribution by assessing the probability that an animal would occur at particular point in space (Worton 1989, Seaman and Powell 1996). We used a fixed-kernel model (Ranges V software; Kenward and Hoddard 1996) and measured kilometers of shoreline within fixed-kernel estimates using ARC INFO (ESRI, Redlands, California, USA; Blundell et al. 2001). We excluded the few otters that moved between oiled and "nonoiled" areas from our analyses during 1996-99. We determined an adequate sample size for an individual by obtaining an asymptotic relation between home-range size and cumulative number of locations ($\bar{x}$ = 25 locations/individual – Bowyer et al. 1995; $\bar{x}$ = 19.5 locations/individual Blundell et al. 2001). In 1990, we relocated otters an average of 25 times per individual; in 1997-98 that value was 28 locations. We used 2-way ANOVA with area and gender as factors to test for differences in size of home ranges for otters inhabiting oiled

and “nonoiled” areas from 1996 to 1999. We entered otter ID as a factor to control for multiple years of telemetry data for some individuals. As a further measure of recovery, we compared home-range data collected in 1990-92 for otters in Herring Bay with more recent (1997-99) data for otters in that same area. For continuity, we used that same methodology for home-range calculations (Bowyer et al. 1995) to compare between phases of study.

Age Structure

The status of a population can be evaluated by quantifying recruitment (i.e., number of young successfully added to the population – McCullough 1979). Because assessing reproduction in river otters is difficult, we used age structure as an indirect method of investigating differences in recruitment between oiled and “nonoiled” areas. To determine whether that indirect measure of recruitment would be indicative of stationary or increasing populations, we also compared our data with a well-established population of river otters in Maine, USA (Docktor et al. 1987, Maine Dept. of Inland Fisheries and Wildlife Files, Orono, Maine, USA). We used a Mann-Whitney test (Zar 1999) to establish differences in age structure in populations of otters in Jackpot and Herring bays as well as between populations in Prince William Sound, Alaska, and those in Maine.

Estimating Population Numbers

Radiotracers. – In 1990, we estimated population size of river otters in oiled and “nonoiled” areas using a mark-recapture approach with radiolabeled tracers (Testa et al. 1994). Five unique tracers were used; unique combinations of several radiotracers were encased into a mold of silicone resin and implanted into the peritoneal cavity of individual otters. The implantation was considered the marking event. Samples of feces subsequently were collected from latrines and stored individually in whirlpaks and frozen for later analysis. Samples were analyzed for presence and absence of radiotracers (Testa et al. 1994 provide details) and used as recapture events. Data were analyzed with a Bayesian model to derive population estimates (Testa et al. 1994).

Microsatellite DNA. – To enumerate otter populations in Herring and Jackpot bays (Fig. 2), we used a minimum-number alive approach (Slade and Blaire 2000), based on DNA microsatellites extracted from blood and feces of river otters. Those estimates were derived from otters trapped and feces collected at latrine sites along continuous coastlines of our study areas. Consequently, our definition of populations was constrained by that sampling protocol, and does not differentiate between resident and transient animals. Nonetheless, we assume that movements of animals in and out of our designated study areas did not differ between sites. We recognized, however, that using minimum-number alive would underestimate the population (Nichols 1986), but would provide a reliable index to population density (Slade and Blair 2000).

Microsatellites are hypervariable regions of short repeats within DNA. Because microsatellites are within noncoding regions, there is little selective pressure and those regions can change rapidly in size (Tautz 1989). With polymerase chain reaction (PCR) and specific microsatellite primers, those regions can be amplified and their sizes compared between

individuals. When the appropriate suite of microsatellite markers is surveyed, a unique pattern, or fingerprint, identifying each individual arises (Crawford et al. 1991, Craighead et al. 1995). We identified individual otters from signatures of microsatellite DNA extracted from feces and blood and enumerated all unique patterns representing individuals in each area. In instances where the same DNA signature was identified in blood and feces, fecal samples were discarded.

We collected blood samples from each otter trapped in May-July 1997 in Herring and Jackpot bays. Concurrently, fresh samples of feces were collected from 42 latrine sites along the approximately 55 km of shoreline in each study area (every 4th latrine site). Similarly, DNA was extracted from blood samples of otters captured in 1996 and 1998 in those areas. Blood samples were kept frozen and fecal samples were either frozen or preserved in 100% ethanol. For live otters, DNA was extracted from white blood cells, whereas fecal DNA was extracted from intestinal cells shed within feces (Höss et al. 1992). Selected portions of DNA were amplified from small amounts of tissue with PCR (Mullis and Faloona 1987).

A library of 11 specific primers was developed for individual identification of river otters (Dallas and Piertney 1998, Fleming et al. 1999). Of those 11 primers, 9 provided complete separation of all 110 individual river otters captured in Prince William Sound from 1996 to 1998 (Blundell et al. *in review* a, *in press* b). DNA was not successfully available for only 1 of 111 otters for which we had blood samples. A complete DNA microsatellite profile was developed for each individual and then compared with similar profiles developed for each fecal sample.

Population Reconstruction and Projections. – Populations of river otters inhabiting Herring and Jackpot bays were reconstructed based on age structure of live-captured individuals in 1997; age was determined from cementum annuli of teeth extracted from otters. We used the conventional method of accumulating cohorts derived from capture to determine population size (Hesselton et al. 1965, Lowe 1969, McCullough 1979, Hilborn and Walters 1992, Bowyer et al. 1999, Lancia et al. 1994, Bender and Spencer 1999). This method assumes equal catchability, that survivorship and fecundity are fixed, and thereby that the population exhibits a stable-age distribution (Eberhardt 1985). Although we have no independent method to assess whether these assumptions were met, and data for other mustelids indicate changes in age distribution with resource availability (R. W. Flynn and M. Ben-David, unpublished data). Nevertheless, this technique provides a robust index to large changes in population size (Hesselton et al. 1965, Lowe 1969, Lancia et al. 1994), and can be used to compare populations inhabiting adjacent geographical areas. In addition, reconstructing populations within, rather than across years helps minimize biases associated with a stable age distribution (Bowyer et al. 1999). Moreover, such analysis provides an important independent evaluation of our conclusions regarding population status based on other methods with a weight-of-evidence approach.

To determine whether the population in Herring Bay (oiled area) declined or increased between the early and late phase of our study, we used standard methods (Caughley 1977) to calculate the intrinsic rate of increase (r) and the annual growth rate (λ) from 2 estimates. We used average population size in 1990 (Testa et al. 1994) and minimum-number alive in 1997 to calculate those parameters. Similarly, we calculated those values using data from population reconstruction based on age structure in 1997 and population estimates from 1990. To evaluate whether the rate of increase of otters in our study was at a maximum, we estimated r_{max} for river

otters by using the age structure of a population of 254 otters described by Tabor and Wright (1977), and data from Docktor et al. (1987) on corpora lutea for 114 female otters to determine age-specific fecundity. We assumed a 1:1 sex ratio (Toweill and Tabor 1982). Calculations were based on the life-table approach described by Caughley (1977). Similar comparisons for our reference sites (Esther Passage and Jackpot Bay) would be inappropriate because of potential differences in habitat quality between areas.

Survival

In 1997-99, we used radiotelemetry to determine numbers and timing of mortality for individual otters inhabiting oiled and "nonoiled" areas. We calculated Kaplan-Meier survival estimates using a staggered-entry model described by Pollock et al. (1989). We obtained survival estimates for Herring Bay (oiled) and Jackpot Bay ("nonoiled") and compared survivorship using the log-rank test (Pollock et al. 1989). This analysis excluded otters that traveled between study areas.

Synthesis

Data collected on river otters in both phases of the study included different variables, which may be independent from each other, and yet their combined effect provides for a weight of evidence in exploring injury and recovery. To evaluate this combined effect we used meta-analysis of combined probabilities (Sokal and Rohlf 1981). For data collected in the early phase, we used body mass, haptoglobin levels, diet, home-range size, selection of tidal slope as the variables of interest. For the later phase, we used body mass, haptoglobin levels, diet, home range size, selection of tidal slope, age structure, and survival. We selected those variables because they provided independent measures for the status of river otters in Prince William Sound: physiological damage (e.g., body mass, and haptoglobin levels), avoidance of oil (e.g., tidal slope), and behavioral responses to damage (e.g., diet, home range size). In the later phase, we also incorporated 2 variables that represented population responses (e. g., age structure and survival). We compared results from this meta-analysis with 1 performed by Taylor et al. (2000*a*) on physiological responses in the early phase of the study.

RESULTS

Exposure to Oil

Carcass Counts. – The small number of river otter carcasses (n = 12) located along shorelines of Prince William Sound immediately following the spill cast doubt upon how severely this mustelid might have been injured. We evaluated the utility of using carcasses of river otters as an index to mortality by examining the locations in which our radio-implanted otters perished. From 1990 to 1992, we located the carcasses of 8 otters equipped with radiotransmitters in Herring Bay and Esther Passage. Of those 8 individuals, only 2 animals died in locations where their carcasses might have been detected during beach surveys (i.e., $\leq$10 m from the shore). The remainder of mortalities was well away from the shore, or inside dens or

other cavities along the beach. Even those 2 animals that did not die in cavities likely would not have been located by walking along the shore, because 1 was in an area with steep cliffs where searching on foot would have been impractical, and the other was concealed by dense vegetation.

From 1996 to 1999, we located the remains of 19 additional telemetered otters, including 10 released from the Alaska Sealife Center in 1999 (Ben-David et al. 2002). Of those animals, only 2 perished near the shoreline where those searching beaches might have detected them. Again, most otters died far away from beaches, below ground, or in deep rock crevices. The fate of those river otters would have been unknown without the aid of radiotelemetry. Indeed, during our 7 years of research, including intensive surveys of shorelines for other purposes, we encountered only 2 carcasses of unmarked river otters. Clearly, number of carcasses of river otters counted immediately following *EVOS* are a gross underestimate of the actual mortality; beach surveys were not a useful index to effects of that catastrophe on river otters.

Hydrocarbons in Tissues. – Of the 12 carcasses of river otters recovered from searches of oil-contaminated shorelines in Prince William Sound immediately following the spill, and 8 additional carcasses recovered from 1989 to 1990, only 2 specimens were suitable for complete toxicological analyses. Values (ppb) of phenanthrene, of 1,600 and 1,700, and naphthalene of 13,000 and 74,000 from bile indicated metabolites of hydrocarbons were present in those river otters. Analysis of lung tissue from 1 additional otter revealed polyaromatic hydrocarbons (PAH) values of 28,000; lower PAH values for liver (455), kidney (132), and brain (311) indicated that death in that animal likely occurred prior to elevation of PAHs in tissues other than lungs. Results indicated that river otters died from acute effects of oiling, but the magnitude of that loss was uncertain because of the inability to locate mortalities and appeared small in comparison with some marine mammals killed by the oil spill (Loughlin et al. 1996).

Hydrocarbons on Otter Fur. – To establish whether river otters continued to be exposed to oil 8 years after EVOS, we used GC-MS assays to detect the presence of hydrocarbons on pelage of otters (Duffy et al. 1999b). Analyses revealed that Pentacosane occurred significantly more often on the pelage of otters inhabiting oiled compared with “nonoiled” areas in 1997 (2 sample *t*-test with unequal variance, $P < 0.001$; Duffy et al. 1999*b*), but Phenanthrene and Hexacosane did not ($P > 0.05$; Duffy et al. 1999*b*).

Morphometrics and Biomarkers

Morphometrics. – Data on morphometrics, blood chemistry, and fecal porphyrins revealed patterns suggestive of initial physiological damage followed by subtle and potentially chronic effects from oiling. In 1990-91, river otters from oiled areas had significantly lower body mass (adjusted for length of body, sex, and age class) compared with otters inhabiting “nonoiled” areas ($P = 0.04$; Fig. 3). For example, male otters in oiled areas of Knight Island were, on average, 1.13 kg lighter than their counterparts living in “nonoiled” areas along Esther Passage from 1989 to 1990. Data from otters captured in 1992 indicated that differences in body mass between otters inhabiting oiled and “nonoiled” areas were no longer substantial (Fig. 3). Those results, however, should be interpreted with caution due to small sample sizes. Subsequent data collected from otters in 1996-98 exhibited similar convergence of body mass between oiled and

"nonoiled" areas (Fig. 3). River otters in both areas gained weight in 1998, and no significant differences occurred between oiled and "nonoiled" areas ($P = 0.71$). Change in mass of otters inhabiting oiled areas consistently increased (>1 kg) with time ($r_s = 1.0$, $P < 0.05$), indicating likely recovery on oiled areas.

Blood Panels. – Initial analyses of blood parameters from 1990 to 1991 revealed that mean values for haptoglobin (Hp) were elevated (MRPP, $P = 0.04$) in 8 otters from oiled areas of Knight Island (361 mg Hb-bound/100 ml) compared with 6 otters inhabiting the "nonoiled" Esther Passage (306 mg Hb-bound/100 ml; Fig. 4A). In samples collected in 1992, Hp values did not differ significantly between otters from oiled and "nonoiled" areas (Fig. 4A), but sample sizes were small (Appendix A). In 1991, IL-6 *ir* was elevated in otters from oiled sites compared with "nonoiled" sites (Fig. 4B), but in 1992, IL-6 *ir* values were below detectable levels (Duffy et al. 1994*b*). In 1996-98, a more sensitive assay was used permitting the detection of lower levels of IL-6 *ir* than were possible in the early years; nonetheless, no difference occurred in levels of IL-6 *ir* between oiled and "nonoiled" areas in our later studies (Fig. 4B).

Stepwise logistic regression for data collected in 1991, with 26 blood values (Table 2; Duffy et al. 1994*a*), sex, length, body mass, and age class of otters as potential independent variables, selected only 3 parameters. Aspartate aminotransferase (AST), Hp, and IL-6 *ir* correctly classified 86.4% of 22 river otters as coming from oiled (coded 0) and "nonoiled" (coded 1) areas of Prince William Sound (Duffy et al. 1994*a*):

$$\log\left(\frac{\pi(x)}{1-\pi(x)}\right) = 5.1280 - 0.2886\ Hp - 0.1043\ AST - 0.1724\ IL\text{-}6\ ir$$

Moreover, AST brought information to the model concerning other serum enzymes-AST was positively correlated with both creatine kinase (CK; $r = 0.86$) and alanine aminotransferase (ALT; $r = 0.52$). We refrained from using a similar model in 1992 because of small sample sizes.

With data collected from 1996 to 1998, we again examined levels of Hp and IL-6 *ir* in the serum of river otters inhabiting oiled and "nonoiled" areas of the Sound for comparison with earlier analyses. Values of Hp in otters captured in 1996 at previously oiled sites were higher than at "nonoiled" sites ($P = 0.01$; Fig. 4A), but no difference was detected between sites from 1997 to 1998 ($P > 0.08$). Mean levels of Hp in samples collected from 1996 to 1998 were lower than during 1990-92 (Fig. 4A). No differences were detected in values of IL-6 *ir* between oiled and "nonoiled" sites from 1996 to 1998 ($P > 0.05$; Fig. 4B).

In those later analyses, we attempted to model the response of river otters to exposure to oil using logistic regression and the same independent variables that were influential in classifying otters as inhabiting oiled or "nonoiled" shorelines in 1991. That identical model,

$$\log\left(\frac{\pi(x)}{1-\pi(x)}\right) = 0.5124 + 0.0010\ AST - 0.0027\ Hp + 0.337\ IL\text{-}6\ ir$$

was not significant ($P > 0.13$) and classified only 58% of 112 otters correctly. Moreover, the liver enzyme AST in blood serum was elevated in otters on "nonoiled" areas in 1996-97 (Table 3), an outcome contrary to our expectations.

Endothelial *P*450-1A, an enzyme synthesized in response to exposure to hydrocarbons, was elevated in river otters captured in oiled compared with "nonoiled" areas in 1996 (Table 4), indicating continued exposure. Data on *P*450-1A collected in 1997 from those same sites, and data from 1998, which were gathered throughout western Prince William Sound, were nearly identical for otters living in oiled versus "nonoiled" areas (Table 4). Despite this apparent evidence of continued exposure as demonstrated by values of *P*450-1A from 1996, adjusted body mass as well as haptoglobin levels were indicative of recovery (Figs. 3 and 4).

Principal components analysis (PCA) on blood data from 1996 to 1998 (Tables 3 and 5) captured much of the variability (42%) in the 28 blood characteristics with 3 principal component (PC) axes. PC1 likely was created by the loading of blood parameters associated with diet (Table 6), whereas PC2 probably was indicative of general health, as identified by loadings of several liver enzymes (Table 6). PC3 likely reflected physiological responses to trapping and handling interacting with climatic conditions. When 95% confidence ellipses were plotted, illustrating the eigen vectors based on scores for individual otters relative to oiling and year, we observed a separation between years on PC1 and PC3, and a less-pronounced separation on PC2 (Fig. 5). PC1 and PC3 ostensibly reflect nutritional and physiological conditions, respectively, and are not associated with oiling, whereas PC2 probably would distinguish between areas if responses to oil were occurring (Fig. 5). In 1997, our trapping occurred during unusually warm and dry weather for the Sound. Therefore, the deprivation of food and water while an otter was in a trap probably resulted in greater physiological strain during 1997. PC3 separated 1997 from 1996 and 1998; 1996 and 1998 exhibited little separation on this axis (Fig. 5). In 1996 and 1997, PC2 distinguished between oiled and "nonoiled" areas, but liver enzymes were unexpectedly elevated in the "nonoiled" area (Table 4); that difference disappeared in 1998 (Fig. 5). MANOVA revealed that variables contributing to separation on PC1 (Table 6) did not differ between areas ($P > 0.1$), but differed between years ($P < 0.003$). Liver enzymes influencing PC2 differed between areas ($P < 0.03$), whereas bilirubin (total and direct) and sodium did not ($P > 0.13$); ALT, direct bilirubin, and sodium were different between years ($P < 0.04$). Variables loading on PC3 (Table 7) did not differ by area ($P > 0.5$). The year effect on that axis was significant only for HDL, calcium, and serum creatinine ($P < 0.01$). Moreover, a strong year by area interaction ($P = 0.007$) for Hp indicated PC3 did not reflect exposure to oil.

Linear regression between adjusted body mass (Table 7) and haptoglobin revealed a weak but positive relation ($r^2 = 0.053$, $P = 0.014$) for all otters (Fig. 6A). When each area (oiled or "nonoiled") was assessed individually, that relation was significant for otters in "nonoiled" areas ($r^2 = 0.214$, $P = 0.0004$) but not oiled areas (Figure 6A; $r^2 = 0.007$, $P = 0.85$). Similarly, the relation between adjusted body mass and *P*450-1A (Fig. 6B) exhibited a weak positive relation for all otters ($r^2 = 0.051$, $P = 0.02$), but no relation was observed in "nonoiled" areas ($r^2 = 0.014$, $P = 0.4$); a positive relation ($r^2 = 0.083$, $P = 0.03$) occurred only in oiled areas.

Fecal Porphyrins. – In addition to blood and tissue biomarkers, we assessed the concentration of porphyrins in feces of river otters. Porphyrins are involved in heme synthesis, a biosynthetic pathway that can be disrupted by exposure to hydrocarbons as well as other challenges (Taylor et al. 2001). Initially, we examined porphyrins in feces of river otters from oiled (Knight Island) and "nonoiled" (Esther Passage) areas in the Sound during 1990 (Blajeski et al. 1996). River otters inhabiting oiled areas exhibited higher mean (± SE) levels of total

porphyrins in their feces (48.2 ± 2.45 nmol/g dry wt, n = 117) compared with animals from "nonoiled" areas (34.5 ± 3.42 nmol/g dry wt, n = 84; $P < 0.001$). Moreover, proportion of feces with coproporphyrins present was higher ($P < 0.05$) on oiled (26%) than "nonoiled" (11%) areas (Blajeski et al. 1996). Unfortunately, our initial analysis lacked resolution to distinguish between coproporphyrin I and coproporphyrin III. Coproporphyrin III is a critical link in the biosynthetic pathway for the synthesis of the heme molecule, whereas coproporphyrin I is not (Taylor et al. 2000*b*). Therefore, we repeated the porphyrin analysis specifically for coproporphyrin III (Taylor et al. 2000*a*). Median concentrations of coproporphyrin III in feces from Herring Bay (oiled) did not differ from those at Esther Passage ("nonoiled") in 1990 (P = 0.09), but a trend for elevated levels in the oiled area was detected (Fig. 7). Those levels were higher in 1990 compared with those detected in 1996 in both Herring Bay (oiled) and Jackpot Bay ("nonoiled"), which did not differ from each other (Fig. 7). The reduction in coproporphyrin III in the feces of river otters in Herring Bay from 1990 to 1996 is a strong evidence of recovery (Fig. 7).

Diet and Prey Availability

Prey Remains in Feces. – Bony fishes dominated the diet of river otters (>35%) in the Sound during 1989-90. Those results, derived from analysis of prey remains in feces, underestimated the prevalence of bony fishes in the diet because of differential digestibility of fishes compared with other foods such as bivalves (Bowyer et al. 1994). We later confirmed this outcome with analysis of diet (80% marine fishes) based on stable isotopes (Ben-David et al. 1998*b*). Changes in either the species richness or diversity of otter diets were few between oiled (Knight Island) and "nonoiled" (Esther Passage) areas in winter 1989 prior to the oil spill or during the summer immediately following the contamination of shorelines with oil (Bowyer et al. 1994). By summer 1990, (>1 year after *EVOS*), however, substantial differences existed in the diets of otters inhabiting oiled compared with "nonoiled" areas (Bowyer et al. 1994). Moreover, changes in diets of otters in summer 1990 resulted mostly from a reduction in prey species on the oiled area (Bowyer et al. 1994). MANOVA revealed that perciform fishes (sand lances, gunnels, and ronquils) declined in diets of otters on oiled areas between pre-spill 1989 and post-spill 1990, as did archaegastropod mollusks (keyhole limpets and Margarite snails), whereas those groups increased on "nonoiled" sites during that period ($P < 0.001$). Conversely, Malacostraca (crustaceans) increased in the oiled area but declined in the "nonoiled" area (Bowyer et al. 1994).

Stable Isotope Analysis. – In 1996-97, we determined diets of river otters using stable isotope analysis of hair samples (n = 64). Isotopic values of otters from Jackpot Bay ("nonoiled") compared with Herring Bay (oiled) were different in both $\delta^{13}C$ and $\delta^{15}N$ (Fig. 8A; overall model, P = 0.02; year effect, P = 0.001; area effect, P = 0.22). That difference, however, was driven by a shift in diet for some otters in Jackpot Bay. Several individuals in that area foraged primarily on freshwater fishes. Based on telemetry locations, we excluded all otters that occurred mainly in freshwater habitats (n = 6) from our analysis of diet. With those otters removed, differences in diet between otters living in oiled versus "nonoiled" areas were no longer present (Fig. 8B; P = 0.20). Comparison of isotopic values relative to our earlier data from prey remains in feces should be interpreted with caution because of differences in methodologies. Nonetheless, isotopic data indicated that differences in diets of river otters no longer occurred between oiled

and “nonoiled” areas in 1996-97 (Fig. 8). Stable isotope analyses ($\delta^{13}C$) for data collected from 1996 to 1998 indicated that interannual changes in diet were not consistent between areas (Fig. 9).

Prey Availability. – During the early phase of our studies, assessment of prey availability was not included in our research design because of a lack of funding. In 1996-97, however, we assessed density of marine fishes on oiled and “nonoiled” areas. In addition, we recorded the distribution of fishes relative to latrines used by river otters as well as at random sites (Table 8). Abundance of marine fishes (≥8 cm in total length; excluding Gadidae) sampled along underwater transects did not differ between oiled and “nonoiled” areas (Fig. 10; $P = 0.87$), or between type of site (i.e., random or latrine, $P = 0.22$). Interactions were not significant for type by area, type by year, or a 3-way interaction between those variables ($P = 0.57$, 0.65, and 0.77, respectively). Nonetheless, there was a difference between years ($P = 0.02$) as well as a year by area interaction ($P = 0.01$), indicating that annual changes in prey availability were not consistent throughout Prince William Sound.

Use of Landscape

Habitat Use and Selection. — Another way in which the oil spill was expected to modify behavior and ecology of river otters was through damage to habitat. Contamination of shorelines altered use and selection of habitats by this mustelid. Because river otters concentrated their activities on and around latrines (Rock et al. 1994, Testa et al. 1994, Ben-David et al. 1998*b*), we compared a suite of habitat characteristics at latrines with those at random sites along shorelines in both oiled (Knight Island) and “nonoiled” (Esther Passage) areas in 1990 (Bowyer et al. 1995). We evaluated 128 latrine sites and 210 random sites in Herring Bay, and 113 latrine sites and 180 random sites in Esther Passage. Logistic regression identified 5 of 19 habitat characteristics that were influential in discrimination between used and available sites (i.e., otter latrines versus random sites), and between oiled and “nonoiled” areas (Table 9). All of those models were significant ($P < 0.001$) and between 80 and 83% of sites were classified correctly. River otters selected habitats differently between oiled and “nonoiled” areas (Fig. 11), even though availability of habitats was similar (Bowyer et al. 1995; Table 9). Otters on both study areas selected (use > availability) old-growth forests, but otters inhabiting oiled areas did so more strongly; selection for brush did not differ between areas (Fig. 11; Bowyer et al. 1995). Likewise, otters on both areas avoided (use < availability) steep vegetated slopes (Fig. 11). Most notable was that otters selected steeper tidal slopes (e.g., below mean high-tide line) on oiled compared with “nonoiled” areas (Fig. 11; $P = 0.001$). We interpreted that outcome to mean that otters in the oiled area avoided shallower tidal slopes where oiling was most severe and likely persisted the longest (Bowyer et al. 1995). Likewise, selection for large rocks by otters on the oiled area (Fig. 11) probably reflected an avoidance of oil accumulation on finer substrates (Bowyer et al. 1995). Indeed, our initial analysis of differences in river otter use of latrines within Herring Bay immediately following the spill indicated that otters used latrine sites that were free of oil more often than latrines subjected to heavy oiling (Bowyer et al. 1995; Table 9).

In 1996-97, we evaluated 67 latrine sites and 32 random sites in Herring Bay, and 93 latrine sites and 64 random sites in Jackpot Bay. Logistic regression identified 6 of 10 habitat

characteristics that were important in distinguishing between latrines and random sites (i.e., used and available), and between oiled and "nonoiled" areas (Table 9). Those models classified between 80 and 87% of sites correctly and all were significant ($P < 0.001$). River otters on both study areas selected vegetated slopes that were not steep (Fig. 11), and selected sites with more understory (brush) and greater exposure; selection for those characteristics was more pronounced in the oiled than "nonoiled" area. Otters on both sites avoided (use < availability) gravel and small rocks (Fig. 11). Old-growth forest was selected for by otters in the overall model and in the model including only habitat evaluations in the "nonoiled" area, but that variable did not enter the model for the oiled area. Although a MANOVA comparing overall differences in habitat selection between areas was significant ($P < 0.001$), the direction of selection for all variables was similar in both areas (Fig. 11), an outcome that differed markedly from our earlier studies (Fig. 11). In addition, the variable that represented avoidance of oiled beaches (tidal slope) did not enter the model in 1996-97 ($P = 0.21$).

Home Range. – We examined home ranges (measured in kilometers of shoreline) for river otters inhabiting oiled (Knight Island) and "nonoiled" (Esther Passage) areas of Prince William Sound during summer 1990. Tracking of radiotelemetered individuals revealed that otters inhabiting oiled areas of the Sound had larger home ranges ($P < 0.05$) than those living in "nonoiled" areas (Fig. 12; Bowyer et al. 1995), indicating that otters on oiled areas needed to travel greater distances to find suitable tidal habitats.

Data from otters living in oiled (Herring Bay) and "nonoiled" (Jackpot Bay) areas collected from 1997 to 1999 indicated no difference in home-range size between areas (ANOVA, overall model, $P = 0.1$; area effect $P = 0.62$, sex effect $P = 0.02$; interaction $P = 0.69$; Fig. 12). That result held whether we used the older method developed by Bowyer et al. (1995; Fig. 12) or a probabilistic method using fixed-kernel estimates (Fig. 13). During the later phase of our study, several male river otters (11% of 37 otters) moved between oiled and "nonoiled" sites in Herring and Jackpot bays. That phenomenon did not occur in our earlier study (1990-92), ostensibly because of the long distance between Herring Bay and Esther Passage. Those individuals that moved between study sites were excluded from analyses of home range. Data from several individuals that moved between oiled and "nonoiled" areas in 1996-98 had the potential of obscuring some differences in biomarkers, and likely made that analysis conservative.

We also compared home-range size in Herring Bay in 1990 with data from 1997-99. The size of home ranges declined between study periods when compared with identical methods (Bowyer et al. 1995; Fig 12). Home-range sizes for males tended to be larger than for females in all years; differences were significant only in the more recent data (Fig. 13; $P = 0.03$).

Demography

Fecal Deposition. – River otters scent mark at latrine sites located along the coastline where this mustelid gathers to engage in social and other activities (Rock et al. 1994, Testa et al. 1994, Ben-David et al. 1998*b*). We used deposition of feces at latrines in 1991 to obtain a crude estimate of differences between otter populations in oiled and "nonoiled" areas (Duffy et al. 1994*a*). We observed that river otters inhabiting oiled areas abandoned the use of about 15% of 339 latrines, whereas otters living in "nonoiled" areas abandoned <4% of 113 latrines ($P < 0.01$;

Duffy et al. 1994*a*). This 3-fold difference in abandonment of latrines led us to believe that otter populations continued to decline in oiled regions compared with "nonoiled" areas.

We observed interannual and seasonal changes in use of latrine sites by otters. In 1996, new latrine sites (n = 67 of which 28 were active) had been established in Herring Bay and abandonment of historic sites was noted (n = 11 or 9%). Greater levels (>90% of 78 latrines) of abandonment of historic sites were observed in Esther Passage in August 1996. Seasonal variation in use of latrine sites was assessed by comparing fecal deposition at latrines during 2 surveys within the same year. In a survey conducted in May 1996 in Herring Bay (oiled), 41% of 121 latrine sites (including new latrines) were classified as active (≥10 recent feces); by August 1996, only 14% remained active. A similar pattern occurred in Jackpot Bay ("nonoiled"): 31% of 85 latrines were active in the May survey, and 5% were active in the August survey. Those data indicated that the social organization of river otters likely influenced their use of latrine sites and, consequently, interpreting such changes in the use of latrines as solely demographic could be misleading.

Population Estimates. – Population estimates for river otters inhabiting oiled areas (Herring Bay) and "nonoiled" sites (Esther Passage) were obtained by implanting otters with radiotracers and performing a mark-recapture analysis on their feces in 1990 (Testa et al. 1994). Using a Bayesian model, we obtained estimates of 36 to 42 otters/100 km of shoreline in Herring Bay and 32 to 44 otters/100 km in Esther Passage, but 95% confidence limits for the densities of river otters overlapped between oiled and "nonoiled" areas throughout summer and early autumn (Testa et al. 1994). Pre-spill estimates of otter density in Herring Bay, however, were not available (Testa et al. 1994).

In 1997, we enumerated the minimum number of river otters inhabiting oiled and "nonoiled" study sites by summing the number of unique live captures with the number of additional unique individuals identified via analysis of DNA microsatellites from feces. We developed a full microsatellite profile for a subset of those samples (Herring Bay n = 40, Jackpot Bay, n = 30). Of those DNA profiles, one matched that of an otter trapped in 1996 in Herring Bay that was not recaptured in 1997. Two more matched the DNA profiles of animals from Jackpot Bay that were present on the study area as determined by radiotelemetry.

The minimum number of otters living in Herring Bay totaled 13 captured animals and 24 additional individuals identified from DNA in feces (37 otters/80 km), or 46 otters/100 km of shoreline. Thirteen otters were captured in Jackpot Bay ("nonoiled" site) and 8 additional otters were identified from DNA analysis (21 otters/80 km), yielding an estimated density of 26 river otters/100 km of shoreline. The minimum number of animals alive in both areas indicated that during spring 1997, river otters in Herring Bay were more numerous than in Jackpot Bay. Recaptures of individuals with either method were too few to provide reliable mark-recapture estimates.

We also reconstructed populations on both oiled and "nonoiled" areas in 1997 from captured individuals of known age using the conventional method of summing across age classes. Our point estimates, which provide a robust index, were 52 otters in Herring Bay (65 otters/100 km), and 29 otters (36 otters/100 km) in Jackpot Bay.

By comparing the population estimate of river otters obtained by Testa et al. (1994) for June 1990 in Herring Bay (42 otters/100 km) with the minimum number of otters known to be

alive in that area in 1997 (46 otters/100 km), we obtain an intrinsic rate of increase, $r = 0.013$ =([ln46-ln42]/7). Additionally, a finite growth rate of $\lambda = 1.013$ ($= e^{0.013}$) was obtained for that 7-year period. If we use the estimate from the reconstructed population at Herring Bay in 1997 (65 otters/100 km), $r = 0.064$ and $\lambda = 1.064$. Thus, 2 independent methods place the annual growth rate for river otters in Herring Bay (oiled area) at between 1.3 and 6.4%; we caution, however, that the enumerated value is within the 95% confidence interval calculated by Testa et al. (1994).

Age Structure. – Age structure of river otters captured in Herring Bay and Jackpot Bay exhibited few differences in 1997 (Fig. 14a). Moreover, all otters in that sample were recruited into the population after *EVOS* in 1989 (i.e., the oldest individual was 8 years old). A comparison of the combined age distributions of animals from Prince William Sound (Alaska) with river otters trapped from a population in Maine, USA, revealed no significant differences (Fig. 14b). The tendency toward more middle-aged otters in Alaska, and greater numbers of younger animals in Maine may have resulted from trapping of otters in Maine for fur.

Survivorship. – Survivorship of river otters, estimated by Kaplan-Meier analyses based on radiotelemetry at one-half month intervals from late June 1997 to January 1999, did not differ between oiled and "nonoiled" areas (Fig. 15a; $P > 0.20$). Otters on both areas were protected from sport and subsistence harvest, and overall survivorship was high (>0.8 over 20 months). Even the lower 95% confidence interval was >0.7 for that same period (Fig. 15b).

Synthesis

Meta-analysis of combined probabilities from several independent tests revealed differences between oiled and "nonoiled" areas in the early phase of the study (Table 10, $\chi^2_{8} = 40.06$, $P < 0.0001$). In contrast, no such differences between oiled and "nonoiled" areas were detected in the later phase (Table 10, $\chi^2_{14} = 15.93$, $P > 0.4$). That the combined probabilities in the later phase of the study exhibited no differences between oiled and "nonoiled" areas indicates recovery from effects of *EVOS*.

DISCUSSION

Evaluating Injury with Carcass Counts

The initial method of determining injury immediately following *EVOS* was by counting the number of carcasses recovered (Dean et al. 1994, Ford et al. 1996, Piatt and Ford 1996). Our data indicated that applying that criterion to all species without considering their drastically different life-history characteristics clearly was inappropriate. River otters, which spend much of their time in terrestrial habitats and make extensive use of holes and dens (Bowyer et al. 1995), seldom died in locations where they would have been detected by those searching beaches for carcasses. The near absence ($n = 12$) of river otter carcasses recovered from the beaches of Prince William Sound was one reason this mustelid was not listed originally as an injured resource by *EVOS* Trustees Council, in part because of the legal need to establish the economic value of

injured animals under the federal Comprehensive Environmental Resources Conservation and Liability Act (CERCLA; Spies et al. 1996). Integration of physiological, ecological, and behavioral data from our early studies confirmed negative effects of *EVOS* on river otters, which led to their recognition as an injured species in 1993. Incorporating biomarkers, diet, and landscape use, with demographics, offered a reliable approach to further assess initial injury and subsequent recovery. Our later research offered evidence that effects of *EVOS* on river otters had diminished; consequently, these mustelids were listed as recovered in 1999.

Exposure to Oil

Hydrocarbons were detectable immediately post spill as indicated by high concentrations of phenanthrene and napthalene in the tissues of carcasses recovered from beach surveys the first 2 years following the spill. Our more recent data from GC-MS analysis of pelage swabs of otters in 1997 indicated exposure to oil continued, but to a lesser degree. Concentrations of hydrocarbons on pelage of river otters (Duffy et al. 1999*b*) were considerably less than those reported for carcasses of sea otters collected in 1989, immediately post-spill (Ballachey and Kloecker 1997, Duffy et al. 1999*b*). That outcome is consistent with the decline of oil in sediments in Prince William Sound (Braddock et al. 1996, O'Clair et al.1996, Short et al. 1996). Although tempting to do so, inferring the dosage of oil assimilated by an otter based upon the concentration of oil on the pelage should not be done, because the swab might not have been wiped through the area of highest concentration on the animal. Moreover, the time of initial exposure to oil relative to the time of capture, how much oil the animal groomed from its fur, or how much oil might have been removed by otters mutually grooming are unknown (Ormseth and Ben-David 2000).

Biomarkers

Our early studies indicated elevation in levels of Hp, IL-6 *ir*, and several liver enzymes in otters living in oiled areas compared with those in "nonoiled" areas (Table 2; Duffy et al. 1993, 1994*a*, 1994*b*). By 1992, enzyme levels diminished and no differences were detected between areas. Although suggestive of recovery, those results were not conclusive because of small sample sizes, the absence of baseline data on blood chemistry of river otters in the wild (Duffy et al. 1993), the potential movements of otters between oiled and "nonoiled" areas, and the changing distribution of oil throughout the Sound (Stringer et al. 1992). The predictive model we developed with logistic regression clearly indicated differences in blood chemistry of otters inhabiting oiled and "nonoiled" areas in Prince William Sound during 1991. Nonetheless, Duffy et al. (1994*a*) cautioned that none of those biomarkers were specific to hydrocarbon exposure, and that elevated levels of biomarkers in river otters from oiled sites might result from interactions with other stressors associated with oiling, such as loss of body mass (Duffy et al. 1993, 1994*a*). Indeed, our data on adjusted body mass supported such observations (Fig. 3). Although no single result from our biomarker research from 1989 to 1992 provided an overwhelming case that river otters were damaged by *EVOS*, considered in concert, those results offered strong evidence that river otters were suffering subtle and chronic effects from the spill.

In our later studies, both Hp (Fig. 4a) and *P*450-1A (Table 4) were elevated in the oiled area in 1996 compared with the "nonoiled" area. Liver enzymes, however, exhibited the opposite trend (Table 3). Moreover, levels of Hp in both areas were substantially lower than in previous years. This decline in Hp is further supported by our data from fecal porphyrins; elevated values in the oiled area occurred in 1990 but that difference disappeared in 1996, and overall levels were lower in the later phase (Blajeski et al. 1996, Taylor et al. 2000*a*). Smith and El-Far (1980) documented a relation between malnutrition and liver porphyrins. Our field data indicated a similar trend. In the early studies, otters from oiled areas had comparatively low body mass and porphyrins extracted from feces collected in those same areas were elevated (Fig. 7; Taylor et al. 2000*a*). Those trends disappeared in the later phase of the study, indicating recovery.

Analysis of induction of the cytochrome *P*450-1A enzyme offers a sensitive method to detect exposure to xenobiotics, including hydrocarbons (Woodin et al. 1997, Ben-David et al. 2001*c*). Although differences in *P*450-1A among areas may represent variation in exposure to hydrocarbons, the enzyme certainly did not evolve in river otters to help detoxify crude oil, and therefore should not be considered as responding exclusively to hydrocarbon exposure (Ben-David et al. 2001*c*). Much remains to be discovered about the conditions that might induce this enzyme. If levels of *P*450-1A, measured across differing sex and age classes of river otters, were elevated, and the primary difference was location of capture (i.e., oiled or "nonoiled"), this biomarker would provide reasonable inference of oil exposure. In 1997-98, however, no differences in Hp (Fig. 4a) or *P*450-1A (Table 4) were detected between oiled and "nonoiled" areas.

In our earlier studies, when values for biomarkers were high, changing timing of capture relative to mating season and area (i.e., oiled or "nonoiled") revealed no alteration in the overall pattern of biomarkers between areas. In our later studies, however, differences disappeared when otters were sampled in the "nonoiled" areas during the mating season. We hypothesize that otters in previously oiled areas were continuously exposed to low levels of oil, but significant elevation of biomarkers only occurred when additional stressors such as the mating season were added to challenges faced by those mammals. The year in which no other notable factors (e.g., mating season or site-specific phenomena) were evident was 1998, when all samples were collected from 3 oiled (n = 27 otters) and 3 "nonoiled" (n = 24 otters) sites during the mating season. In that year, no biomarker or other index suggestive of exposure to oil or stress entered any diagnostic model, ostensibly because all otters were experiencing similar environmental conditions. Therefore, biomarkers may be informative, but caution should be used when interpreting such data. Overall weight of evidence should be used in evaluating damage or assessing risk.

Principal components analysis (PCA) clearly distinguished between years of data collected in the later phase of our research. We expected differences, a priori, in the outcomes of our analyses using a multi-year approach with pooled data, because we sampled the same 2 populations in 1996 and 1997, and different populations in 1998 (except for the inclusion of Herring Bay; 20% of the total samples for that year). Others have reported interannual variation in blood values of river otters (Tocidlowski et al. 2000). In our analysis, PC2 was characterized by liver enzymes (Table 7), a response we expected from exposure to residual oil in the environment (Duffy et al. 1993, 1994*a*). Separation between otters residing in oiled versus "nonoiled" areas (Fig. 5), however, was primarily because of higher values for those liver

enzymes in individuals from our “nonoiled” area (Jackpot Bay) in 1996 and 1997 (Table 4). Therefore, data from 1998 are most informative in terms of recovery, because river otters were sampled from throughout western Prince William Sound, and blood values should not reflect the localized condition in Jackpot Bay or elsewhere. Moreover, timing of sampling in 1998 prevented confounding effects associated with season. In 1998, there was no difference on PC2 between oiled and “nonoiled” areas (Fig. 5). PC1, which likely reflected diets of otters, did not differ between areas, but did so between years. Those differences may reflect the distribution of fishes at various locations in Prince William Sound in different years (Table 9, Fig. 12). Indeed, Fuller and Sievert (2001) noted that changing availability of prey would be expected to alter diets of carnivores. Our conclusion is further supported by interannual variation in diets between areas revealed by stable isotope analysis (Fig.11). Our interpretation of why PC3 distinguished 1997 from 1996 and 1998 was the likely effects of weather on otters in traps in 1997 (an El-Nino year; Khole 2000). Other factors may be involved in loadings on PC3, but stress from weather and trapping is the most obvious explanation. Thus, we conclude that oiling played no role in differences we observed in blood parameters during the late phase of our study (Fig. 5), and that river otters probably have recovered from extreme effects of *EVOS*.

We expected negative relations between adjusted body mass of otters and haptoglobins or *P*450-1A levels if oil persisted in the environment at high levels. Linear regression of those parameters revealed no such pattern (Fig. 6). That outcome indicated that nearly a decade after the spill, either oil was absent or persisted at levels too low to cause noticeable physiological changes in river otters in western Prince William Sound. Alternatively, those particular biomarkers may not become elevated unless exposure of river otters to hydrocarbons is coupled with additional environmental challenges. Indeed, captive experiments conducted at the Alaska Sealife Center in Seward, indicated that biomarker responses of river otters to exposure to hydrocarbons in low doses were nonlinear, and opposing physiological processes co-occurred in oiled animals, which made interpretation of those biomarker data difficult (Ben-David et al. 2001*b*, *c*). No detectable relation between adjusted body mass and biomarkers in river otters in the Sound during 1998 provides additional support for our conclusion that these animals have recovered from the more pronounced effects of *EVOS*.

Although our results indicate recovery of river otters from effects of *EVOS*, we wish to alert future investigators to potential difficulties with interpretation of similar data. There is a long and successful history of interpreting blood values for domestic animals in veterinary medicine (Kerr 1989). Comparisons between captive and free-ranging animals, however, may be more difficult to make. Baseline data on the "normal" range of values for free-ranging animals seldom are available, and differences in diet, activity, social organization (Warren and Kirkpatrick 1978, Lochmiller et al. 1986, Messier et al. 1987, Gau and Case 1999), or stress resulting from captivity (Ben-David et al. 2001*b*) can make comparisons with animals held in zoos or research facilities problematical. Likewise, capture and handling of wild mammals may affect particular blood variables (Seal et al. 1978, Boonstra et al. 1998, Keech et al. 1998). For example, values of AST, GLU, BUN, and LDH in our free-ranging otters were higher at capture than were mean values recorded for captive river otters (Davis et al. 1992, Reed-Smith 1995, Ben-David et al. 2001*b*). Values of those parameters in our free-ranging otters, however, were similar to those recorded for other wild-caught otters (Serfass et al. 1993). Until recently, traditional biomarker profiles for river otters, whether free ranging or captive, did not include

many of the biomarkers we evaluated in our study (e.g., fecal porphyrins, IL-6, haptoglobin, Cytochrome *P*450-1A1). Lack of baseline data from free-ranging animals for those biomarkers, and the complications of effects of captivity and capture (Ben-David et al. 2001*b*, *c*) may lead to ambiguity in interpretation of such data.

Another difficulty related to biomarkers stems from trying to interpret data for the population as if it were an individual animal with a characteristic set of blood parameters indicative of a particular condition or disease process. Not all animals in a population would be expected to exhibit elevated blood values (or values of other biomarkers) from exposure to oil or from some other factor such as infection. Even if 50% of the population responded in the expected manner and exhibited levels that would be interpreted as clinical damage in an individual, the population mean would be much lower. Finding even a small difference in biomarkers likely has biological importance. Moreover, more than 1 toxicological or disease process might occur in the same population, which would tend to obscure the expected profile of blood parameters or other biomarkers, and make a diagnosis similar to that of an individual difficult and inappropriate.

A critical factor in interpreting blood-related data is the nature of the dose-response curve (Fig. 16). If exposure or capture of all individuals is not simultaneous, some animals may be sampled at the peak of the biomarker response (Fig. 16, sample 2), whereas others will be sampled during the waning phase of the dose-response curve (Fig. 16, sample 3). Consequently, 2 individuals subjected to the same challenge but sampled at different stages in their response curves would have markedly different levels of biomarkers, even though both animals underwent an identical physiological process. Clearly, valid comparisons of biomarkers for free-ranging animals must be made at the population level.

Deciding which biomarkers to consider, or which blood variables should be most informative, is not simple. Including every possible blood characteristic, and sorting among the myriad of those potential variables is a daunting task, both theoretically and statistically. Our use of multivariate statistics (MANOVA, logistic regression, and principal components analysis) allowed for simultaneous consideration of nonindependent and autocorrelated variables. Our approach also reduced biases associated with analysis of extreme values (i.e., outliers; Fadely 1997). Outlier analysis may underestimate the overall population response, because that approach results in consideration only of those individuals that exhibit a peak response at sampling (Fig. 16). Nonetheless, multivariate analyses do not alleviate the need for selecting appropriate biomarkers that exhibit a specific, quantitative, predictable, dose-response relation between the contaminant and the physiological response (Vanden Heuvel and Davis 1999). Selection of such biomarkers should be consistent with models based on physiological theory (Ben David et al. 2001*b*, *c*).

Diet and Prey Availability

Our earlier studies based on analyses of prey remains in feces indicated a difference in diets of river otters inhabiting oiled and “nonoiled” areas (Bowyer et al. 1994). Declines we observed in invertebrates would be expected because bivalves and limpets, which are sessile as adults, occurred in habitats that received heavy oiling (Bowyer et al. 1994). Our data on habitat selection clearly demonstrated that otters avoided oiled shorelines in 1990 (Table 9; Fig. 11).

Some changes in diets of otters may have resulted from lower prey availability in oiled areas. For example, studies of benthic communities in the Sound approximately 1 year following *EVOS* revealed that a suite of invertebrates, including gastropods, bivalves, and crabs, were reduced in several shallow (<20 m) subtidal habitats that were oiled (Jewett et al. 1995). Conversely, a number of demersal fishes (e.g., cod, greenling, sculpin, ronquil, and pricklebacks) were higher in abundance in shallow oiled habitats than at "nonoiled" sites 1 year after *EVOS* (Jewett et al. 1995), which is consistent with our observation that those fishes were more prevalent in the diets of otters from oiled areas in 1990 (Bowyer et al. 1994). The delay in dietary changes in otters living in the oiled area until summer 1990 could have resulted from oil moving from intertidal to subtidal habitats during that time (Bowyer et al. 1994). Nearshore (<30 m depths) demersal fishes exhibited continuing exposure to oil through the first 3 years after the spill (Collier et al. 1996, Laurand and Haldorson 1996). Overall reductions in bony fishes in the diet of otters on oiled sites (Bowyer et al. 1994) was likely most important to their ecology, and may have resulted in the lower body mass observed in otters inhabiting oiled areas (Fig. 3).

Increases in crustaceans in diets on oiled sites may have reflected either lower availability of fishes (the preferred diet of otters; Larsen 1984, Stenson et al. 1984, Bowyer et al. 1994), or alternatively a reduction in the diving and foraging abilities of otters as a result of direct exposure to hydrocarbons (Fig. 1; Ben-David et al. 2000). Indeed, Kruuk (1995) observed that European otters that were less efficient in their foraging ability fed more on crabs, whereas otters with better foraging ability or potentially in better health, existed on a diet of marine fishes.

Although comparing results from stable isotope analysis with those of prey remains in feces cannot be done directly (Ben-David and Schell 2001), our observation that stable isotope ratios of river otters captured in Herring and Jackpot bays in 1996-97 were similar indicated that no dietary differences occurred between those 2 areas. Several species of fishes in this system have similar isotopic signatures (Ben-David et al. 1998*b*), thus otters feeding on different amounts or species of fishes could have similar isotopic values (Ben-David 1997*a*, 1997*b*; Blundell et al. 2002). Nonetheless, crabs have a different isotopic signature than any of the fishes and an increased consumption of crabs would have resulted in different isotopic signatures in otter tissues (Ben-David et al. 1998*b*).

Our analyses of SCUBA transects in 1996-97 revealed similar distributions and density of fish species in both study areas but differences in fish densities between years (Table 8, Fig. 10). This observation fits well with the annual changes we observed in stable isotope values of otter hair (Fig. 8), lending further support to the conclusion that lack of differences in isotopic values between areas indicates similar diets for otters in oiled and "nonoiled" areas in 1996-97. Thus, we conclude that in contrast to our earlier analyses, we observed no evidence of differences in diets of otters inhabiting oiled and "nonoiled" areas in 1996-97, providing further evidence of recovery. Indeed, evidence exists that intertidal communities are recovering from the effects of *EVOS* (Skalski et al. 2001, Page et al. 2002).

Use of Landscape

We demonstrated differences in habitat selection by otters inhabiting oiled and "nonoiled" areas in the early part of our study (Fig. 11; Bowyer et al. 1995). The variables that were diagnostic in identifying latrine sites, and thus habitat selection by otters, indicated otters

avoided oiled beaches; otters on oiled areas selected steep tidal slopes and large rocks where oil did not accumulate (Bowyer et al. 1995; Fig. 11). Although differences in habitat selection between oiled and "nonoiled" sites still occurred in 1996-97, none of those variables could be associated with avoidance of oiled beaches. In addition, direction of selection (i.e., avoidance of vs. selection for) was identical for oiled and "nonoiled" sites, only the magnitude differed between areas (Fig. 11). No difference occurred between oiled and "nonoiled" areas in the mean value of tidal slopes at latrines. Furthermore, this value was identical to the mean tidal slope selected for by river otters in Esther Passage in 1990 (Table 9). That those differences between oiled and "nonoiled" areas diminished in our recent studies, is another measure of recovery (Fig. 11).

Larger home ranges on oiled compared with "nonoiled" areas in the early years of our research (Fig. 12) supported our observation that some shoreline habitats (Table 9) were avoided on oiled areas (Bowyer et al. 1995). That home-range sizes in Herring Bay declined between 1990 and 1997-99 (Fig. 13) indicated that otters during the later years no longer needed to avoid oiled shores. That outcome agrees with our observation that selection of habitat characteristics related to avoidance of oiled beaches (tidal slopes and rock size) no longer differed (Fig.11). Therefore, previous avoidance of oiled shores likely resulted in increased home-range size for river otters, which in turn contributed to the dietary differences we observed in 1990 (Bowyer et al. 1994). Otters traveling over large areas likely were forced to forage on more sedentary prey to compensate for reduced foraging efficiency (Fig. 1; Bowyer et al. 1994, Ben David et al. 2000), as manifested by the differences in diet between oiled and "nonoiled" areas in the early phase of our study.

Demography

Although our early studies indicated a 3-fold difference in the abandonment of latrines on oiled compared with "nonoiled" areas (Duffy et al. 1994*a*), our more recent research showed that other factors, in addition to population density, were involved in that process. We caution that abandonment may provide a biased index to population size. Other studies of otters used feces deposition at latrine sites as an index of population size (Crawford et al. 1979, Strachan et al. 1990, Serfass et al. 1993), although the use of fecal deposition at latrines has been controversial (Mason and Macdonald 1987, Kruuk and Conroy 1987). Clearly, more research on the role of social behavior and its effects on the deposition of feces by otters and other mustelids is needed. Scent-marking behavior among mustelids may vary across and within species in relation to social systems, habitat, and population density (Hutchings and White 2000).

In our early studies, no differences in population estimates were detected between oiled and "nonoiled" areas (Testa et al. 1994). We cautioned, however, that estimates for river otters prior to the oil spill were unavailable. Furthermore, no measurable decline in otter numbers on the oiled site (Herring Bay) was detected through 1 season but that observation did not rule out the possibility that substantial mortality might have occurred in that area prior to obtaining that estimate. Alternatively, our estimates could have been conducted too early and substantial mortality could have occurred after we conducted our sampling in 1990.

Mortality of river otters after 1990 could have been the result of interactions between direct physiological damage from chronic exposure to oil, decreases in diving and foraging

efficiencies (Tarasoff et al. 1972, Fish 1994; Ben-David et al. 2000), and increases in energy demands from the need to avoid oiled beaches (i.e., larger home ranges; Kruuk 1995, Powell et al. 1997). These combined processes may have resulted in the observed reduction in body mass of otters from oiled areas (Fig. 3), associated increases in levels of fecal porphyrin (Smith and El-Far, 1980; Taylor et al. 2000*a*), and an increase in the probability of mortality. Supportive data on reductions in body condition of sea otters following *EVOS* are available (Rotterman and Monnett 2002).

Calculated densities of river otters in Herring Bay in 1997 based on population enumeration result in an estimate of 1 animal per 2.2 km of shoreline. In contrast, density estimates obtained from the population reconstruction are 1 animal/ 1.5 km of shoreline. Both estimates are higher than those reported for river otters in freshwater systems (1 otter/ 2.7 - 5.8 km of waterway; Melquist and Hornocker 1983, Reid et al. 1987). We believe it unlikely that such densities of otters could be maintained under continuous exposure to hydrocarbons even in marine systems, where availability of forage is higher than that of freshwater habitats (Kruuk 1995).

Our enumeration of populations for oiled and "nonoiled" areas in 1997 indicated that densities of otters were lower in Jackpot Bay ("nonoiled") than in Herring Bay (oiled). Furthermore, the minimum number of river otters alive in Herring Bay in 1997 was within the upper values of the confidence interval of earlier (1990) estimates. Another analysis (population reconstruction from age structure) yielded even higher values for otters in that area than previously estimated. Comparing the estimates from 1990 with the minimum number alive and the population reconstruction in Herring Bay in 1997 indicated a growth of 1.3 - 6.4% per year. Moreover, most of those otters (animals <6 years old; 12 of 13) were recruited following *EVOS* (Fig. 14). Numerous studies on mustelids demonstrate reductions in fecundity and high mortality of neonates in animals exposed to hydrocarbons or similar compounds such as Polychlorinated Biphenyl (PCB; Bleavins et al. 1980). A reduction in fecundity as a result of hydrocarbon exposure of the mother also was pronounced in a second generation of captive mink (Mazet et al. 2001). Effects of hydrocarbons on reproduction in river otters are not well studied, and outcomes for otters may differ from other mustelids (Wren 1991). Nonetheless, recruitment of river otters for most cohorts on oiled areas following the spill (Fig. 14) is opposite of that expected from lingering effects of oil, and likely indicates recovery.

We derived a theoretical value of $\lambda = 1.24$, and $r = 0.212$ for river otters. This estimate likely approaches the reproductive potential (r_{max}) for the species. We hypothesize that the rate of recovery for river otters in Herring Bay is less than maximal, potentially because we estimated number of otters in 1990 before a decline occurred. Moreover, recovery could have occurred before we began sampling in 1997. Indeed, the r_{max} we calculated for river otters would have allowed 20 otters in 1991 (i.e., one-half the number of otters estimated in 1990) to reach a population size of 58 animals by 1996. Our data on age structure and survivorship indicate that recruitment and survival were not depressed in the later phase of our study. Ultimately, we would expect otters to approach some pre-spill equilibrium with their environment (data that are not available), which would explain our relatively low calculated rates of annual increase (1.3-6.4%).

Our analysis of population growth based on recruitment assumed no immigration or emigration occurred in Herring Bay between 1990 and 1997. That assumption, however, may be

invalid. In a companion study, Blundell et al. (2002*b*) investigated relatedness (Queller and Goodnight 1989) and gene flow (Cornuet et al. 1999) using DNA microsatellite analysis on blood samples collected from river otters captured in 1996-98. That analysis indicated that relatedness among river otters in Prince William Sound was generally low (average relatedness coefficient *R* ranged between 0.05 and 0.14), but animals in Jackpot Bay were more closely related to each other (average $R = 0.14$) than animals captured in Herring Bay (average $R = 0.06$; Blundell et al. 2002*b*). This lower relatedness among animals in our oiled site may have resulted from colonization of Herring Bay by migrants from other locations in Prince William Sound.

The lack of difference in our indirect measures of recruitment (i.e., age structure; Fig. 14) is supported by our estimates of survivorship, which did not differ between areas (Fig. 15). Our sample sizes for survivorship analysis were smaller than recommended (Pollock et al. 1989), likely reducing our ability to detect differences between areas. That survivorship was uniformly higher on the oiled area, however, indicated that effects of oiling were not manifested by high mortality in that population. In addition, estimates of survival for both areas were similar to those reported for river otters in Oregon (about 75%; Tabor and Wright 1977), and wild European otters in Shetland (about 85%; Kruuk and Conroy, 1991). Survivorship of our otters was also similar to that of wild-caught river otters reintroduced in North America (46 - 91%; Erickson and McCullough 1987, Greiss 1987, McDonald 1989), and wild caught and re-introduced European otters in Sweden (79%; Sjoasen 1996). Thus, all our demographic parameters indicate that the initial damage to river otters from the oil spill had diminished by the end of our study, and otters likely have recovered, regardless of whether recruitment of individuals into the oiled area was a result of reproduction or emigration.

Potential Routes for Intermittent Exposure

Because the physical properties of ingested oil affect assimilation of hydrocarbons by animals (Ormseth and Ben-David 2000), understanding the route of exposure to oil hydrocarbons is important. Ormseth and Ben-David (2000) reported that ingestion of crude oil as nondispersed molecules (e.g., from an animal grooming its coat after swimming through an oil slick) resulted in increased passage rate of digesta and reduced assimilation of hydrocarbons. In contrast, ingestion of oil in the form that might occur in prey tissues may result in greater assimilation of hydrocarbons by the predator (Ormseth and Ben-David 2000). Thus, clarifying the potential routes of exposure is imperative to understanding how response to that exposure may manifest itself in the studied populations.

The most likely source of remaining oil in Prince William Sound was the 8-16% of 39,000 metric tons buried in marine sediments by a storm on the 3rd day following the spill (Wolfe et al. 1994). Oil is degraded by aerobic microorganisms (Braddock et al. 1995, 1996), which would have little opportunity to detoxify crude oil until it was uncovered and released by tides, currents, and winds in the Sound. Oil buried only 15 cm below ground persisted for 20 years without degrading substantially in terrestrial systems (Collins et al. 1993). Moreover, ultra violet light may enhance the toxicity of weathered oil, including some polysyclic aromatic compounds, beyond that observed under laboratory conditions; toxicity may be increased markedly in intertidal zones (Barron and Ka'Aihue 2001) where river otters concentrate their activities (Bowyer et al. 1995). Thus, while grooming, river otters may ingest previously buried

and resuspended oil that accumulated on their fur. European otters spent substantial time grooming (Kruuk 1995), and our observations suggest the same for river otters. Oil recovered from the pelage of river otters in 1997 (Duffy et al. 1999*b*) supports this as a potential route of exposure.

Another potential route of exposure in otters may be through the consumption of prey. The presence of crude oil in mussel (*Mytilus* spp.) beds throughout western Prince William Sound was noted from 1990 to 1998 (Short et al., 1996, Carls et al., 2001; J. Short, NOAA, Juneau, Alaska, pers comm). Indeed, mussels occurring in oiled areas continue to exhibit metabolic signs of stress 10 years following the spill (Downs et al. 2002). This source, however, is not likely to be the primary route for exposure for river otters because invertebrates compose only a small portion of otter diets (Larsen 1984, Stenson et al. 1984, Bowyer et al. 1994). Moreover, otters inhabiting areas with oiled mussel beds did not respond with elevated levels of Hp or IL-6 *ir* in 1992 (Duffy et al. 1994*b*). Alternatively, otters may be exposed to oil through consumption of fishes. The extent of that exposure, however, will depend on the ability of fishes to metabolize hydrocarbons (Woodin et al. 1997), and the time elapsed between the exposure of fishes and the ingestion of those fishes by otters. Recent investigation documented the occurrence of *P*450-1A in masked greenling (*Hexagrammos octogrammus*) collected in Herring Bay (Jewett et al.2002). Whether hydrocarbons that occur in fishes are passed up the food chain and whether such exposure would be of sufficient magnitude to elicit *P*450-1A response in otters are uncertain and merit further investigation.

Synthesis

Taylor et al. (2000*a*) performed meta-analysis to examine the response of a suite of physiological variables from river otters collected in the early phase of the study, and concluded that the weight of evidence indicated river otters were injured by the spill. We likewise used that method to combine probabilities from a wide array of response variables collected in both phases of our study. Those analyses indicated that the initial injury no longer could be detected in the later phase (Table 10), indicating recovery of river otters from effects of *EVOS*. This is particularly evident when considering that our reference sites were lightly oiled rather than "nonoiled" (Stringer et al. 1992). Although diagnostic biomarkers, including Hp and IL-6 *ir*, fecal porphyrins, and body mass, all were consistently higher on oiled compared with "nonoiled" areas in our early studies (Figs. 4, 7, and 3, respectively), data for otters inhabiting presumably "nonoiled" sites also exhibited a pattern consistent with initial exposure to, and recovery from the effects of *EVOS*. Consequently, our data are best interpreted as differences in response of river otters to severity of exposure to petroleum hydrocarbons. Accordingly, our comparisons provide a conservative analysis of damage and recovery of river otters.

Injury to river otters from *EVOS* could have been caused directly from toxicity of petroleum hydrocarbons or indirectly from damage to the nearshore ecosystem (Fig. 1). Nonetheless, both pathways may be interacting. Ben-David et al. (2000, 2001*b*, *c*) determined that chronic exposure to low doses of weathered crude oil, under controlled conditions, resulted in physiological damage, especially reduction in hemoglobin levels (and associated hematocrit and red blood-cells), reduction in white blood-cells, and elevation in several liver enzymes, Cytochrome *P*450-1A1, and IL-6 *ir*. Further, this physiological damage (especially the reduction

in hemoglobin) led to an increase in energetic costs of terrestrial locomotion (up to 40%), a decrease in aerobic dive limit (from 51 to 45 sec), and a potential increase in foraging time (up to 64%), because of a decrease in total length of submergence during each foraging bout (Ben-David et al. 2000). Thus, physiological damage from exposure to crude oil could result in a decrease in body condition in free-ranging river otters. Indeed, we documented a reduction in body mass (controlled for age and sex classes) for otters live-captured in oiled areas of the Sound in the early phase of the study.

Furthermore, constraints imposed by oiling on diving behavior of otters likely will alter their diets. We would expect otters to concentrate on prey that potentially have a high rate of capture (i.e., prey that are slow moving, easily detected, or abundant). Again, we documented that change in diets of otters from oiled shores resulted mostly from a reduction in prey species and an increase in consumption of slow moving prey such as crustaceans (Bowyer et al. 1994). Nonetheless, changes in prey availability or avoidance of oiled beaches (i.e., selection of tidal slope) with an associated increase in home-range size could have caused similar changes in those behavioral responses of otters (Fig. 1).

Ben-David et al. (2002) demonstrated that levels of hemoglobin, which were indicative of incomplete rehabilitation in oiled captive river otters, were related to post-release survival of those individuals. Indeed, in that study, animals with lower levels of hemoglobin perished soon after release and more experimental animals died of starvation than wild otters during a period of potential food shortage (Ben-David et al. 2002). Thus, physiological damage from oiling can negatively affect survival of oiled free-ranging river otters, regardless of prey availability. That we detected no differences in age structure and survival in otters from oiled and “nonoiled” areas of our study indicates that river otters in Prince William Sound have recovered from effects of *EVOS*.

Initial injury and subsequent recovery of river otters from *EVOS* likely resulted in cascading effects in the terrestrial system in Prince William Sound. Number of animals on the landscape would have determined the amount of nutrients transported from sea to land. For example, Ben-David (unpublished data) calculated that a density of 1 otter/2.7 km of shoreline (Reid et al. 1987) would result in an average deposition of 754 kg/ha/year of marine derived nitrogen at latrines sites. In contrast, a density of 1 otter/1.3 km of shoreline (Testa et al. 1994) would result in an average deposition of 1,567 kg/ha/year nitrogen at latrines sites, a >100% increase in fertilization of terrestrial vegetation. Otter latrines are distributed along substantial stretches of coastline (Testa et al. 1994, Bowyer et al. 1995, Ben-David et al. 1998*b*), and hold the potential to influence far greater areas of the terrestrial environment near the coast than point sources of nutrient input surrounding streams (Ben-David et al. 1997*b*, 1998*a*). Changes in fertilization from feces of river otters may have a substantial effect on community composition of the beach fringe forest (Ben-David et al. 1998*b*). Nonetheless, even smaller changes in otter densities, such as those we observed, may substantially alter the terrestrial ecosystem. Further research on interactions between pollution, populations, and landscape use by individuals and their effects on the land-margin system are warranted.

MANAGEMENT IMPLICATIONS

Use of biomarkers, including blood panels with numerous variables, to assess the well being of free-ranging fish and wildlife populations has become widespread (Peakall 1992, Stegeman et al. 1992, Akins et al. 1993). Undeniably, biomarkers have several important advantages as a method for assessing the status of wildlife populations. First, most of these measures can be obtained with nondestructive sampling. Second, biomarkers may yield subtle information concerning the status of the population that cannot be obtained from gross necropsy or, in some instances, even sophisticated laboratory procedures (Zentano-Sabin et al. 1997). Third, some methods, such as analysis of fecal porphyrins, do not require capture or handling of individuals. Finally, biomarkers can be quantified and do not require subjective assessments based on experience to determine the health of individuals.

We documented that interpretations based on the physiological state of individuals may not be appropriate for making inferences about populations. Epidemiologists long ago embraced the science of population ecology in understanding the spread of diseases (Anderson and May 1985, Bacon 1985), and we agree with Caswell (1996) that the time is at hand for those who examine blood values and other biomarkers of individuals from wild populations to make a similar transition. We offer several standard methodologies with strong empirical underpinnings to aid in that process. We do not claim to have solved all of the existing difficulties with this complex problem - much remains to be accomplished. Nonetheless, we believe our approach offers an important first step in understanding how such data should be analyzed and interpreted.

We do not believe that a single biomarker will be sufficient to monitor the health of populations. Different causations can induce or elevate multiple biomarker systems (Ben-David et al. 2001*b*). Differences between and among species, sexes, age classes, reproductive status, physical condition, and other variables can complicate interpretation of biomarkers. For this reason alone, several systems for assessing status of populations are desirable, especially when biomarkers vary in their sensitivity to a particular stimulus or challenge. Likewise, that some biomarkers are more general (e.g., haptoglobin) and others more specific (e.g., *P*450-1A) is a further reason to use them in concert. Difficulties in knowing which biomarkers to use to answer a specific question is the reason that so many different biomarkers are employed, and highlights the need for methods that allow for reductions in the dimensionality of those data, such as PCA.

We caution that successful interpretation of biomarkers may rely as much on an appropriate sampling design, including considerations of scale, as on physiological responses of animals. Without a sampling design to address a specific hypothesis, the likelihood of obtaining reliable knowledge (*sensu* Romesburg 1981) is nil. The particular objectives of the study will dictate the biomarkers required. Moreover, we advocate the use of sentinel and keystone species, such as river otters, to assess effects of environmental pollution. Seldom is it possible to determine the consequences of a calamity such as the *Exxon Valdez* oil spill on all links in an ecosystem. Use of sensitive and important components of the ecosystem allow for the initial assessment of pollution and provide a barometer for recovery

Finally, integrating individual-based and population-level studies was essential to our understanding of processes and responses of a sentinel and keystone species to environmental pollution. We linked the use of biomarkers with the ecology and behavior of river otters (Fig. 1). To our knowledge, such an integration of disciplines to answer questions about effects of

environmental pollution is rare. We often gained insights from 1 approach when others failed to provide clear-cut answers. Our study design allowed us to document chronic effects from *EVOS* on river otters when other studies were entrained exclusively on acute outcomes that could be related directly to mortality (Peterson 2001). We now know that such a narrow view of environmental pollution is short sighted, and hope that future studies of catastrophes such as EVOS, will incorporate a broader, more long-term ecosystem-based approach in their initial design.

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Table 1. Published literature indicating a sensitivity of mustelids to pollutants.

Pollutants				
Pesticides	Heavy Metals	Cesium 137	PCBs	Crude Oil
Clark et al. 1981	Wren et al. 1980	Clark et al. 1981	Bleavins et al. 1980	Duffy et al. 1993, 1994*a*, 1994*b*, 1996
Halbrook et al. 1981	Clark et al. 1981	Halbrook et al. 1981	Clark et al. 1981	Williams et al. 1995
Henny et al. 1981	O'Connor and Nielson 1981		Halbrook et al. 1981	Blajeski et al. 1996
Elliott et al. 1999	Sheffy and Amant 1982		Henny et al. 1981	Ben-David et al. 2000, 2001*a*, 2001*b*, 2001*c*
Sample and Suter 1999	Wren 1984, 1985		Harding et al. 1999	Mazet et al. 2000, 2001
	Francis and Bennet 1994		Engelharat et al. 2001	Beckett et al. 2002
	Halbrook et al. 1994, 1996			
	Kruuk et al. 1997			
	Gutleb et al. 1997, 1998			
	Evens et al. 1998			
	Harding et al. 1998			
	Dansereau et al. 1999			
	Duffey et al. 2000			
	Ben-David et al. 2001*a*			

Table 2. Means (± SE) of selected blood variables from river otters inhabiting oiled and "nonoiled" areas of Prince William Sound, Alaska, 1991 (adapted from Duffy et al. 1994*a*).

Blood Variables	Oiled (n = 11)		"Nonoiled" (n = 11)	
	$\bar{x}$	SE	$\bar{x}$	SE
Interleukin (IL-6 *ir*, pg/mL)	48.3	13.8	17.3	11.3
Interleukin (IL-1 *ir*, pg/mL)	13.3	6.6	10.1	6.1
Haptoglobin (Hp, Hb binding dl/100 mg)	156.9	27.9	30.0	15.6
Alanine Aminotransferase (ALT, IU/L)	152.7	8.8	138.5	14.6
Asparate Aminotransferase (AST, IU/L)	437.2	70.0	418.1	67.0
Lactate Dehydrogenase (LDH, IU/L)	146.2	25.2	154.0	43.1
Creatine Kinase (CK, IU/L)	3,038.6	820.8	1,885.8	516.4
Hemoglobin (Hb, g/dL)	16.3	0.6	15.7	0.6
Packed Cell Volume (PCV, ml/mm^3)	42.9	1.6	44.1	1.6

Table 3. Means (± SE) of blood-serum chemistry for river otters captured in oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, during 1996–98. Otters were captured in Herring Bay (oiled) and Jackpot Bay ("nonoiled") in 1996–97. During 1998, otters were live-trapped throughout oiled and "nonoiled" areas of the Sound. Abbreviations and units for blood values are provided in Appendix B.

	1996				1997				1998			
	Oiled (n = 20)		"Nonoiled" (n = 19)		Oiled (n = 11)		"Nonoiled" (n = 11)		Oiled (n = 27)		"Nonoiled" (n = 24)	
Blood Variables	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
ALB	3.01	0.05	2.91	0.06	2.92	0.07	2.96	0.07	3.05	0.05	3.09	0.05
ALK PHOS	147.85	15.04	175.53	15.43	145.09	17.11	129.36	17.11	182.22	8.68	139.79	9.21
ALT	111.0	8.96	170.05	19.85	127.82	15.20	190.0	41.51	187.81	15.78	176.88	13.68
AST	308.4	48.45	694.63	205.9	286.27	44.47	727.64	185.01	465.78	78.17	418.92	74.21
BUN	48.25	4.13	43.16	4.24	38.45	4.53	47.64	4.53	51.33	3.08	43.50	3.26
Ca	8.95	0.09	8.63	0.09	8.16	0.24	8.51	0.24	8.87	0.08	8.86	0.08
CL	113.45	0.93	112.42	0.95	114.36	1.01	114.09	1.01	111.93	0.71	112.25	0.76
CHOL	182.40	10.90	224.05	11.18	193.64	12.28	209.36	12.28	170.81	10.29	166.04	10.91
CHOL/HDL	1.84	0.81	1.87	0.08	2.82	0.16	2.93	0.16	1.93	0.07	1.83	0.07
Dir bili	0.05	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.07	0.01	0.08	0.01
GGT	25.55	8.16	38.00	8.37	22.09	4.26	34.18	4.26	30.48	5.23	27.79	5.55
GLOB	4.40	0.12	4.67	0.12	4.42	0.16	4.77	0.16	4.16	0.09	3.83	0.10
GLU	145.45	11.11	120.26	11.40	120.00	10.96	103.64	10.96	162.74	9.56	136.79	10.14
HDL	98.70	4.61	121.58	4.73	70.00	3.45	71.64	3.45	87.56	3.56	89.13	3.78
Hp	50.08	10.01	11.77	10.27	9.53	13.47	42.22	13.47	19.30	7.81	22.38	8.28

Table 3. Continued

Blood Variables	1996				1997				1998			
	Oiled (n = 20)		"Nonoiled" (n = 19)		Oiled (n = 11)		"Nonoiled" (n = 11)		Oiled (n = 27)		"Nonoiled" (n = 24)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
IL-6 *ir*	2.00	1.15	2.88	1.18	0.00	0.19	0.27	0.19	0.82	0.29	0.75	0.31
LDH	200.05	43.64	319.84	44.78	174.91	63.06	346.09	63.06	187.96	25.74	221.33	27.30
LDL	71.90	7.92	89.00	8.12	112.73	9.54	124.73	9.54	72.19	6.36	65.92	6.74
PHOSPH	5.98	0.38	6.30	0.39	5.63	0.55	7.23	0.55	5.39	0.26	4.62	0.27
K	4.21	0.07	4.10	0.07	4.25	0.08	4.28	0.08	3.93	0.06	3.95	0.07
SCREAT	0.73	0.083	0.483	0.083	0.61	0.06	0.69	0.06	0.25	0.01	0.26	0.01
Na	152.15	0.82	150.89	0.84	149.55	0.69	151.45	0.69	150.00	0.60	149.50	0.63
TP	7.40	0.12	7.58	0.12	7.34	0.19	7.73	0.19	7.20	0.09	6.92	0.09
T. bili	0.28	0.02	0.32	0.02	0.31	0.01	0.32	0.01	0.30	0.01	0.33	0.01
TRIG	58.90	6.81	62.26	6.99	54.64	12.04	65.18	12.04	55.89	9.48	55.29	10.50
UA	2.81	0.26	2.36	0.26	3.16	0.43	3.46	0.43	2.24	0.19	1.83	0.20
VLDL	11.80	1.37	13.00	1.41	10.91	2.44	13.00	2.44	11.07	1.89	11.00	2.00

Table 4. Values for staining index of endothelial *P*450-1A (ranges from 0 to 12) for 114 river otters inhabiting oiled and "nonoiled" areas of Prince William Sound, Alaska, USA. Otters were captured in Herring Bay (oiled) and Jackpot Bay ("nonoiled") in 1996–97. During 1998, otters were live-trapped throughout oiled and "nonoiled" areas of the Sound.

	Area					
	Oiled			"Nonoiled"		
Year	*n*	$\bar{x}$	SE	*n*	$\bar{x}$	SE
1996	20	3.2	0.47	19	1.2	0.48
1997	12	4.7	0.61	12	4.0	0.61
1998	27	0.9	0.40	24	0.7	0.43
Years pooled	59	2.4	0.38	55	1.6	0.28

Table 5. Values for complete blood counts for river otters inhabiting oiled and "nonoiled" areas of Prince William Sound Alaska, USA, during 1996–98. Otters were captured in Herring Bay (oiled) and Jackpot Bay ("nonoiled") in 1996–97. During 1998, otters were live-trapped throughout oiled and "nonoiled" areas of the Sound. Abbreviations and units for blood values are provided in Appendix C.

Blood Variables	1996				1997				1998			
	Oiled (n = 20)		"Nonoiled" (n = 19)		Oiled (n = 12)		"Nonoiled" (n = 11)		Oiled (n = 23)		"Nonoiled" (n = 21)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Hct	43.07	1.36	42.09	1.36	40.23	0.96	43.10	1.00	47.18	0.84	46.98	0.92
Hgb	15.33	0.45	14.59	0.45	13.73	0.27	14.64	0.29	15.40	0.23	15.72	0.24
MCH	17.25	0.14	16.67	0.14	17.44	0.24	16.69	0.25	17.51	0.14	17.21	0.15
MCHC	35.68	0.24	34.69	0.24	34.18	0.44	34.01	0.46	33.12	0.24	33.51	0.25
RBC	8.91	0.28	8.75	0.28	7.89	0.19	8.78	0.20	8.80	0.14	9.14	0.15
RDW	33.64	1.83	31.37	1.83	19.43	2.14	26.48	2.24	28.07	1.92	31.32	2.01
WBC	11.12	1.57	11.97	1.57	10.57	1.30	14.99	1.36	11.17	1.06	10.33	1.11
Lymph	10.54	1.66	14.38	1.66	17.58	2.02	10.55	2.11	11.28	1.77	8.81	1.94
Neuts	87.38	1.68	84.54	1.68	81.33	2.16	86.73	2.25	80.80	1.76	86.33	1.92
Mono	1.62	0.40	0.77	0.40	0.83	0.37	1.82	0.39	5.37	0.82	4.33	0.89
Eos	0.31	0.23	0.15	0.23	0.25	0.24	0.36	0.25	2.40	0.55	0.48	0.60
Baso	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.05	0.03

Table 5. Continued

Blood Variables	1996				1997				1998			
	Oiled (n = 20)		"Nonoiled" (n = 19)		Oiled (n = 12)		"Nonoiled" (n = 11)		Oiled (n = 23)		"Nonoiled" (n = 21)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Bands	0.15	0.13	0.15	0.13	0.00	0.24	0.55	0.25	0.00	0.03	0.05	0.03
PLAT	466.15	31.18	398.46	31.18	458.50	33.96	329.18	35.47	366.48	10.57	357.24	11.06

Table 6. Loading values for principal component analysis (PCA) on blood values of river otters from Prince William Sound, Alaska, USA. These parameters were identified by S-Plus software as being the most influential variables in the first three principal components (PCs). The first three PCs accounted for 43% of the variability in data sets.

	Loading Values		
Variable	PC1	PC2	PC3
Low Density Lipids	0.338	—	—
Cholesterol	0.335	—	—
Albumin/Globulin Ratio	-0.312	—	—
Globulin	0.302	—	—
Cholesterol/High Density Lipid Ratio	0.287	—	—
Phosphorous	0.256	—	—
Aspartate Aminotransferase (AST)	—	0.403	—
Alanine Aminotransferase (ALT)	—	0.353	—
Lactate Dehydrogenase (LDH)	—	0.293	—
Total Bilirubin	—	0.292	—
Direct Bilirubin	—	0.261	—
Sodium	—	0.255	—
Triglyceride	—	—	0.382
Very Low Density Lipids (VLDL)	—	—	0.373
High Density Lipids	—	—	0.351
Serum Creatinine	—	—	-0.302
Calcium	—	—	0.316
Haptoglobin (Hp)	—	—	-0.284

Table 7. Adjusted body mass and total length corrected for sex and age classes (ANCOVA) of river otters captured in oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, 1996–98.

Sex and Age Classes	Oiled					"Nonoiled"				
	Body Mass (kg)			Total Length (mm)		Body Mass (kg)			Total Length (mm)	
	n	$\bar{x}$	SE	$\bar{x}$	SE	*n*	$\bar{x}$	SE	$\bar{x}$	SE
Males										
Yearlings	6	7.3	0.3	1,218.0	30.2	6	7.3	0.6	1,171.0	27.9
Adults	36	9.1	0.2	1,287.0	12.2	34	9.8	0.2	1,287.0	8.7
Females										
Yearlings	0	—	—	—	—	0	—	—	—	—
Adults	18	8.2	0.2	1,261.0	1.7	17	8.0	0.2	1,232.0	13.7

Table 8. Densities (fish/100 m^2) of marine fishes ≥8 cm in length at river otter latrine sites and randomly selected sites in Jackpot Bay and Herring Bay, Prince William Sound, Alaska, USA, July 1996 and 1997.

	1996								1997							
	Herring Bay				Jackpot Bay				Herring Bay				Jackpot Bay			
	Latrine		Random		Latrine		Random		Latrine		Random		Latrine		Random	
Group	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Green-lings	7.8	1.6	8.3	2.1	2.5	0.8	2.4	0.8	6.2	2.3	5.6	1.6	4.2	2.0	1.6	0.6
Prickle-backs	6.7	2.5	7.6	2.1	0.7	0.4	3.1	1.4	4.0	2.9	3.8	1.3	6.8	3.7	6.6	2.2
Gunnels	2.9	1.3	3.3	1.3	0.3	0.3	1.6	0.7	2.2	1.8	0.2	0.2	0.7	0.5	1.3	0.8
Cod	1.9	0.9	2.0	0.6	2.4	1.7	1.4	0.8	267.0	117.9	68.7	5.7	4.1	1.7	8.7	3.5
Rock-fishes	1.3	0.7	0.0	0.0	4.2	2.2	1.6	1.1	1.3	0.7	0.0	0.0	2.2	1.3	0.8	0.8
Sculpins	0.7	0.4	0.4	0.3	0.3	0.3	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.7	0.5
Ronquils	0.7	0.4	0.4	0.3	4.3	2.2	2.4	1.4	0.2	0.2	0.7	0.7	6.8	2.3	4.0	1.9
Others	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 9. Use (latrine sites) and availability (random sites) of selected shoreline habitats for river otters on "nonoiled" (Esther Passage) and oiled (Herring Bay) areas, during summer 1990 (adapted from Bowyer et al. 1995), and on "nonoiled" (Dangerous Pass) and oiled (Herring Bay) areas 1996–97, Prince William Sound, Alaska, USA. For 1990, only habitat characteristics that were selected from a suite of 19 variables by logistic regression are presented here; the complete list of those variables is provided by Bowyer et al. (1995).

Habitat variables	"Nonoiled" 1990				Oiled 1990				"Nonoiled" 1996–97				Oiled 196–970			
	Random (*n*=180)		Latrine (*n*=113)		Random (*n*=210)		Latrine (*n*=128)		Random (*n*=61)		Latrine (*n*=89)		Random (*n*=32)		Latrine (*n*=67)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Tidal Slope (°)	23.9	1.19	18.7	1.06	25.2	1.00	27.7	1.04	23.1	1.8	17.5	1.5	15.2	2.5	18.6	1.8
Vegetated Slope (°)	37.7	1.34	26.8	1.09	36.0	1.11	29.3	0.80	46.7	2.0	29.9	1.7	36.9	2.8	31.9	2.0
Old-growth/overstory (ranked on a 0–4 scale)	1.5	0.08	2.3	0.08	1.1	0.06	2.8	0.10	1.9	0.2	2.1	0.09	1.5	0.2	2.5	0.1
Brush/understory (ranked on a 0–4 scale)	1.4	0.07	1.0	0.07	0.6	0.05	0.5	0.05	1.9	0.1	2.1	0.09	2.3	0.2	2.9	0.1
Bedrock (ranked on a 0 –4 scale)																
Large Rock (ranked on a 0–4 scale cover)	1.5	0.10	0.9	0.10	1.4	0.10	1.9	0.14	0.6	0.1	0.5	0.1	0.4	0.2	0.6	0.2
Small Rock (ranked on a 0–4 scale cover)									0.8	0.1	0.5	0.1	0.9	0.2	0.2	0.1
Gravel (ranked on a 0–4 scale cover)									1.1	0.1	0.8	0.1	0.6	0.2	0.007	0.1
Sand (ranked on a 0–4 scale cover)									0.3	0.1	0.4	0.1	0.001	0.1	0.003	0.1
Exposure (ranked on a 1–3 scale cover)									1.1	0.1	1.1	0.1	0.9	0.1	1.4	0.1

Table 10. *P*-values for variables included in the meta-analysis of data collected on river otters in Prince William Sound, Alaska, USA, in 1996–98.

Variable	*P*-value early phase	*P*-value later phase
Age structure	—	0.36
Body mass	0.04	0.62
Diet	0.001	0.20
Haptoglobin	0.04	0.52
Home range	0.05	0.97
Tidal slope	0.01	0.21
Survival	—	0.20

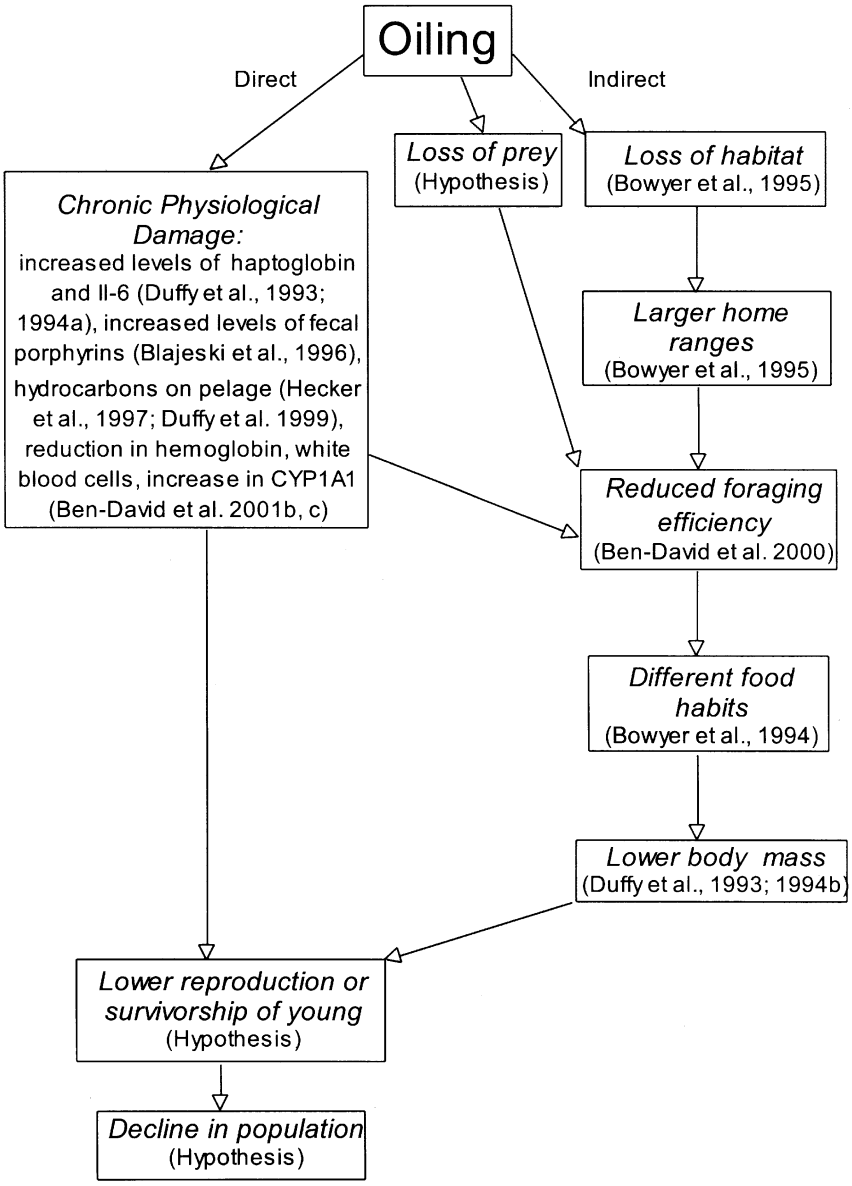

Figure 1. Potential effects of the *Exxon Valdez* oil spill on river otters in Prince William Sound, Alaska, USA.

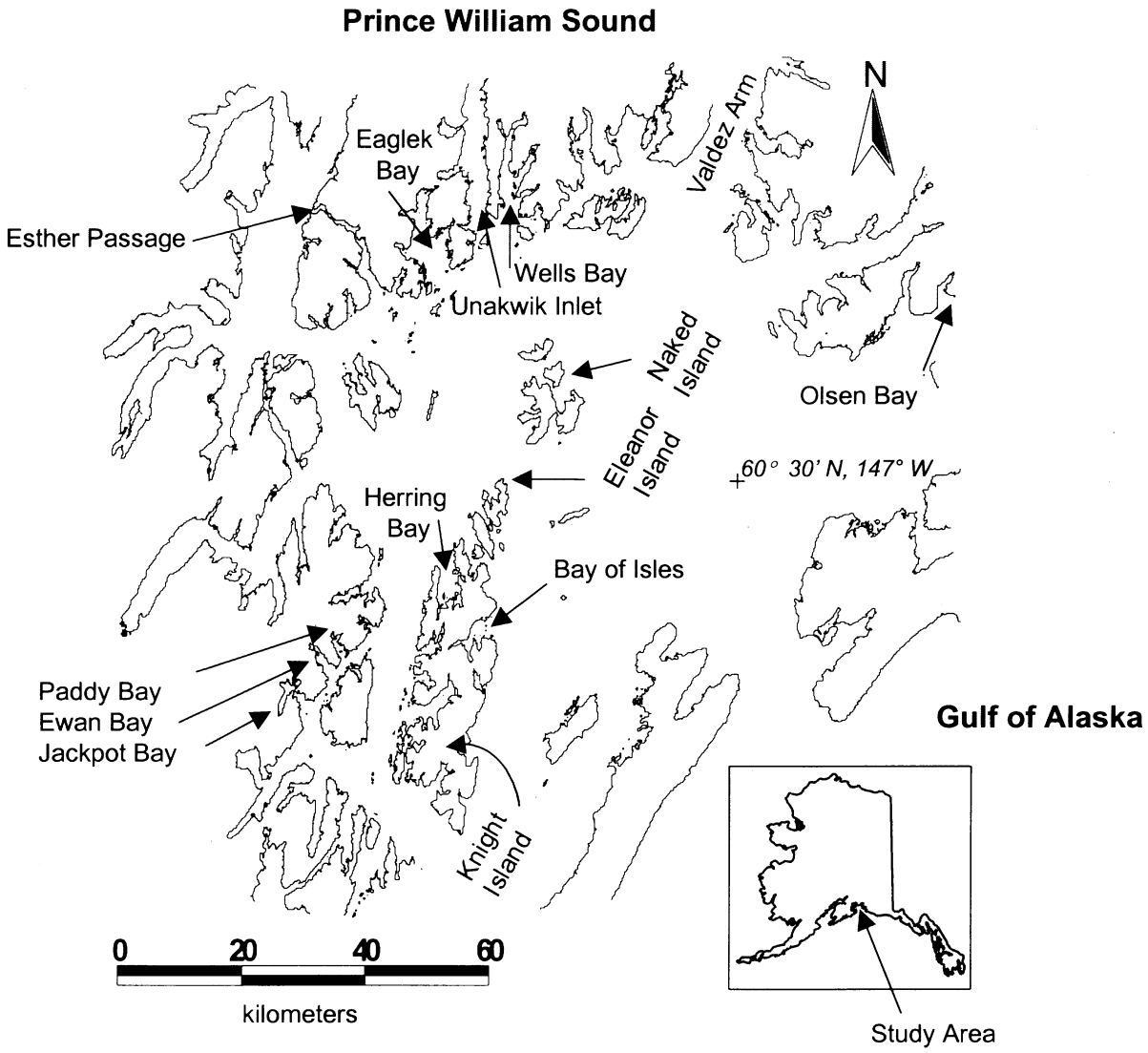

Figure 2. Areas of Prince William Sound, Alaska, USA, where river otters were live-captured from 1990 to 1998.

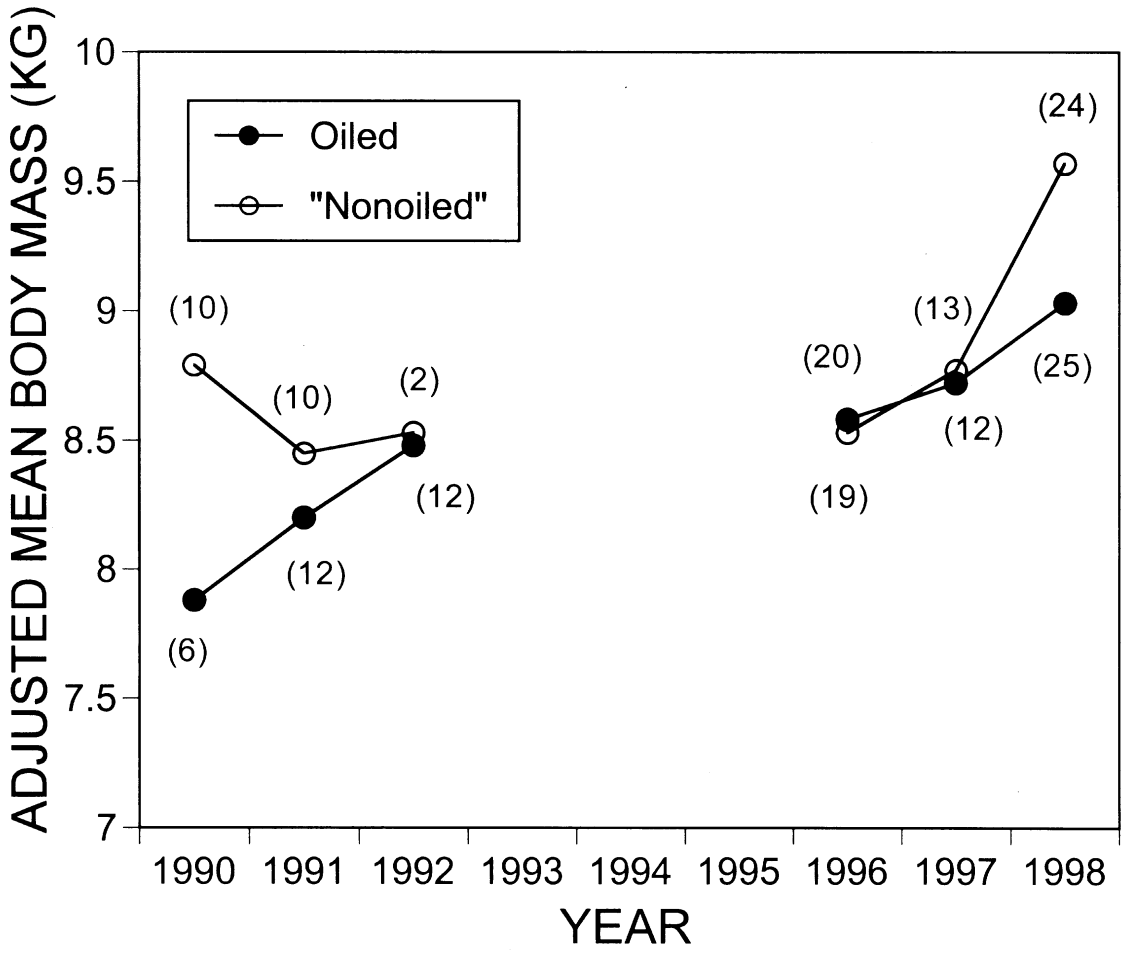

Figure 3. Mean body mass of river otters adjusted for sex, age class, and total body length with ANCOVA, inhabiting oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, following the *Exxon Valdez* oil spill. Sample sizes are provided in parentheses. Data are missing between 1993 and 1995. ANCOVA indicated significant differences ($P = 0.04$) between otters living in oiled and "nonoiled" areas only during 1990 and 1991. Data from 1990 to 1992 are from Duffy et al. (1993, 1994*b*).

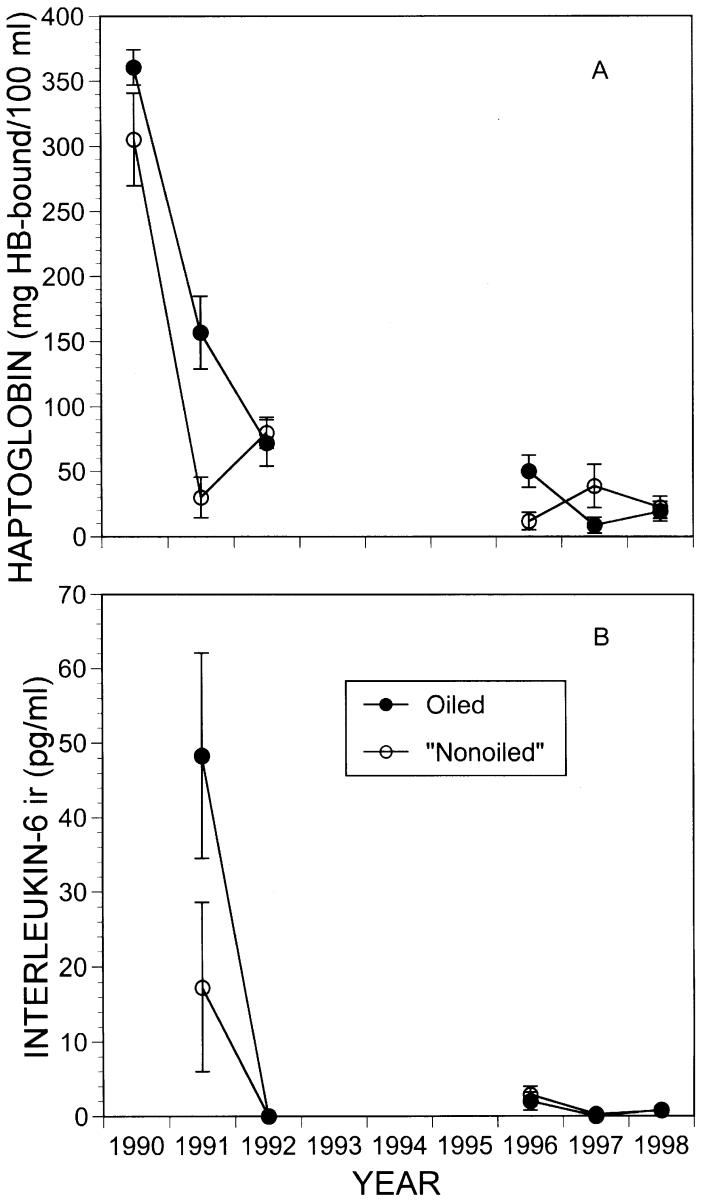

Figure 4. Mean (± SE) haptoglobin (*A*; an acute-phase protein) and interleukin-6 *ir* (*B*; a cytokine) values for river otters inhabiting oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, following the *Exxon Valdez* oil spill. Sample sizes are provided in Figure 3. Data are missing between 1993 and 1995. In 1996–98 a more sensitive assay for IL-6 *ir* was used. ANOVA indicated significant differences ($P < 0.05$) between oiled and "nonoiled" areas in haptoglobin values in 1990, 1991, and 1996; interluken-6 *ir* differed ($P \leq 0.05$) only in 1991.

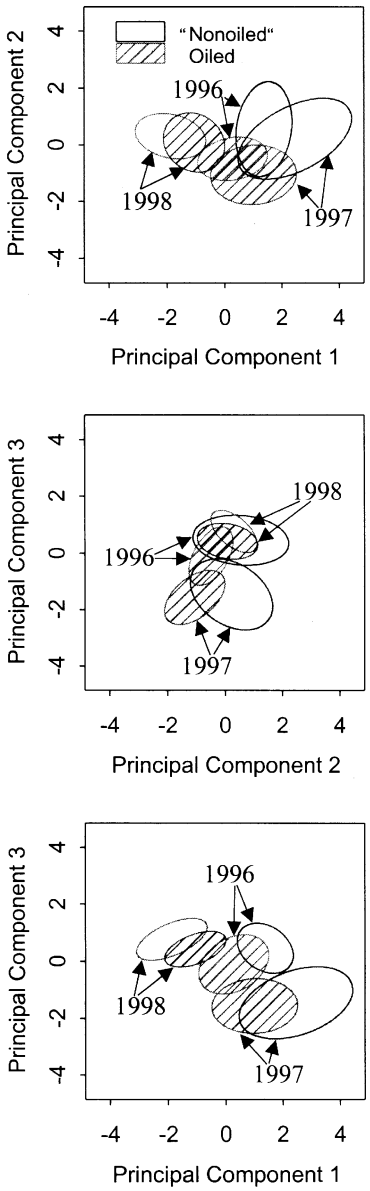

Figure 5. Principal component analysis (PCA) on blood variables of river otters captured in oiled and "nonoiled" areas in Prince William Sound, Alaska, USA, during 1996–98. Ellipses represent 95% confidence intervals for oiled and "nonoiled" areas for each year. PC1, which explained 21.1% of the variability in 28 blood values, was related to diet. PC2 (12.3%) represented the health of otters, and PC3 (9.4%) included blood values influential in explaining the response of otters to trapping conditions. Data from 1996–97 are from Herring (oiled) and Jackpot ("nonoiled") bays; in 1998 otters were captured throughout oiled and "nonoiled" areas of the Sound.

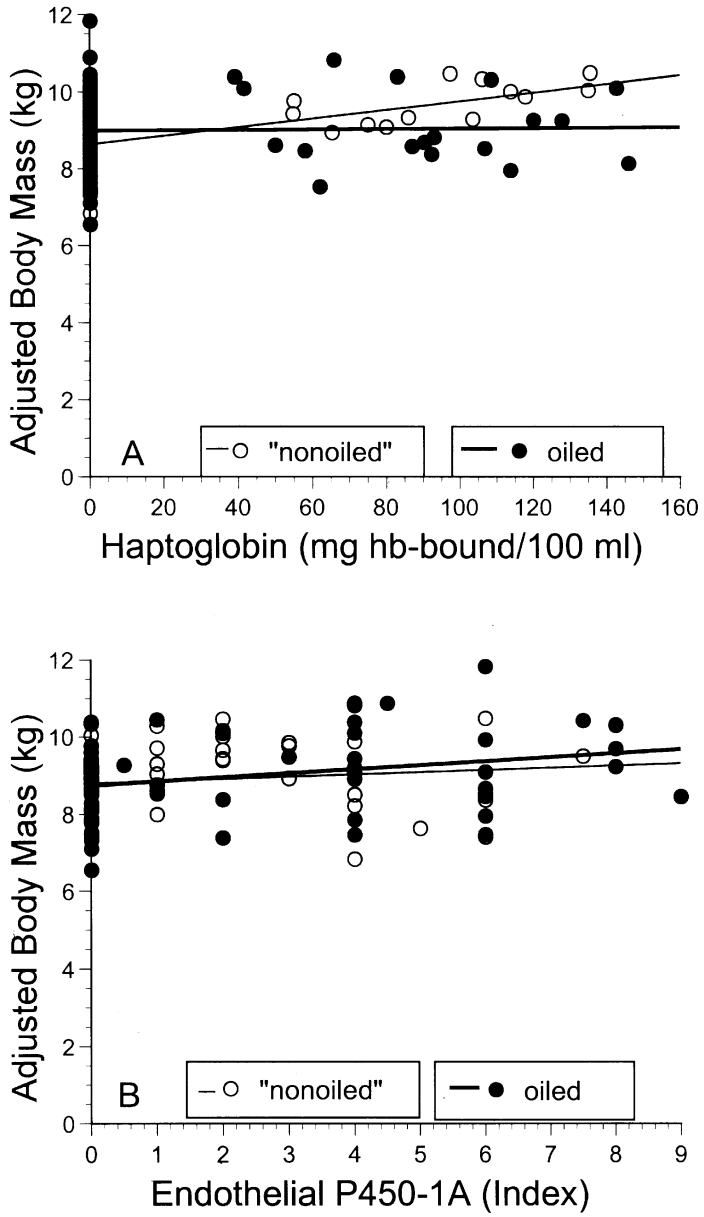

Figure 6. Linear regressions between adjusted body mass and haptoglobin (*A*) revealed a significant positive relation ($r^2 = 0.053$, $P = 0.014$) for all otters. When treatment was assessed individually, that positive relation was significant for otters in "nonoiled" areas ($r^2 = 0.214$, $P = 0.0004$), but not oiled areas ($r^2 = 0.007$, $P = 0.85$). The relation between adjusted body mass and *P*450-1A (*B*) exhibited a significant positive relation for all otters ($r^2 = 0.051$, $P = 0.02$), but no relation was observed in "nonoiled" areas ($r^2 = 0.014$, $P = 0.4$), and a positive relation ($r^2 = 0.083$, $P = 0.03$) was observed in the oiled area. Data were collected in Prince William Sound, Alaska, USA, from 1996 to 1998.

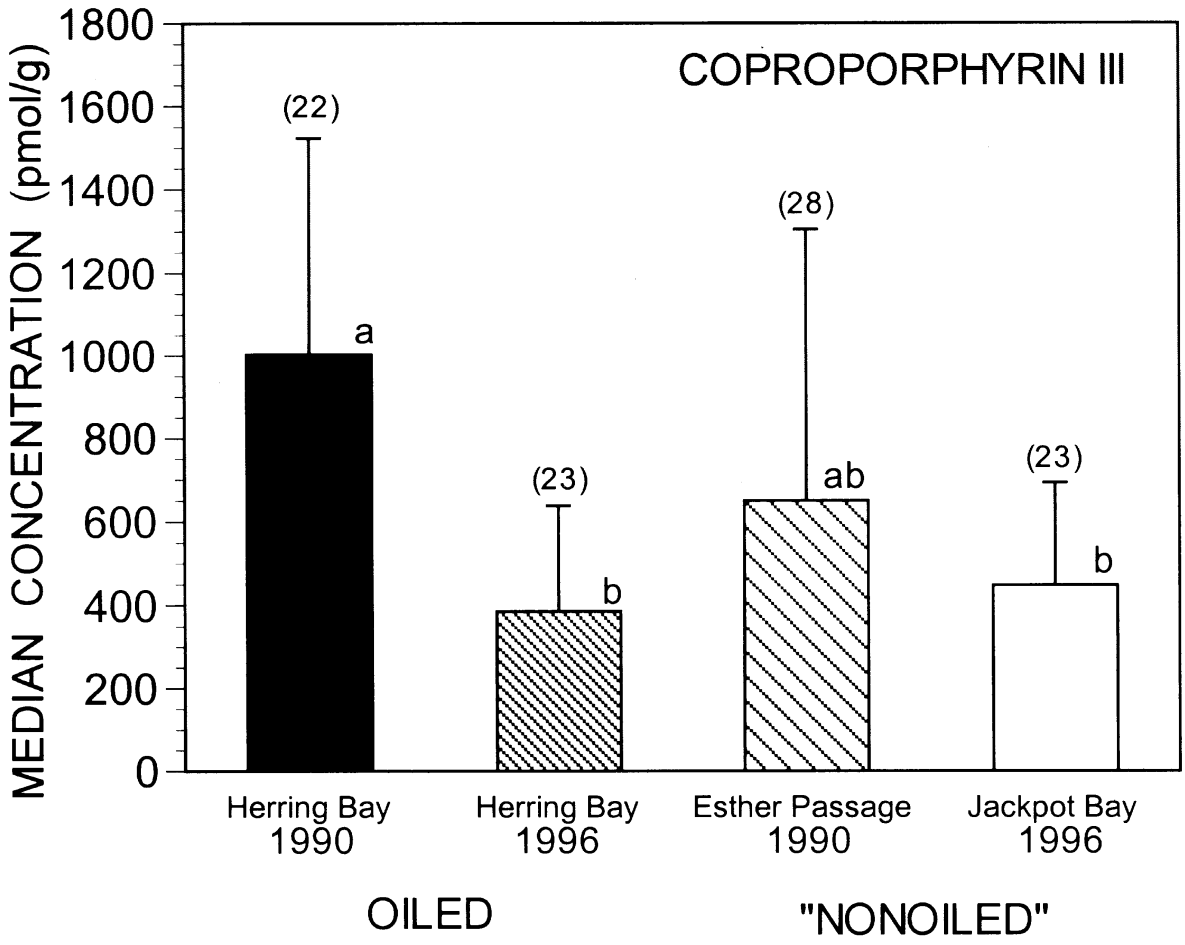

Figure 7. Median concentrations of coproporphyrin III in the feces of river otters from oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, 1 year following the *Exxon Valdez* oil spill (1990) and 6 years later (1996). Sample sizes are provided above error bars, which depict one-half the interquartile distance. Letters above bars that differ indicate significantly different ($P < 0.05$) concentrations as determined by planned contrasts from a one-tailed ANOVA on ranked data (modified from Taylor et al. 2000*a*).

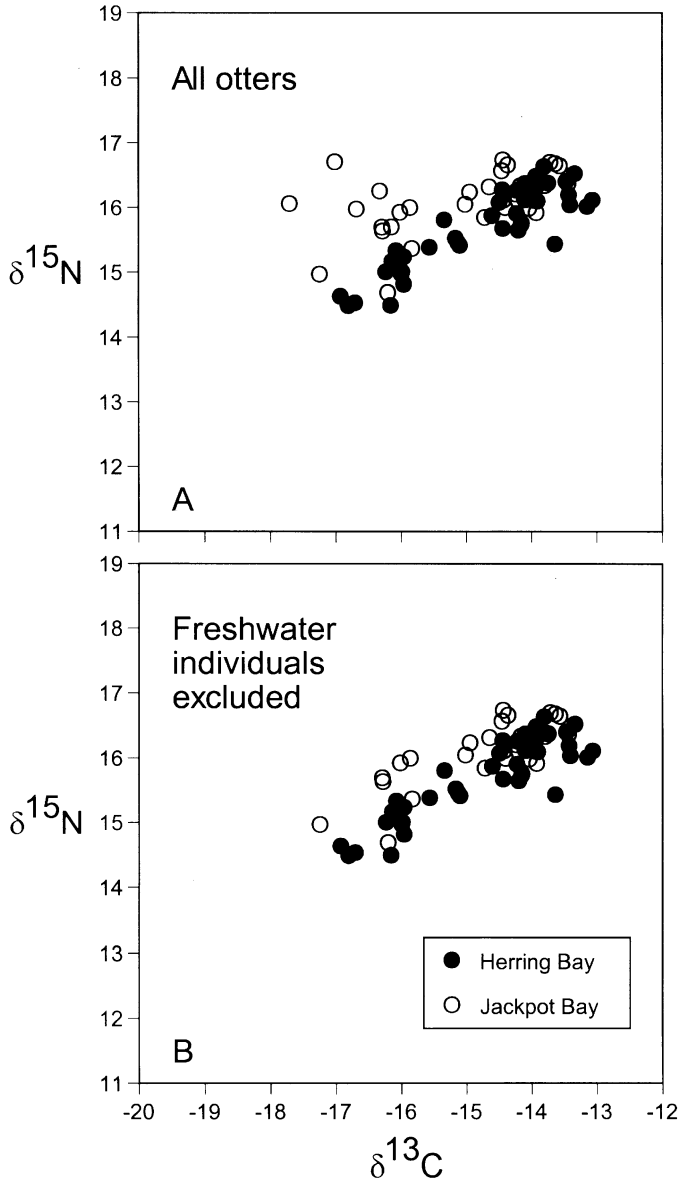

Figure 8. Stable isotope values of the guard hairs of individual river otters reflecting their diets and, hence, trophic position on oiled (Herring Bay) and "nonoiled" (Jackpot Bay) areas of Prince William Sound, Alaska, 1996–97. MANOVA with $\delta^{13}C$ and $\delta^{15}N$ as dependent variables with all otters (n = 64) included (*A*), produced a significant (P = 0.02) overall model with a year (P = 0.001) but no area (P = 0.22) effect. When 6 river otters that used fresh-water habitats (determined from radiotelemetry) were withheld from analysis (*B*), the overall model was no longer significant (P = 0.20).

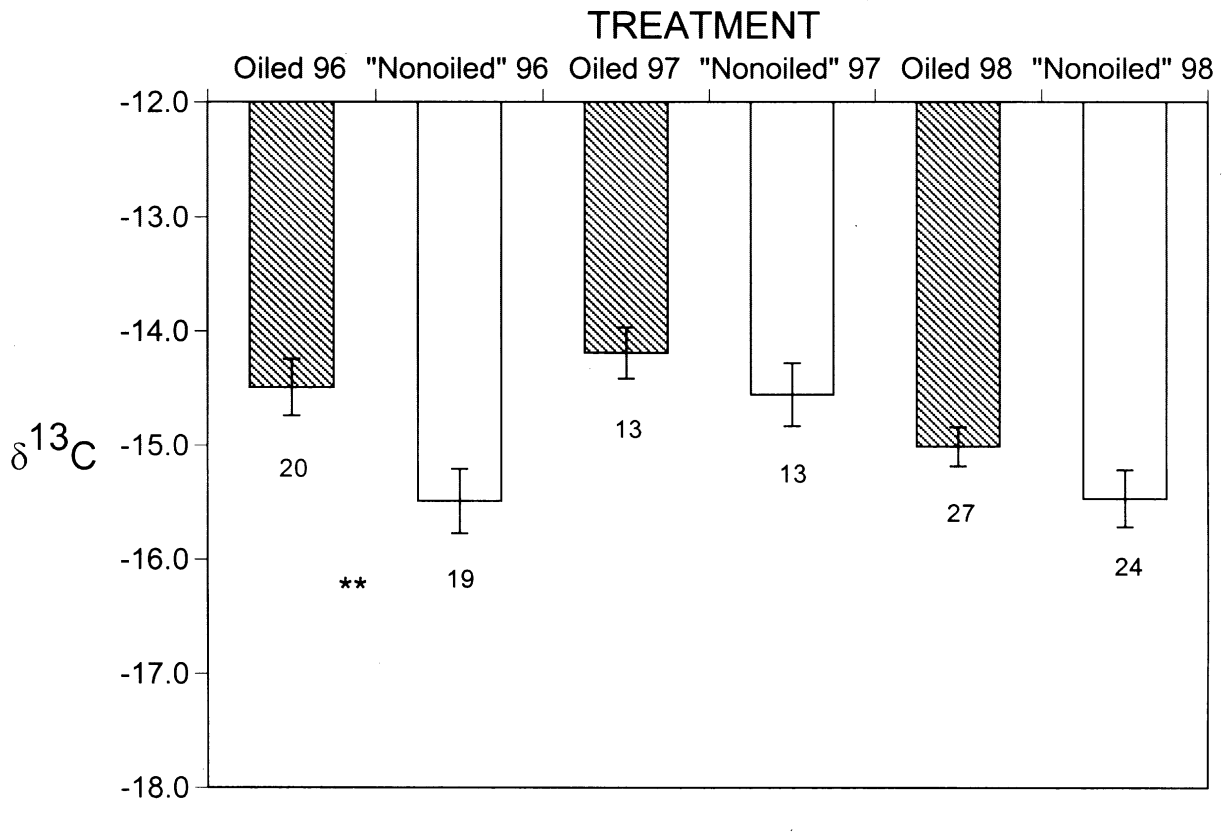

Figure 9. Values (mean ± SE) of $\delta^{13}C$ for river otters plotted against area and year. Sample sizes are provided below error bars. Significant differences ($P \leq 0.001$) are represented by **. Analysis revealed an area effect ($P = 0.004$) and a year effect ($P = 0.005$), but no area by year interaction ($P = 0.4$), indicating that dietary changes between years were inconsistent between oiled and "nonoiled" areas in Prince William Sound, Alaska, USA, 1996–97. These differences in abundance may reflect variable patterns in distribution of fishes at specific locations in different years, with no relation to *EVOS*.

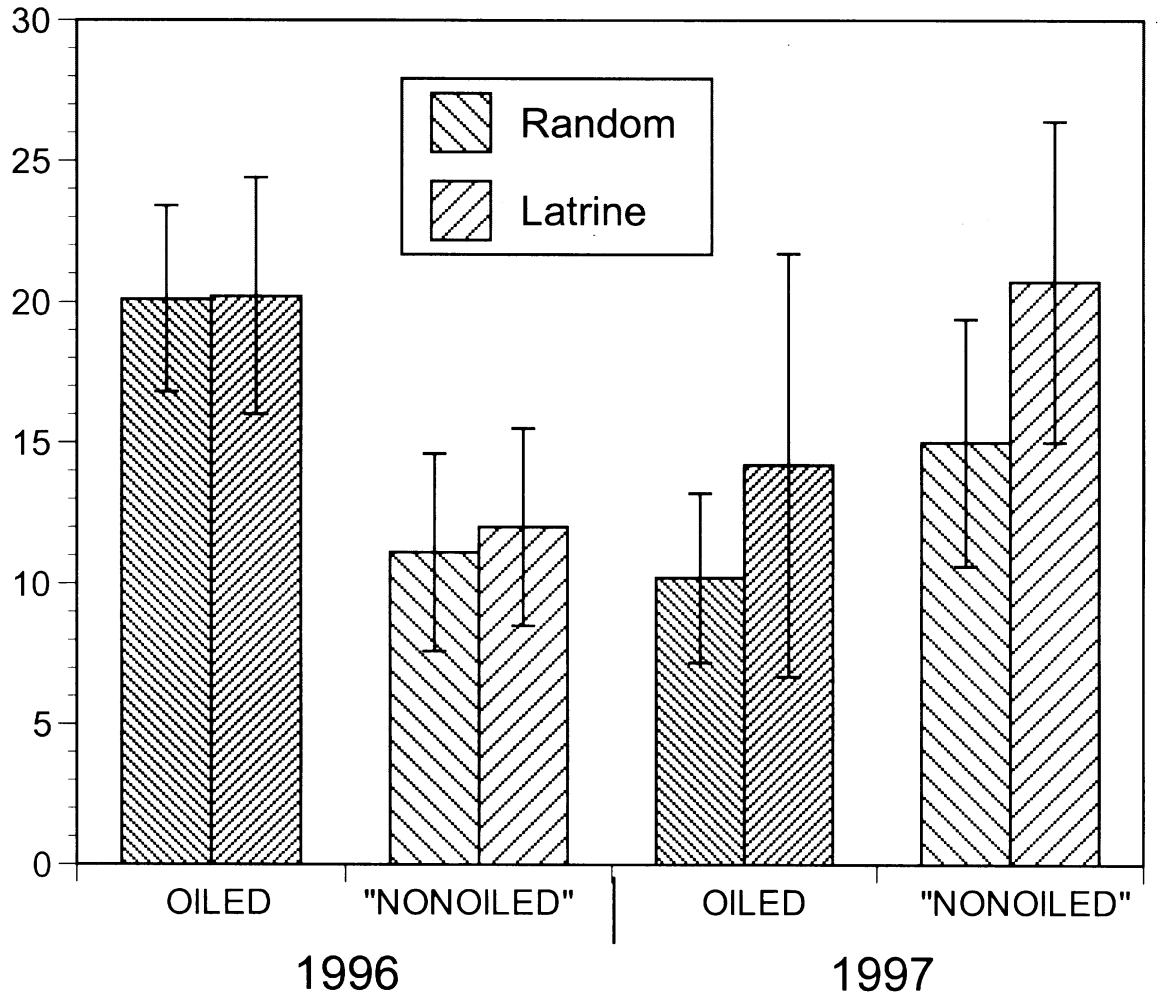

Figure 10. Mean (± SE) density of demersal fishes ≤8 cm in total length (excluding cod) sampled at both latrine sites of river otters and at randomly selected sites on oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, summer 1996–97. ANOVA indicated a year effect ($P = 0.02$) and a year by area interaction ($P < 0.01$). No differences ($P > 0.05$) occurred between areas, or between latrines and random sites.

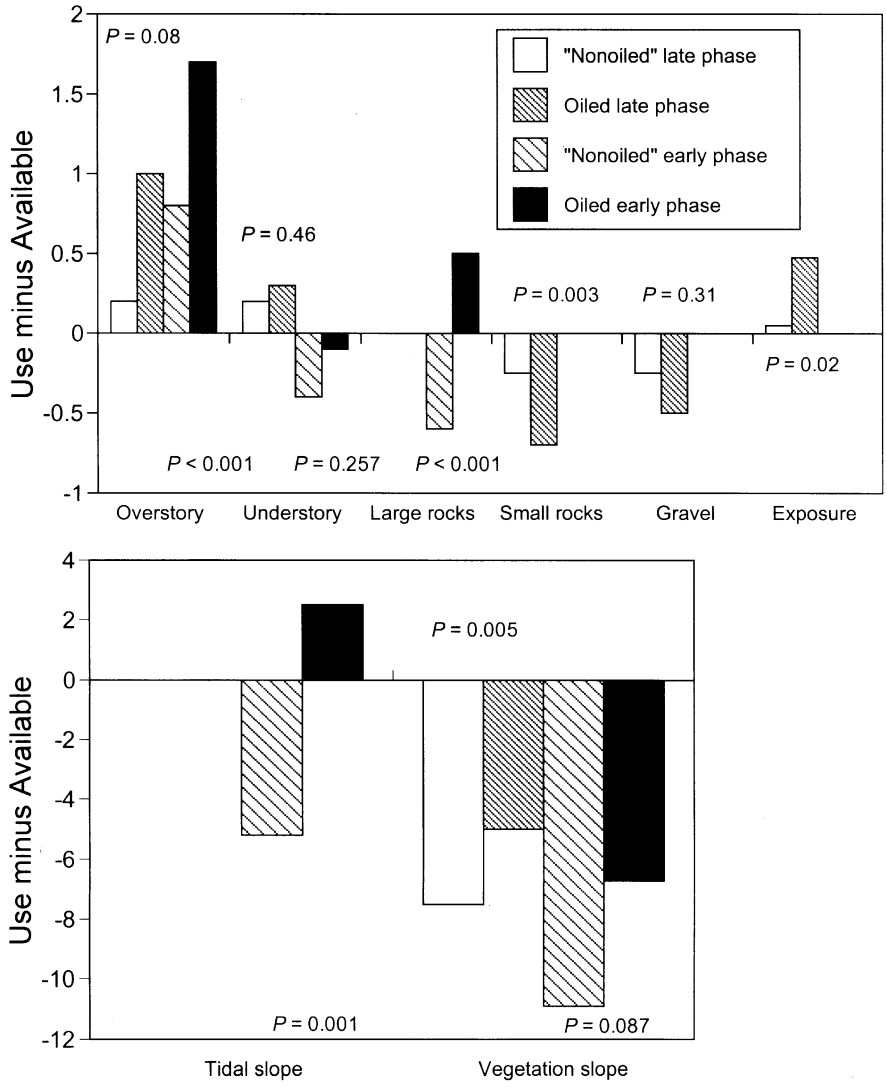

Figure 11. Used (latrine sites) minus available (random sites) shoreline habitats on "nonoiled" (Esther Passage) and oiled (Knight Island) study areas in Prince William Sound, Alaska, USA, during summer 1990 and 1996–97. Filled bars represent data from Herring Bay 1990, loose etching represents Esther Passage in 1990, tight etching represent Herring Bay in 1996–97, and clear bars represent Jackpot Bay 1996–97. Positive values indicate selection for a habitat feature, whereas negative ones represent avoidance. Analysis was conducted on untransformed data and not on the selection indices (i.e., used minus available), which are presented for descriptive purposes only. Selection of habitat variables for the MANOVA was based on results from logistic regression. *P*-values represent post-hoc comparisons following MANOVA. Values above bars correspond with late-phase data, whereas values below represent early phase data. Although differences in habitat selection between oiled and "nonoiled" sites still occurred in 1996–97 ($P < 0.001$), the direction of selection on oiled and "nonoiled" areas was no longer different.

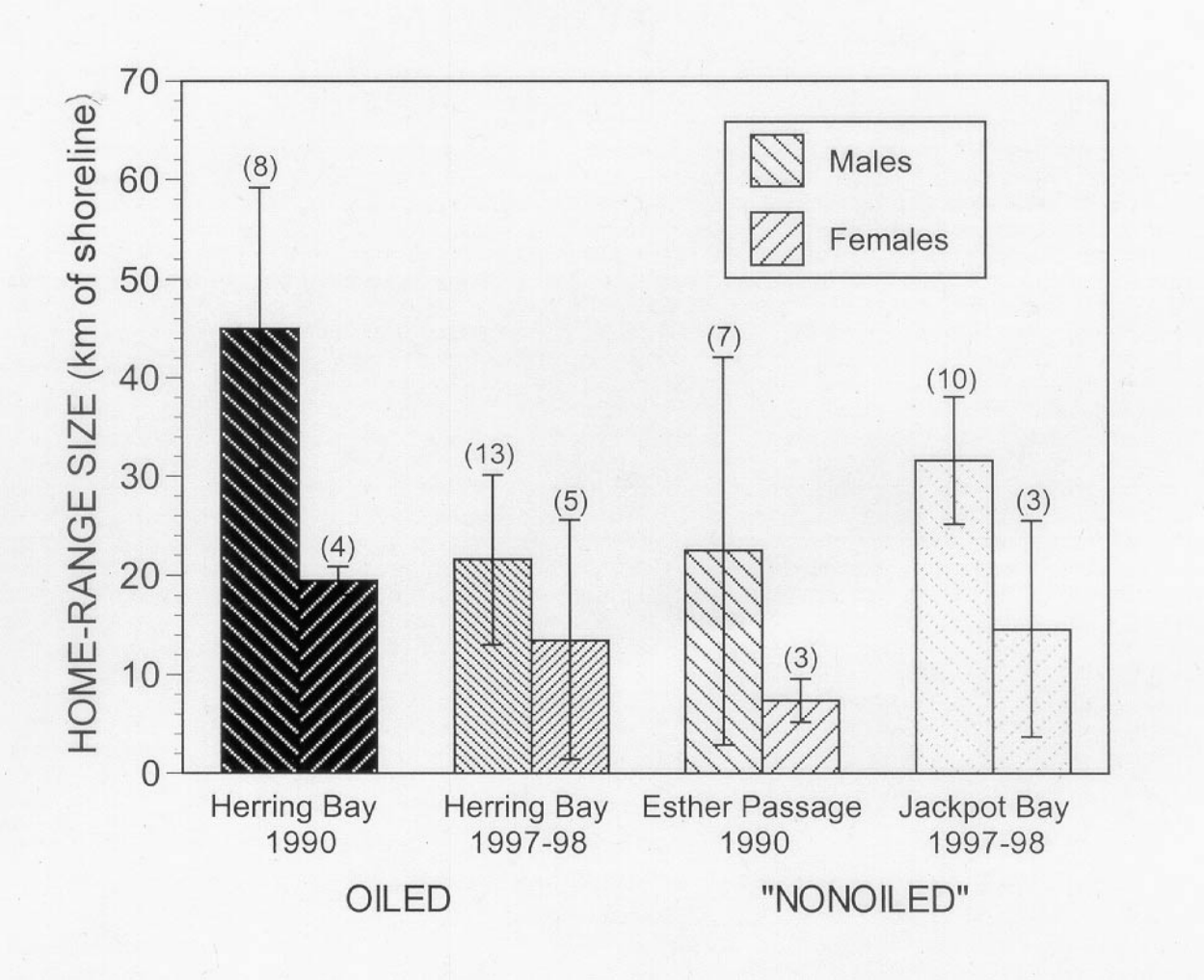

Figure 12. Home-range size ($\bar{x}$ ± 95% confidence limits) for river otters calculated with the conservative method of Bowyer et al. (1995) for oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, in 1990 and again in 1997–98. Sample sizes are provided in parentheses. A 2-way ANOVA on home-range size with area and gender as main effects on data collected in the early phase of the study indicated home ranges were larger on oiled vs "nonoiled" areas (area $P < 0.05$, sex $P < 0.1$, interaction $P > 0.75$; Bowyer et al. 1995). Note the marked decline in the size of home ranges for otters living in the oiled area (Herring Bay) between 1990 and 1997–98.

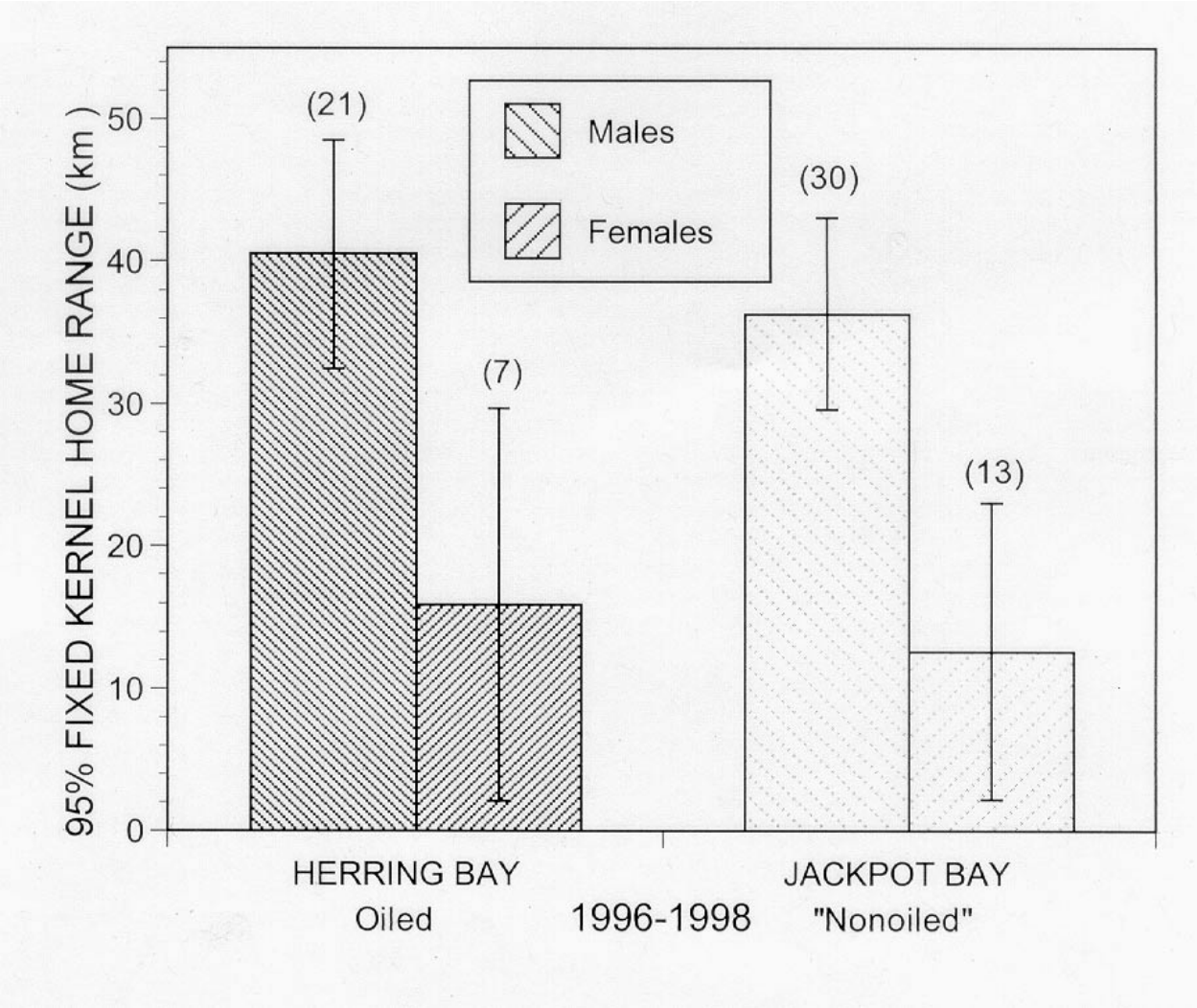

Figure 13. Mean (± SE) home-range size for river otters using marine habitats in Herring Bay (oiled) and Jackpot Bay ("nonoiled"), Prince William Sound, Alaska, USA, from 1996 to 1999. Ranges V software was used to calculate 95% fixed-kernel estimates of area. ARC-INFO was used to measure kilometers of shoreline within the isolines. Size of home ranges for otters in oiled and "nonoiled" areas did not differ (MANOVA blocked by otter to control for multiple years of data for the same individual; $P = 0.7$), and no differences occurred between years ($P = 0.7$). Home-range size was significantly larger for males compared with females ($P = 0.03$).

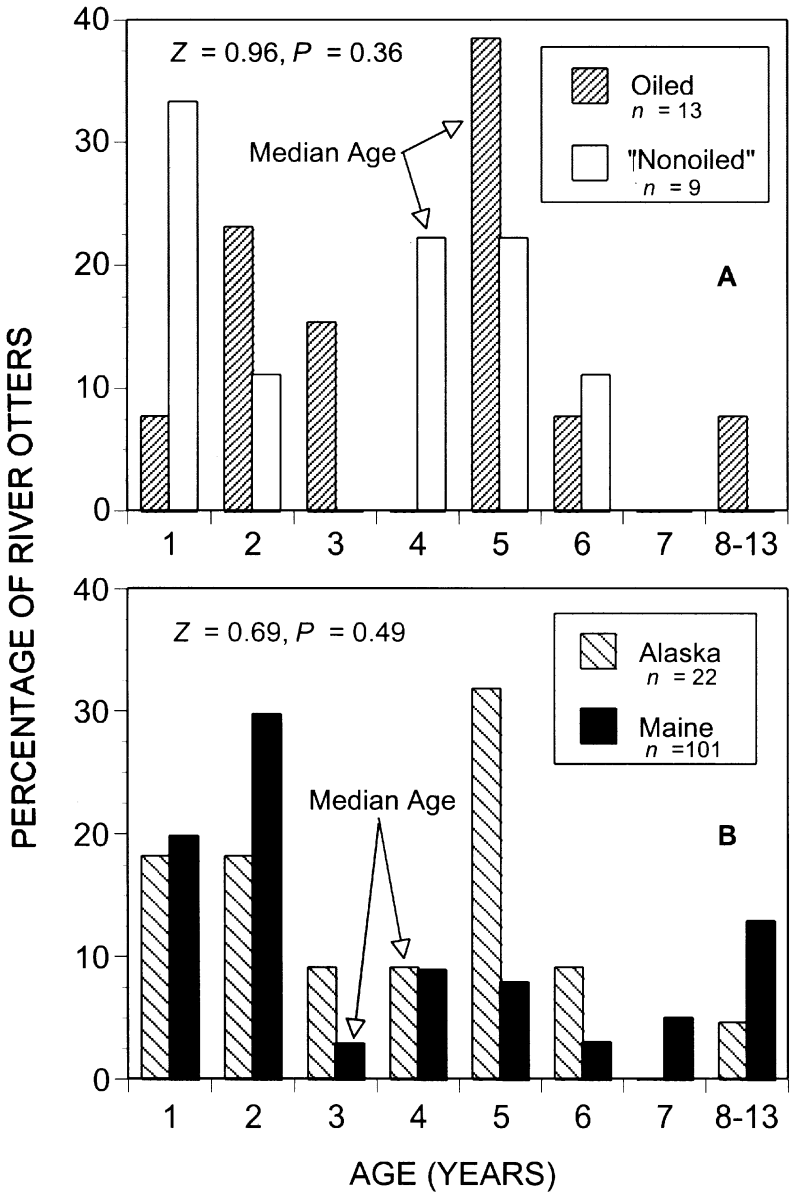

Figure 14. Age structure of river otters inhabiting oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, in 1997 (*A*), and a comparison of these same animals from Alaska, with river otters harvested in Maine, USA, during 1982 (*B*). *Z*-scores and *P*-values indicating no differences in medians were derived with the Mann-Whitney test. The zero age class was omitted because those individuals were still in dens when trapping was conducted in Alaska. Data for Maine are from Docktor et al. (1987) and files of the Maine Department of Inland Fisheries and Wildlife.

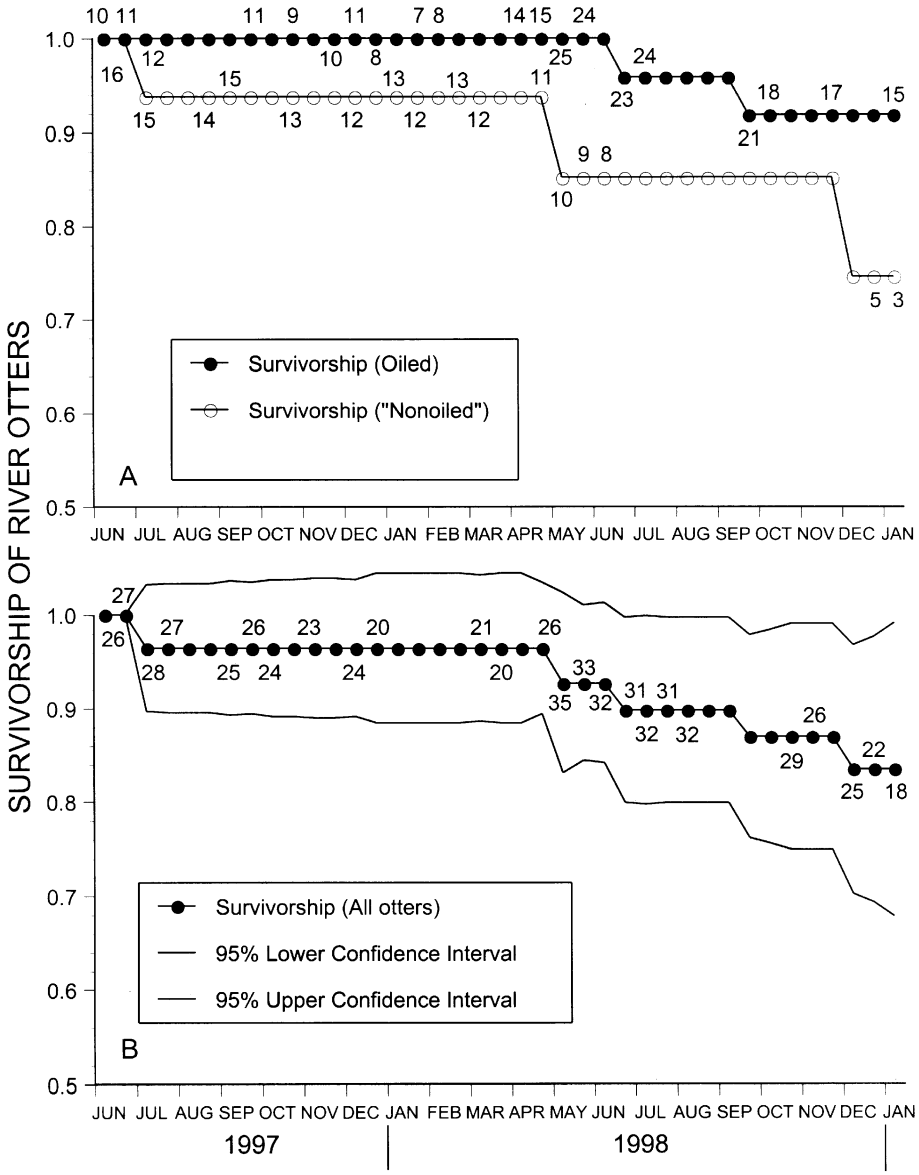

Figure 15. Survivorship of river otters with radiotransmitters inhabiting oiled and "nonoiled" areas of Prince William Sound, Alaska, USA, by one-half month intervals from late June 1997 to early January 1999 (*A*), and combined survivorship of otters with 95% confidence intervals for that same period (*B*). Numbers adjacent to circles represent changes in otters at risk used for calculating survivorship from the Kaplan-Meier, staggered-entry model (Pollock et al. 1989). The log-rank test indicated no significant difference ($P > 0.20$) in survivorship of river otters inhabiting oiled and "nonoiled areas" (*A*).

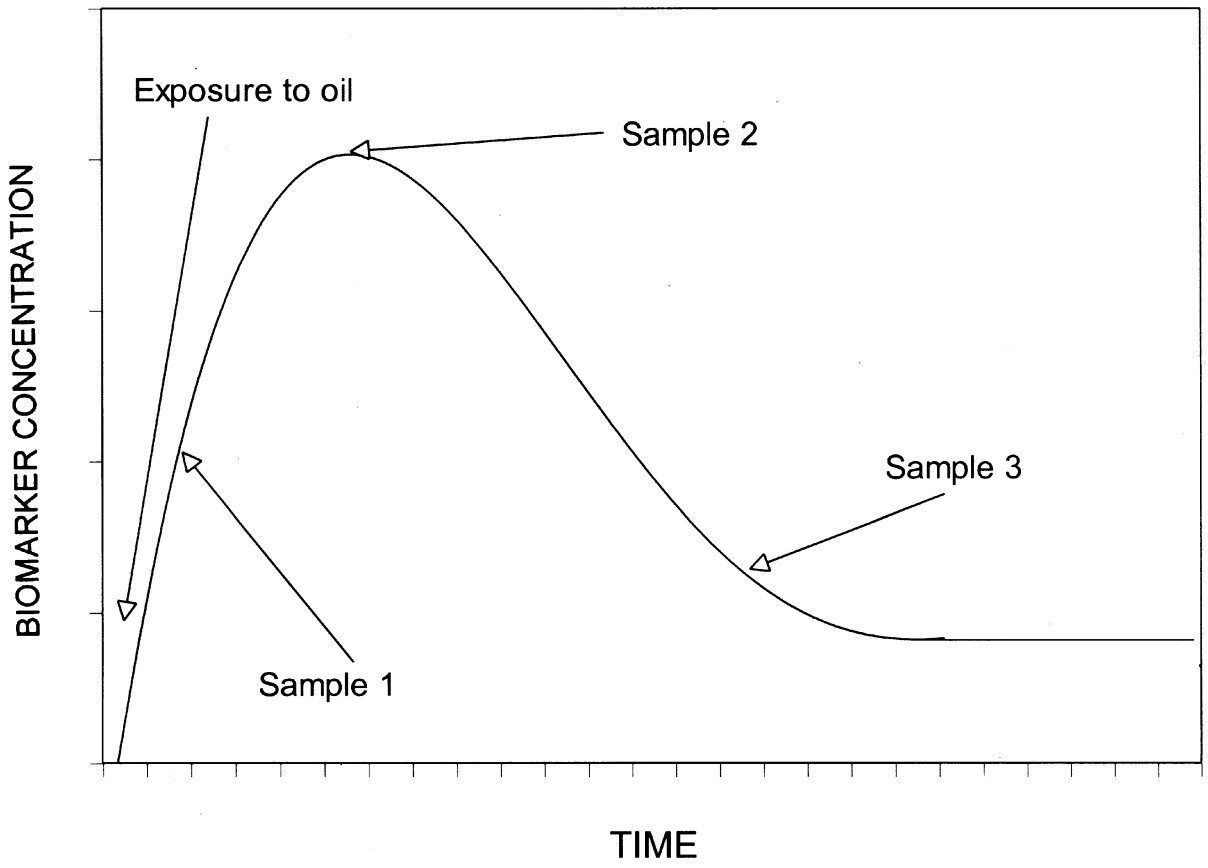

Figure 16. A hypothetical dose-response curve for concentration of a biomarker in an individual animal. Note that the time of collection of each sample (Samples 1–3) relative to the initial exposure will determine the value of the measured response. This dependency on timing of sampling renders interpretation of field data collected from a population as if it were an individual, largely meaningless.

Appendix A. Sample sizes of live-captured river otters

In 1989, we captured 7 otters (3 males, 4 females) in Herring Bay (oiled), and no otters in the "nonoiled" area. In 1990, 7 otters were captured in Herring Bay (all males) and 9 otters (7 males, 2 females) were captured in Esther Passage ("nonoiled"). In 1991, 12 otters (4 males, 8 females) were captured in oiled areas and 11 otters (2 males, 9 females) were captured in "nonoiled" areas. In 1992, we captured 10 individuals (9 males, 1 female) in oiled areas and in "nonoiled" areas 2 animals were captured (1 male, 1 female). In 1996, we captured 20 otters (12 males, 8 females) in Herring Bay, and 19 individuals (13 males, 6 females) were captured in the Jackpot Bay area ("nonoiled"). In 1997, 13 otters were captured in each of those areas with identical sex ratios (5 females and 8 males). In 1998, we captured 27 otters (22 males, 5 females) in oiled areas and 24 otters (18 males, 6 females) in "nonoiled" areas. During handling or shortly thereafter in 1989-90, 7 otters died but no histopathology reports were available. Two individuals captured in Jackpot Bay died during processing in 1997; post-mortem histopathology revealed that the juvenile male had pleuritis and the adult female had an abscessed ovary. These were the only mortalities of 111 individual otters captured 132 times from 1996 to 1998.

During 1989-91, a total of 27 otters were implanted with radiotransmitters, but due to premature failure of transmitters or mortality only 22 otters (15 males, 7 females) were available for radio-tracking (Testa et al., 1994; Bowyer et al., 1995). In 1996, we implanted 17 otters (12 males, 5 females) with transmitters in Jackpot Bay; no otters were telemetered in Herring Bay in that year. In 1997, 8 additional otters were equipped with radio-transmitters in Jackpot Bay (5 males, 3 females), and 12 otters were implanted with transmitters in Herring Bay (8 males, 4 females). In 1998, 9 otters received transmitters in Herring Bay (8 males, 1 female).

Appendix B. Abbreviations and units for blood-serum variables measured in river otters in Prince William Sound, Alaska, USA.

Variable Name	Abbreviation	Units of Measurement
Alanine Aminotransferase	ALT	U/L
Albumin	ALB	g/dL
Albumin/Globulin Ratio	AG Ratio	—
Alkaline Phosphatase	ALK PHOS	U/L
Aspartate Aminotransferase	ALT	U/L
Blood Urea Nitrogen	BUN	mg/dL
Calcium	Ca	mg/dL
Chloride	Cl	mEq/L
Cholesterol	CHOL	mg/dL
Cholesterol/High Density Lipid Ratio	CHOL/HDL	—
Creatine phosphokinase	CPK	IU/L
Direct Bilirubin	Dir Bili	mg/dL
Gamma Glutamyl	GGT	U/L
Globulin	GLOB	g/dL
Glucose	GLU	mg/dL
Haptoglobin	Hp	mg hb-bound/100 ml Hemoglobin [hb]
High Density Lipids	HDL	mg/dL
Interleukin-6 immunoreactive	IL-6 *ir*	pg/mL
Lactate Dehydrogenase	LDH	U/L
Low Density Lipids	LDL	mg/dL
Phosphorous	P	mg/dL
Potassium	K	mEq/L
Serum Creatinine	SCREAT	mg/dL
Sodium	Na	mEq/L
Total Bilirubin	T. Bili	mg/dL
Total Protein	TP	g/dL
Triglycerides	TRIG	mg/dL
Uric Acid	UA	mg/dL
Very Low Density Lipids	VLDL	mg/dL

Appendix C. Abbreviation and units for whole blood variables measured in river otters in Prince William Sound, Alaska, USA.

Variable Name	Abbreviation	Unit of Measure
White Blood Cell Count	WBC	Th/cmm (thousand)
Red Blood Cell Count	RBC	m/cmm (million)
Hemoglobin	Hb	g/dL
Hematocrit = Packed Cell Volume	Hct or PCV	%
Mean Corpuscular Volume	MCV	Fl
Mean Corpuscular Hemoglobin Concentration	MCHC	g/dl
Mean Corpuscular Hemoglobin	MCH	Pg
Red Cell Distribution Width	RDW	%
Platlet Count	PLAT	Th/cmm
Differential		
Segmented neutrophils	neuts	%
Lymphocytes	lymphs	%
Monocytes	mono	%
Eosinophils	eos	%
Basophils	baso	%

Chapter 6. Pigeon Guillemot

(*Cepphus columba*) Perspective

Pigeon Guillemot (*Cepphus columba*) Perspective: Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the *1989 Exxon Valdez* Oil Spill

Long-term Direct and Indirect Effects of the *Exxon Valdez* Oil Spill on Pigeon Guillemots in Prince William Sound, Alaska[1,2]

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ABSTRACT

We conducted a study to determine mechanisms constraining population recovery of Pigeon Guillemots following the 1989 T/V *Exxon Valdez* oil spill. We asked whether recovery was limited by continuing exposure to residual oil, limitations imposed by prey availability, or other causes. Our approach was to compare demographic, physiological, and behavioral parameters between an oiled site pre- and post-spill, and between the oiled site and an unoiled site post-spill. Adult mass, body condition, and nestling survival were significantly lower at the oiled site post-spill compared to pre-spill. After the spill guillemots increased in number at the unoiled site and chicks fledged at significantly heavier weights than at the oiled site, where populations remained depressed. Elevated CYP1A, LDH, and AST enzyme activities detected in adult guillemots a decade after the spill at the oiled site suggest that continued exposure to residual oil may have limited population recovery, although reduced availability of sand lance, a preferred forage fish, may have also played a role. Previous studies conducted at the oiled site demonstrated that

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[2]Document citation has not been revised to reflect overall final restoration report citation.

guillemot chick growth and reproductive success were positively related to the percentage of high-lipid forage fish, such as sand lance, in the chick diet. Aspects of sand lance life history and the pattern of *Exxon Valdez* oil deposition strongly suggest that sand lance were impacted by the spill, although we lack direct evidence of this, and reductions in this species' abundance may have also resulted from natural causes. Our study suggests that the recovery of a top-level generalist predator may be constrained by both direct effects (continued exposure to residual oil) and indirect effects (reduced availability of a key prey species) following a large-scale perturbation. Furthermore, it demonstrates that recovery following oil spills may take considerably longer for certain species than the few years that have been proposed as typical for marine birds.

Key words: *Exxon Valdez* · oil spills · marine birds · *Cepphus columba* · Pigeon Guillemot · Prince William Sound · blood parameters · reproductive performance

INTRODUCTION

Because organisms are often killed outright and en masse following exposure to oil, it is well recognized that oil spills can have immediate adverse effects on wildlife populations (Bourne et al. 1967, Dunnet 1982). Nonetheless, quantifying population-level impacts can be very difficult. Pre-spill population estimates are often unavailable, and initial impact mortalities due to oiling are typically hard to quantify (Parrish and Boersma 1995, Piatt and Ford 1996). Estimating the duration of sustained injury, and identifying mechanisms that constrain population recovery following initial impacts is more challenging still, as this requires that both pre-perturbation and current population status be known.

When pre-spill population estimates are available they are often not comprehensive estimates. For marine birds and mammals, for example, pre-spill population estimates are typically based on counts of breeding adults at their colonies. Assessing injury based upon these counts may underestimate impacts, however, as mortalities of subadults and non-breeders may not be accounted for, and mortalities of breeders may be masked when non-breeders fill vacancies at the colony. Non-breeder replacement may explain why colony-based studies typically identified oil-spill effects on seabirds as short-lived (Birkhead and Hudson 1977, Stowe 1982, Boersma et al. 1995), even though the projections of empirical population models suggest that effects should be longer lasting (Samuels and Lanfear 1982). Empirical support for the notion that colony-based studies in themselves present inadequate estimations of population injury comes from east Britain where the death of 30,000 auks in late winter had no detectable effects on nearby breeding populations in the subsequent spring (Harris and Wanless 1984).

A better approach for assessing injury to marine bird populations involves comparing geographically broad-based population surveys made before and after a perturbation. Surveys of this type were conducted for marine birds in Prince William Sound (PWS) before and after the 1989 T/V *Exxon Valdez* oil spill (hereafter *EVOS*). Using a before-after-control-impact design with paired sampling (Osenberg et al. 1994), Murphy et al. (1997) compared pre- and post-spill (through 1991) densities of birds along oiled and unoiled shorelines in PWS. Their analysis revealed that of all marine birds in PWS, the impacts of the spill on abundance and distribution were most pronounced for Pigeon Guillemots (*Cepphus columba*). Murphy et al. (1997) stated

"The Pigeon Guillemot was the one species that...showed persistent declines in overall abundance relative to pre-spill baseline, [and further was the marine bird species that] showed the greatest negative impacts and the fewest signs of recovery". Irons et al. (2000) performed analyses similar to those of Murphy et al. (1997), but based on surveys that covered a wider geographic area, over a longer time span. The results of Irons et al.'s (2000) analyses corroborated those of Murphy et al. (1997), but further demonstrated that spill effects continued through 1998, nine years after the oiling event.

Given clear evidence that guillemot populations in oiled areas of PWS were negatively impacted and not recovering, we initiated a study to determine whether recovery was limited by continuing exposure to residual oil, prey availability, or other causes. Our approach was to compare demographic, behavioral, physiological, and dietary parameters between an oiled site pre- and post-spill, and between the oiled site and an unoiled site post-spill. Our study subsumes data previously collected and analyzed by Oakley and Kuletz (1996). Oakley and Kuletz (1996) assessed the effects of *EVOS* on Pigeon Guillemots by comparing components of reproductive success measured at an oiled site in the two years immediately following the spill with measures drawn from the same site a decade prior. They found that overall productivity was significantly lower after the spill, but did not attribute the difference to an oil spill effect. Instead, increased post-spill predation on guillemot nests was suggested as the cause (Oakley and Kuletz 1996). Their study did, however, show that chick growth rates tended to be lower following the spill, leaving open the possibility that residual oil continued to affect the birds.

In this paper we suggest that the recovery of a top-level generalist predator may be constrained by both direct effects (continued exposure to residual oil) and indirect effects (reduced availability of a key prey species) following a large-scale perturbation, and further, that recovery following oil spills may take considerably longer for certain species than the few years that have been proposed as typical for marine birds. Our study fits into a larger context of work that reports on effects of *EVOS* on a wide assemblage of marine organisms (reviewed by Peterson 2001) including invertebrates (Fukuyama et al. 2000), fishes (Jewett et al. *in press*), mammals (Bowyer et al. 1995, Ben-David et al. 2001, Bodkin et al. 2002, Dean et al. 2002) and birds (Irons et al. 2000, Esler et al. 2002).

BACKGROUND

Following the grounding of the supertanker *Exxon Valdez* on Bligh Reef on the morning of 24 March 1989, ~4.1 x 10^7 L of North Slope crude oil spilled into the waters of PWS. Although approximately 20% of the spilled oil volatilized, and a further 20% left the Sound, 60% of the spilled oil was retained in PWS, either sinking or coating shorelines (Wolfe et al. 1994). Oil spread from the spill site south-west across PWS, first hitting the central island groups (Naked and Knight islands), and then mainland shorelines, and adjacent islands (Galt et al. 1991, Neff et al. 1995) (see inset map of Fig.1). The *Exxon Valdez* spill differed from other large spills (e.g., the T/V *Braer* spill) because it occurred in an area protected from large seas by barrier islands, and because the bulk of the spilled oil formed a slick that did not disperse into the water column (Kingston 1995). Although oil concentrations declined rapidly in the first few years following the spill (Neff et al. 1995, O'Clair et al. 1996), as recently as 1997, residual oil from

EVOS was still found in many intertidal and subtidal zones of the Sound (Hayes and Michel 1999).

An estimated 250,000 seabirds were killed outright by *EVOS* (Piatt and Ford 1996). Of approximately 30,000 oiled carcasses that were recovered following the spill, ~12% were collected in PWS, with alcids (32%, primarily murres [*Uria aalge*]), sea ducks (26%), and cormorants (16%) dominating the carcass recoveries (Piatt et al. 1990). Various aspects of their life history may make alcids, and guillemots in particular, especially vulnerable to oil spills (King and Sanger 1979). Guillemots typically forage in nearshore benthic environments, which can be significant repositories for spilled oil. They spend large portions of their time resting on surface waters and roosting on inter-tidal rocks, and because they have restricted foraging ranges (Ewins 1993), they may be less able to avoid oiled habitat than seabirds that forage more widely.

METHODS

Study sites

Our oiled study site was the Naked Island group, which includes Naked, Peak and Storey Islands, located in central PWS (Fig. 1). We studied guillemots here for five years prior to the spill (1978 - 1981, and 1984), and eight years post-spill (1989 - 1990, and 1994 - 1999). Naked Island is located approximately 16.5 nautical miles from Bligh Reef, and was the first land mass hit by oil spilled by the *Exxon Valdez*. The near shore habitat of the Naked Island group includes many bays and passages with shallow (<30 m) shelf habitat radiating about one kilometer from shore. The islands are forested to their summits (< 400 m), mostly with sitka spruce (*Picea sitchensis*) and western hemlock (*Tsuga heterophylla*). Guillemots nest semi-colonially along the island's rocky shorelines in cavities beneath tree roots overhanging crumbling cliffs, in rock crevices, and among boulders on talus slopes. Other members of the Alcidae breeding on these islands include Marbled Murrelets (*Brachyramphus marmoratus*), Parakeet Auklets (*Cyclorrhynchus psittacula*), Tufted Puffins (*Fratercula cirrhata*), and Horned Puffins (*F. corniculata*). Populations of all these species have declined appreciably in PWS since the 1970's, presumably due to large-scale changes in forage fish abundance in the region (Agler et al. 1999).

Our unoiled study site was located in southwestern PWS (Fig. 2). Most of our work was conducted on Jackpot Island, a small low-elevation island with a shoreline of low (< 25 m) cliffs and one small bay. We studied guillemots here from 1993 - 1998. A sound-wide survey conducted in 1993 showed that Jackpot Island had the highest density of guillemots in all of PWS (Sanger and Cody 1994). Horned Puffins also nest on the island. In 1998 and 1999 guillemots breeding at Icy Bay were captured for liver biopsies and blood collection. Icy Bay is situated four nautical miles south of Jackpot Island, and was also unoiled.

Population assessment

Guillemot populations were assessed before and after *EVOS* by (1) conducting shoreline surveys as part of a U. S. Fish and Wildlife Service monitoring program designed to estimate the densities of marine birds over the entire PWS, and (2) conducting whole-island censuses to

estimate populations of guillemots at locations where this species was known to concentrate during breeding.

Sound-wide surveys were conducted during June and July over eight years (two prespill:1984-1985; six postspill:1989-1991, 1993, 1996, and 1998). Transects were selected by stratified-random sampling to account for differences in marine habitat. Surveys were conducted from 8-m boats piloted 100 m offshore. One observer scanned continuously with binoculars from each side of the boat, counting all guillemots observed within a sampling window that extended 100 m to either side of, in front of, and above the survey vessel. Intertidal rocks, beaches, and uplands were also scanned for guillemots. In total, 123 transects were sampled. Transects varied in length, but were typically several km long. For analysis transects were grouped into 23 oiled and 22 unoiled clusters. See Irons et al. (2000) for further details on this survey methodology.

We conducted whole-island censuses of guillemots along the shores of Naked, Peak, Storey, Smith, Little Smith, and Jackpot Islands in late May and early June. The specific dates of the surveys varied by year, and were set to coincide with early morning high tides, when guillemot attendance peaks (Vermeer et al. 1993). Years in which counts were made are indicated on Figs. 3A and 3B. Censuses were conducted in a manner identical to that described above for Sound-wide surveys.

Continuing exposure to oil

To determine if guillemots faced continued exposure to residual *Exxon Valdez* oil, we assayed hepatic cytochrome P4501A (CYP1A), a liver enzyme that is rapidly induced in many vertebrate species following exposure to polycyclic aromatic hydrocarbons (PAHs) (Collier and Varanasi 1991). PAHs are a refractory class of petroleum hydrocarbons that have a high potential for exerting toxicity in birds (Leighton 1993). Elevated levels of CYP1A are transient following exposure to rapidly metabolized compounds such as PAHs, and thus are indicative of recent exposure to contaminants (J. Stegeman, personal communication).

CYP1A was assayed following liver biopsies of 26 chicks (14 from the oiled colonies [on Naked and Storey islands] and 12 from unoiled colonies [on Jackpot Island and in Icy Bay]) that were 18-24 days old, and 24 adult guillemots (13 from oiled colonies [on Naked and Storey islands] and 11 from unoiled colonies [in Icy Bay]). The surgeries were performed by an avian veterinarian in a field laboratory during 20-26 July1998 (chicks) and 15-23 June 1999 (adults). Details of the anesthesia and surgery procedures are provided in Degernes et al. (in review).

Blood biomarkers

To determine whether guillemots were adversely affected by continued exposure to residual oil, we assessed blood parameters of adult birds at the oiled and unoiled sites. Blood samples were collected from adult guillemots at the oiled site (Naked and Storey islands) during 14 June - 12 July 1998, and during 15 - 18 June 1999. At the unoiled site, samples were collected from Jackpot Island during 10 July - 5 August 1998, and at Icy Bay during 21 - 23 June 1999. This investigation complemented the work of Seiser et al. (2000), who studied blood parameters in 1997 and found no obvious oil-induced effects on chicks, but recommended that adults be examined further. Seiser et al.'s (2000) recommendation was based largely on the finding that

aspartate aminotransferase (AST) was significantly elevated in breeding guillemots at the oiled site relative to the unoiled site. Elevations of AST, as well as elevations of lactate dehydrogenase (LDH), are symptomatic of liver damage, which commonly results from oil exposure (Campbell 1986). These blood parameters may also become elevated following damage to other tissues, however, including kidney, lung, myocardium, or skeletal muscle (Franson 1981). A recent study of mink (*Mustela vison*) demonstrated that chronic low-level ingestion of food contaminated with Alaska North Slope crude oil resulted in long-term increased LDH activity (Mazet et al. 2000), further suggesting the usefulness of this enzyme for assessing oil exposure. In addition to assaying activity levels of AST and LDH, we assayed creatine kinase and alkaline phosphatase. Serum was analyzed for concentrations of bile and uric acids, corticosterone (1998 samples only), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphorus (P), total CO_2, glucose, total protein, albumin, blood urea nitrogen, and cholesterol. Anion gap, albumin:globulin ratio (A:G ratio), and globulin concentration were calculated. Previous studies of guillemots (Peakall et al. 1980) and other seabirds (Fry and Lowenstein 1985, Leighton 1985, 1993, Khan and Ryan 1991, Peakall and Shugart 1993, Rattner et al. 1996, Newman et al. 2000) suggest that oil exposure may affect these blood parameters.

Approximately 2.0 ml of blood was aseptically obtained from the metatarsal vein using a 23 gauge hypodermic or butterfly needle and 3 ml syringe. Blood was immediately transferred into Microtainer™ serum separator tubes (Becton-Dickinson and Co., Franklin Lakes, NJ, USA) and stored in coolers for < 3 h prior to centrifugation. Samples were centrifuged in a Triac Centrifuge™ (Clay Adams, Sparks, MD, USA) for 15 minutes at 3500 rpm. Sera was transferred with disposable polyethylene pipettes into 1.5 ml plastic microcryovials (Out Patient Services, Petaluma, CA, USA), which were subsequently stored frozen until analyses at the Veterinary Medical Teaching Hospital (University of California, Davis, CA, USA).

Adult body condition

We compared adult body condition at the oiled and unoiled sites because previous studies have shown that oiling adversely affects this parameter in seabirds (Culik et al. 1991, Fowler et al. 1995). Adult guillemot body condition was determined by scaling body mass by body size. Two techniques were used. For the pre- vs. post-spill comparison, we calculated body condition with a simple ratio index (body mass / wingcord^3) (Moeller 1987). We used this analysis method because fewer morphometric measurements were taken on individual birds in pre-spill years. Additional morphometric parameters were assessed in post-spill years, enabling us to compare body condition between sites with a more sophisticated method, a principle components residual index (Reid 1987, Golet and Irons 1999). Calculating post-spill body condition involved: (1) establishing an index of body size through a principle components of tarsus, head-plus-bill, and wingcord lengths of captured adults, (2) developing regression equations between the index of body size and body mass for the study population at large, and (3) applying measurements of our study animals to these equations and using residuals to generate individual body condition estimates. This method of estimating body condition is recommended over other techniques because the metric is independent of an individual's linear size (Piersma 1984, Piersma and Davidson 1991, Jakob et al. 1996).

To establish the body size index, we performed a principle components analysis (PCA, SYSTAT 1997) on measurements of 24 adults (13 from Naked Island, and 11 from Icy Bay) captured during June 15-23, 1999. We weighed and individually color-marked each bird, and measured the tarsus, head-plus-bill, and wingcord lengths. With PCA we generated weighting coefficients that described positive covariance among the linear measurements. These coefficients had variable loadings (tarsus 0.57, head-plus-bill 0.29, and wingcord 0.53), and the first principle component accounted for 48% of the variance in the original measures. Standardized measurements were multiplied by these coefficients and added together to produce a PCA factor score (our body size index). By regressing body mass (grams) on the body size index, we developed a least squares regression ($y = 478.3 + 11.5x$, $n = 24$, $r^2 = 0.19$, $P = 0.034$) that allowed us to predict the mass of a bird given its size. Although this equation has relatively low predictive power, it serves as a useful benchmark for comparing mean levels of condition in groups of individuals.

We calculated the body condition of experimental birds by subtracting the predicted weight of each bird (based on the regression equation) from its actual weight, dividing this difference by the predicted weight, and then multiplying the resulting quotient by 100. This value (our body condition index) represents the percent by which a bird's measured weight differs from what it was expected to weigh, given its size, providing a rough estimate of each bird's level of nutrient reserves.

Prey availability

To determine if the recovery of oil-impacted populations was constrained by prey availability, we performed dive transects at guillemot foraging areas near the study colonies. Demersal fish population densities were estimated in 1996 and 1997. A total of 60 sites were surveyed (15 sites $\cdot$ area^{-1} $\cdot$ year^{-1}). Sites were systematically selected within a 4-km radius of major guillemot nesting areas (Figs. 1 and 2). At each site, we counted demersal fishes along two transects running perpendicular to shore. Transects extended 30 m from shore, or in cases where the shoreline was steep, until a depth of 15 m was attained. The two transects originated 10 m to either side of the shoreline midpoint at each site. Demersal fishes were counted along a 1-m wide swath on each transect while moving aside algae and other vegetation. All fish $<$ 5 cm were identified to the family level, and classified as one of two size classes (1 - 8 cm, and 8 - 15 cm). For comparison purposes, we calculated the average density of fish (number observed per 100 m^2) at each site.

Chick diet

We studied chick diet to determine if recovery of oil-impacted populations was constrained by food availability. Chick diet composition and delivery rates were determined by observing prey items held crosswise in the bills of adult guillemots as they provisioned their young in the nest. Feeding observations were made with binoculars and spotting scopes from land-based blinds at the oiled site (Naked Island) before and after the spill, and at the unoiled site (Jackpot Island) after the spill. Years in which chick diet and delivery rates were determined are indicated in Appendix A1 and A2. We watched from each blind for an average of four full days,

alternating our observation points to ensure that the diet of chicks aged 8 to 30 days was well documented. Because guillemots often pause on the water or on rocks in front of their nests before making deliveries to their chicks, we were usually able to identify the prey items they carried in their bills. During our blind watches prey items were identified to the lowest possible taxon, but for the purposes of this paper, observed prey items were divided into one of two categories based on lipid content. High-lipid fishes included Pacific sand lance (*Ammodytes hexapterus*), Pacific herring (*Clupea pallasii*) and smelt (Osmeridae), whereas low-lipid fishes included gadids (Gadidae spp.), sculpins (Cottidae spp.), blennies (Stichaeidae and Pholidae spp.), and other demersal fishes. We report the percent high-lipid fish in the chick diet because this parameter is positively related to guillemot chick growth and reproductive success (Golet et al. 2000). High-lipid fishes likely confer reproductive advantages to guillemot chicks because they have high energy densities (kJ/g fresh mass) (Barrett et al. 1987, Hislop et al. 1991, Van Pelt et al. 1997, Anthony et al. 2000), high metabolizable energy coefficients (Massias and Becker 1990, Brekke and Gabrielsen 1994), and are not lacking in other nutrients because lipids tend to replace water and not protein (Harris and Hislop 1978, Anthony et al. 2000).

Chick growth and reproductive success

Previous studies have demonstrated that oil exposure can lead to reductions in egg laying (Ainley et al. 1981), chick growth rates (Miller et al. 1978, Butler and Lukasiewicz 1979, Andres 1999), hatching success, and nestling survival (Trivelpiece et al. 1984, Fry et al. 1986). To test for such effects we measured chick growth and reproductive success at the oiled site (Naked and Storey islands) pre- and post-spill, and at the unoiled site (Jackpot Island) post-spill. Years in which chick growth and reproductive success were determined are indicated in Appendix A1 and A2. We visited all known nests at least once every five days from the egg-laying stage until the chick(s) fledged. At hatching we marked the web of the foot of alpha (the first to hatch, or larger chick, of two-chick broods), and beta (the second to hatch, or smaller chick, of two-chick broods) chicks to distinguish them from one another until they were old enough for banding. Chicks were weighed and measured to determine growth rates, calculated as the slope of the regression of mass on age for chicks between 8 and 18 days post-hatch, the linear phase of the growth cycle (Emms and Verbeek 1991, Ewins 1993). Because this growth measure is not influenced by the particular asymptote that individual chicks attain (Hussel 1972, Gaston 1985), it is independent of peak and fledging mass, which we also report. We define peak mass as the highest mass measured, and fledging mass as the last mass measured prior to fledging. Peak and fledging mass have been shown to affect fledging success and subsequent survival (Perrins et al. 1973).

Based on observations made during nest visits we determined reproductive success parameters, including clutch size, hatching success (eggs hatched per egg laid), nestling survival (chicks fledged per egg hatched), overall productivity (chicks fledged per egg laid), and brood size at fledging. We calculated predation rate as the percent of total nests observed that had evidence of predation (e.g., egg shell fragments, blood stains, dead chicks).

Statistics

For most post-spill comparisons we used general linear models (GLMs) to test for "site" (oiled vs. unoiled) effects. We included "year" and "chick type" (separate categories designated for alpha, beta, and single chicks) as categorical random factors in our GLMs when appropriate. For binomially-distributed data we compared multiple logistic regression models, and tested for significance by assessing the deviance (expressed as a likelihood ratio statistic) of saturated models and models lacking particular effects (Agresti 1990). For pre- vs. post-spill comparisons we used individual year means as our sample units. We used the Lilliefors test to assess normality with variables having continuous frequency distributions. In some instances we performed transformations to satisfy assumptions of parametric tests; otherwise we used non-parametric tests (Kruskal-Wallis or Mann-Whitney *U*). For all *t*-tests we assumed unequal variance. Data on fish abundance were log-transformed (log (density + 0.1)) prior to analyses. For contingency table analyses, log-likelihood ratio tests (*G*-tests) were used (Fienberg 1970, Bishop et al. 1975). For *G*-tests involving only two classes, the Williams correction was applied to reduce the likelihood of type-1 errors (Sokal and Rohlf 1995). All tests are two-tailed, and statistical significance was assigned at $P < 0.05$. We report mean values ± 1 SE.

RESULTS

Table 1 presents a summary of the results of pre- vs. post-spill comparisons at the oiled site and post-spill comparisons between the oiled and unoiled sites.

Guillemot populations

Guillemot populations were negatively affected by *EVOS* and, as of 1998, had not recovered to pre-spill levels (see Fig. 3, and Irons et al. 2000). In the first few years following the spill (1989-1993) guillemot densities appeared depressed relative to pre-spill levels along both the oiled and unoiled transects; however, the magnitude of the decline was greater along oiled transects. In more recent years (1996 and 1998), guillemot densities along oiled shorelines continued to decline, and for the first time fell below what was observed along unoiled coastlines, further suggesting that recovery had not taken place.

Whole-island censuses indicate that guillemots at the oiled and unoiled study sites exhibited divergent population trends following *EVOS* (Figs. 4a and 4b). The population at the unoiled site increased significantly during 1993 - 1998, while no significant post-spill trend was observed at the oiled site. The population multiplication rate (λ) was 1.05 at the unoiled site, and 0.98 at the oiled site.

Continuing exposure to oil

Ten years after the spill, adults from the oiled site had significantly higher CYP1A activity in the liver than adults from the unoiled site (oiled: 3.1 ± 0.4 pmol $\cdot$ $min^{-1} \cdot mg^{-1}$, n =12 birds; unoiled 1.9 ± 0.2 pmol $\cdot$ $min^{-1} \cdot mg^{-1}$, $n = 11$ birds, $t = 2.1$, $P = 0.020$, Fig. 5). Nestlings measured one year earlier, however, did not show a statistically significant difference in this

parameter between the two sites (oiled: 4.1 ± 0.4 pmol · min^{-1} · mg^{-1}, n = 12 birds; unoiled 4.7 ± 0.5 pmol · min^{-1} · mg^{-1}, n = 14 birds, t = 2.06, P = 0.38, Fig. 5). These results suggest that adults, but not chicks, were exposed to residual petroleum hydrocarbons at the oiled site a decade after the spill. Although CYP1A activity was elevated at the oiled site, absolute CYP1A activities were low, suggesting that oil exposure was low-level. CYP1A activities were variable among individuals at the oiled site, indicating different levels of oil exposure. This finding matches expectations, given that guillemots have strong foraging site fidelity (Golet et al. unpublished data) and *Exxon Valdez* oil was patchily distributed at the oiled site (Neff et al. 1995, O'Clair et al. 1996, Wolfe et al. 1996).

Elevated CYP1A activity at the oiled site could have been caused by exposure to contaminants that did not originate with *EVOS*; however, we know of no other contaminant sources in PWS that are likely to explain this result. That *EVOS* hydrocarbons were the cause of observed differences in CYP1A activities (and blood biomarkers) is further suggested by a recent report that found no differences in marine vessel traffic between our oiled and unoiled sites (Murphy et al. 1999).

Blood biomarkers

Significant differences were detected in blood parameters of adult guillemots from the two sites post-spill (see Table 2). In 1998, adult guillemots at the oiled site had lower concentrations of Na, Ca, and P, higher concentrations of K, total CO_2, glucose, and corticosterone, and higher activities of LDH compared with guillemots sampled at the unoiled site. In 1999, adult guillemots at the oiled site again had higher glucose concentrations and LDH activity than birds at the unoiled site. An additional difference apparent in 1999 was elevated AST activity among birds at the oiled site.

The most noteworthy differences between sites from the perspective of assessment of injury from the oil spill were the elevated LDH and AST enzyme activities found at the oiled site. Among adults sampled in 1999, significant correlations were found between both LDH and AST activities (Pearson correlation coefficient = 0.79, Bonferroni probability < 0.001), and CYP1A and AST activities (Pearson correlation coefficient = 0.43, Bonferroni probability = 0.047). Simultaneous elevations of these parameters are strongly indicative of a toxicological response, and support the notion that adult guillemots at the oiled site were exposed to residual oil nine and ten years post-spill. Some caution must be exercised in interpreting these results, however, as AST was significantly elevated in only one of the two years.

Adult body condition

Among adult guillemots studied at the oiled site, both body mass (pre-spill: 502 ± 3 g, n = 3 years; post-spill: 478 ± 2 g, n = 4 years, t = 6.1, P = 0.002) and body condition (pre-spill: 9.2 ± 0.1 g/mm^3, n = 3 years; post-spill: 7.9 ± 0.1 g/mm^3, n = 4 years, t = 7.7, P = 0.002) were significantly higher pre-spill than post-spill. Following the spill, however, there were no statistically significant differences in either of these parameters between oiled and unoiled sites (body mass: $F_{1,44}$ = 0.32, P = 0.58; body condition: $F_{1,44}$ = 1.2, P = 0.28).

Chick diet and prey availability

Demersal prey fish availability, as determined by dive transects near guillemot colonies post-spill, was significantly greater overall at the oiled site than at the unoiled site (n = 60 transects, t = 2.87, P = 0.006, Fig. 6). When broken down by size class, the difference was statistically significant for fishes 1 - 8 cm (n = 60 transects, t = 3.12, P = 0.003), but not for fishes 8 - 15 cm (n = 60, t = 1.82, P = 0.075), although the oiled site tended to have higher densities of fish of the larger size class as well. ANOVA analyses of log-transformed fish densities revealed no significant year ($F_{1,56}$ = 0.02, P = 0.90) or year x site interaction ($F_{1,56}$ = 1.09, P = 0.30) effects. These results suggest that availability of demersal prey fish was not lower at the oiled site relative to the unoiled site seven to eight years after *EVOS*.

The percent high-lipid schooling fish (sand lance, herring, smelt) in chick diets was reduced at the oiled site following *EVOS*. Significantly fewer high-lipid prey were delivered to chicks at the oiled site post-spill compared to pre-spill (U = 0.0, P = 0.016, Fig. 7a), and compared to the unoiled site post-spill (n = 4,619 identified prey items, G = 71, P < 0.001, Fig. 7a). Although the availability of high-lipid schooling fishes appeared reduced at the oiled site following *EVOS*, this did not appear to affect meal delivery rates. There were no significant differences in the rate at which chick meals were delivered to the nests at the oiled site pre- vs. post-spill (U = 9, P = 0.73, Fig. 7b). Chick meal deliveries were significantly less frequent (when controlling for effects of "year" and "number of chicks in the nest"), however, at the oiled site post-spill compared to the unoiled site ($F_{1,82}$ = 7.7, P = 0.007, Fig. 7b). The post-spill difference in delivery rates between sites is not evident in Figure 7b because the values presented are yearly means, as opposed to least-squares means. In other words, the post-spill "site" effect is masked in Figure 7b by the effects of "year" and "number of chicks in the nest".

Chick growth and reproductive success

Linear growth rates of chick mass tended to be higher at the oiled site pre- vs. post-spill (U = 5, P = 0.089, Fig. 8a), although no difference was observed post-spill between study sites ($F_{1,173}$ = 0.32, P = 0.57, Fig. 8a). Differences in peak and fledging masses were not significantly different pre- vs. post-spill (peak mass: U = 5.5, P = 0.10; fledging mass: U = 9, P = 0.34; Fig. 8b), although these parameters were both significantly lower post-spill at the oiled site compared to the unoiled site (peak mass: $F_{1,142}$ = 8.7, P = 0.004; fledging mass: $F_{1,137}$ = 11.4, P = 0.001, Fig. 8b).

Differences in chick growth appear to have contributed to differences in reproductive success pre- vs. post-spill at the oiled site. Guillemot productivity tended to be higher before *EVOS* than after (U = 7, P = 0.088, Fig. 9). The trend of higher pre-spill productivity at the oiled site was the result of significantly higher nestling survival pre-spill compared to post-spill (U = 0.0, P = 0.004, Fig. 9), as hatching success was actually lower at the oiled site before the spill than after the spill (U = 32, P = 0.018, Fig. 9). Contributing to the lower post-spill productivity was a significantly higher rate of predation of guillemot eggs and chicks after *EVOS* (40 ± 9 % of nests depredated · nests with eggs^{-1}, n = 7 years) compared to before *EVOS* (6 ± 2 % of nests depredated · nests with eggs^{-1}, n = 5 years, U = 35, P = 0.004). When considering pre- and post-spill years collectively at the oiled site, productivity was found to be significantly related to

predation rate (y = - 0.47x + 0.57, n = 12 years, r^2 = 0.57, p = 0.004). No difference was observed in clutch size between the oiled site pre- (1.7 ± 0.03 eggs · nest^{-1}, n = 5 years) vs. post-spill (1.7 ± 0.03 eggs · nest^{-1}, n = 7 years, U = 14.5, P = 0.81).

Although chicks attained higher peak and fledging masses post-spill at the unoiled site compared to the oiled site, no difference was observed in overall productivity (n = 596 eggs, G = 0.14, P = 0.71, Fig. 9). Predation of guillemot eggs and chicks was significantly higher at the oiled site post-spill than at the unoiled site (23 ± 16 % of nests depredated · nests with eggs^{-1}, n = 367 nests, G = 12.9, P < 0.001); however, nestling survival was not significantly different post-spill between sites (n = 369 chicks, G = 0.26, P = 0.65, Fig. 9). Clutch size was significantly lower post-spill at the oiled site compared to the unoiled site (1.8 ± 0.03 eggs · nest^{-1}, n = 363 nests, G = 6.0, P = 0.015), although post-spill hatching success was higher at the oiled site (n = 609 eggs, G = 40.9, P < 0.001, Fig. 9).

DISCUSSION

In the decade following *EVOS*, guillemot populations in oiled areas did not show any signs of recovery. Ten years is, however, a sufficient amount of time for this species to increase in numbers (given its reproductive rate, age at first breeding, etc.) if conditions are sufficiently favorable (Samuels and Lanfear 1982). That impacted populations have not rebounded indicates that some mechanism(s) other than intrinsic demographic constraints limited post-spill population growth of guillemots in oiled areas. Our analyses suggest two such mechanisms: a direct effect whereby continued exposure to residual oil reduced adult survival, and an indirect effect whereby the oil spill impacted an important forage fish species, thereby reducing fledging mass and subsequent survival.

Continuing exposure to oil

In 1997 adult guillemots at the oiled site had elevated AST activities relative to the unoiled site, suggesting possible liver injury (Seiser et al. 2000). Because the 1997 study had a small sample size, however, these results were considered preliminary. Analysis of the samples we collected over the next two years corroborated the 1997 results. Elevated CYP1A suggests adult guillemots at the oiled site were exposed to residual oil, and a significant positive correlation between CYP1A and AST activity among individual birds strongly suggests that petroleum hydrocarbon exposure caused organ damage. Collectively, these results suggest that continued exposure to residual oil may have directly limited recovery of guillemots by reducing survival of adult birds. Indeed, a relatively small increase in adult mortality is sufficient to explain a lack of recovery (see below). The suggestion that oil exposure may have reduced the survival of adult guillemots inhabiting oiled areas of PWS is made more plausible given findings of concurrent studies conducted on other nearshore vertebrate predators in PWS. Elevated CYP1A activities, suggestive of continued exposure to residual oil, have been detected for Barrow's Goldeneyes (*Bucephala islandica*), Harlequin Ducks (*Histrionicus histrionicus*) (Trust et al. 2000), sea otters (*Enhydra lutris*) (Bodkin et al. 2002), river otters (*Lutra canadensis*) (Ballachey et al. 2000), and masked greenling (*Hexagrammos octogrammus*) (Jewett et al. *in press*). Most noteworthy, in the two species in which adult survival was studied (Harlequin

Ducks and sea otters), significant reductions in over-winter survival were found for populations inhabiting oiled sites (Esler et al. 2000, Monson et al. 2000). Reduced survival in these studies was observed through at least 1998, suggesting relatively long-term mortality effects associated with the 1989 spill.

That adult guillemots, but not chicks, at the oiled site had elevated CYP1A suggests that differences in diet or habitat use resulted in differential exposure to oil. Because chicks are sheltered in nest cavities, their only opportunities for oil exposure are through their food (almost exclusively fish [Golet et al. 2000]) or physical contact with their parents. Adults have greater opportunities for exposure because they have wider dietary breadth (consuming both fish and invertebrates [Eldridge and Kuletz 1980]), and inhabit nearshore areas that were heavily contaminated with *Exxon Valdez* oil. Invertebrate feeders are more likely to ingest toxins than piscivorous species, as invertebrates typically sequester and accumulate toxins while fish metabolize them (Roesijadi et al. 1978, Varanasi et al. 1989).

Although CYP1A was significantly elevated among adults at the oiled site relative to the unoiled site, levels were low at both sites. Absolute levels of CYP1A expression are difficult to interpret, however, because it is not known what level of exposure is needed to elicit a response of the magnitude observed. Also, because CYP1A values were determined only from breeding birds, they may underestimate exposure levels of the oiled population at large. Higher exposure levels may have existed among non-breeders, as oiling has been shown to reduce egg laying in other alcids (e.g., Cassin's Auklet [*Ptychoramphus aleuticus*], Ainley et al. 1981).

High corticosterone and glucose concentrations among adult guillemots at the oiled site are suggestive of active mobilization of energy substrates (Wingfield 1994), which can occur following exposure to oil. Studies of both externally-oiled penguins (Fowler et al. 1995) and oil-fed nestling seabirds (Peakall et al. 1981) demonstrated elevated levels of corticosterone relative to unoiled controls. Experimental studies in Mallards (*Anas platyrhynchos*), however, showed that ingested oil suppressed adrenocortical function (Gorsline and Holmes 1982). Because oil exposure affects birds through multiple pathways (e.g., thermoregulatory and physiological), variable adrenocortical responses may be expected. Additional research is needed to better understand under what circumstances oil exposure leads to increased vs. decreased levels of circulating corticosterone.

Our assessment of effects of oiling on reproductive performance is incomplete (and conservative) as we were unable to determine whether or not exposure to residual oil resulted in greater instances of non-breeding at the oiled colony. Comparisons of breeders are nonetheless informative, as oil exposure can affect factors other than breeding propensity in birds (Leighton 1993). Chick growth and reproductive success patterns observed at the oiled and unoiled sites pre- and post-spill suggest that the level of oil exposure experienced among breeding adults at Naked Island was insufficient to cause reproductive impairment. Although guillemot chick growth rates were higher at the oiled site pre- vs. post-spill, growth rates at the oiled site after *EVOS* compare favorably with values reported in the literature (Drent 1965, Ainley et al. 1990, Emms and Verbeek 1991,Vermeer et al. 1993). Indeed, the difference in chick growth at the oiled site between these two periods appears not to be a function of depressed rates of growth after the spill, but rather was the result of exceptionally high growth rates pre-spill (Golet et al. 2000). Our finding that chick growth rates did not differ post-spill between oiled and unoiled sites provides additional evidence that residual oil exposure was not affecting chicks. Peak and

fledging masses of the chicks were significantly lower following the spill at the oiled site than at the unoiled site; however, it is unlikely that this was a direct result of oil exposure. Oil effects on chick development are more typically manifested when chicks are young (Leighton 1993). Productivity was significantly higher at the oiled site pre- vs. post-spill due to differences in nestling survival, however, the weight of evidence does not suggest that this resulted from oiling effects. Lower nestling survival following the spill was more likely the result of reduced availability of high-lipid forage fish (see below), although high levels of predation on guillemot nestlings post-spill also contributed.

Forage fish availability

By examining population trajectories, chick diet, chick growth, and reproductive success, between the oiled and unoiled colonies pre- and post-spill, insight can be gained as to the mechanistic role that availability of high-lipid forage fish may have played in limiting the recovery of guillemot populations in PWS following *EVOS*.

Chick diet data suggest that availability of high-lipid forage fish was lower at the oiled site following the spill than at both the oiled site pre-spill, and the unoiled site post-spill. Although we did not directly assess high-lipid forage fish availability, recent work has shown that the diet of nestling Pigeon Guillemots reflects the spatial and temporal abundance of these prey in the environment (Litzow et al. 2000). High-lipid fishes are clearly important to guillemots, as the percent of this prey type in the chick diet has been positively related to chick growth rates, nestling survival, and overall productivity (Golet et al. 2000).

In the present study, a decrease in the percent of high-lipid forage fish in the chick diet at the oiled site after the spill was associated with a decrease in post-spill reproductive performance, suggesting that reduced availability of this prey type may have constrained the recovery of impacted guillemot populations following the oil spill. Compared to pre-spill, post-spill nestling survival at the oiled site was significantly lower, and chick growth and overall productivity also tended to be lower.

A comparison of chick diet and reproductive performance between oiled and unoiled sites after *EVOS* further suggests that availability of high-lipid forage fish may have affected recovery. Following the spill, high-lipid fishes formed a higher percentage of the chick diet at the unoiled site compared to the oiled site, and once again the more lipid-rich diet appeared to confer reproductive benefits. The unoiled site is situated adjacent to several bays that are nursery areas for Pacific herring (*Clupea pallasii*) (Norcross et al. 1996), a high-lipid forage fish that typically comprised about 45% of the chick diet of guillemots at this site (see Appendix A2), and that presumably offset the lower demersal forage fish availability (as determined by dive surveys) in this area. Chicks at the unoiled site had significantly higher peak and fledging masses (by 29 and 31 g, respectively) than chicks at the oiled site, and recent work on captive seabirds suggests that the benefits of a high-lipid diets to nestlings may be greater than are appreciated by comparisons of body mass alone. Romano (2000) found that at fledging, chicks fed high-lipid fishes had double the fat reserves of chicks fed isocaloric low-lipid diets, although there were no significant differences between the two groups in fledging masses. Larger body masses and greater fat reserves are thought to enhance fledgling survival probabilities in birds (especially in species such as guillemots where chicks receive no parental care after leaving the nest) presumably

because they buffer the young from periods of low caloric intake that may follow fledging (Perrins et al. 1973, Jarvis 1974, Gaston 1997).

Using a Leslie population projection matrix (Leslie 1945, Krebs 1994), we calculated the reduction in fledgling survival that was required at the oiled site to explain the divergent population trajectories observed at the two sites post-spill. The matrix reduced to:

$$P_F = ([N_x \cdot \lambda] - [N_x \cdot P_A]) / (F_x \cdot P_A^2)$$

In this equation fledgling survival (P_F) is calculated from the population size (N_x), the population multiplication rate (λ), and the number of offspring (F_x) produced at each site (F_x was calculated from measures of clutch size and productivity, see Appendix A1 and A2). The model assumes stable age distributions, sex ratios of adults and offspring equal to 0.5, ten percent of the population is nonbreeding, and age-constant adult (>1 yr) survival (P_A) of 0.90. Although this model is simplistic, it is informative, as it suggests that fledgling survival must be 41% lower at the oiled compared to the unoiled site (0.37 vs. 0.81) to explain the population trends. Although we cannot be certain that the observed difference in mean fledging weights translated into a difference in fledgling survival, this analysis presents us with a plausible mechanism to explain the lack of recovery of guillemots at the oiled colonies.

A lack of recovery may also have been caused by increased levels of adult mortality at the oiled site after the spill (a direct oil-spill effect, see above). If we apply the same analysis technique, but this time hold fledgling survival constant (at 0.75), we can calculate what difference in adult survival is necessary to explain the divergent population trends. A reduction in survival of 7% (from 0.91 to 0.84) at the oiled site is sufficient to explain the observed rate of population growth at the oiled site.

High levels of predation of guillemot eggs and chicks at the oiled site following the spill also appear to have played a role in limiting recovery, but it is unlikely that this was the sole explanation for the lack of population growth at the oiled colonies post-spill. Instances of egg and chick predation are incorporated into productivity parameters, and overall productivity did not differ significantly between the oiled and unoiled sites post-spill. Our finding that the population at the unoiled site increased even though its post-spill reproductive success was not higher than that observed at the oiled colony suggests that factors other than nest predation limited population recovery of oil-impacted colonies. Predation could have limited recovery, however, if more adult birds were killed by predators at the oiled site post-spill.

Although we lack pre-spill data on sand lance abundance, it is likely that this high-quality forage fish species was negatively affected by the spill. Sand lance depend on fine gravel or sandy beaches, habitats that were contaminated extensively by *Exxon Valdez* oil (O'Clair et al. 1996), and that typically retain toxic fractions of crude oil (PAHs) longer than other habitat types (Conan 1982). Sand lance burrow in beach sediments to gain refuge from predators, and seasonally spawn (in a manner that leaves scoured pits) in the same habitat (Robards et al. 1999). Both activities present obvious avenues of oil exposure. Experimental work has shown that sand lance avoid oiled substrates (Pinto et al. 1984), and spend significantly more time in the water column, thereby exposed to predators, when beach sediments are contaminated (Pearson et al. 1984). Furthermore, Stagg and McIntosh (1996) found a significant relationship between petroleum hydrocarbon concentration in the water and CYP1A in *Ammodytes marinus* (the

ecological counterpart of *A. hexapterus* in the Atlantic Ocean), suggesting that *Ammodytes* are exposed to oil when their habitat is contaminated. Low concentrations of Prudhoe Bay crude oil are toxic to these fishes, and significant histological damage results when water-suspended oil droplets pass over their gills (Anderson 1985). Also, because sand lance exhibit strong site fidelity (Hobson 1986), it may take several years for an impacted population to recover, even in the absence of continuing oil spill effects. Observations that sand lance abundance increased from 1995-1999 at the oiled site (E. Brown *unpublished data*) are consistent with the notion that local populations were reduced by *EVOS*.

Reduced availability of high-quality fishes at the unoiled site following the spill could also have resulted from a natural shift in prey communities, as a large-scale regime shift in forage fish species took place in the northern Gulf of Alaska during the late 1970's and early 1980's (Anderson and Piatt 1999). An examination of seabird diets in the Gulf of Alaska does not, however, support the notion that sand lance declined during this period. Indeed, piscivorous seabirds in the northern Gulf of Alaska shifted from a diet that was dominated by capelin in the early years to one that was primarily sand lance in the late 1980's (Piatt and Anderson 1996). Further evidence that sand lance did not decline in abundance during the regime shift comes from studies conducted in Kachemak Bay, Alaska. Robards et al. (1999) analyzed beach seine data collected in 1976 and 1995 - 1996 and found no decline over this interval in sand lance catch per unit effort or percent occurrence.

Recovery of seabird populations following oil spills

Dunnet (1982) concluded that, in general, oil spills do not represent much of a threat to seabird populations. His conclusion, however, was based upon a comparison of natural levels of mortality and oil-induced mortalities, with the former being an order of magnitude greater than the latter. Also the seabirds in the region studied were experiencing a “particularly favourable general environment” with nearly all populations increasing. If, as Dunnet (1982) acknowledged, oil-induced mortalities were greater, or if conditions were in general less favorable for seabirds, then mortalities due to oil pollution could have been much more significant in terms of population dynamics. For guillemots in PWS, oil-induced impacts appeared to have significant and lingering effects on populations.

Our study demonstrates that seabird populations can not be expected to rebound to pre-perturbation levels in the short term following a mass-mortality event. Furthermore, it suggests that recovery times following oil spills may be considerably longer for certain species than a few years, which was proposed as typical for marine birds (Wiens et al. 1996, Day et al. 1997). For recovery to occur, impacted populations must not only replace individuals that are lost due to normal levels of attrition, but they must also replace individuals that were lost in the perturbation event. In the case of Pigeon Guillemots and *EVOS*, recovery of oiled populations did not take place in the decade following the spill, and this may be due to effects that were both direct (continued exposure of adults to lingering oil) and indirect (oil-spill impacts on an important prey species).

To better understand how oil spills impact seabird populations, future research should more closely examine the physiological effects of oil on both seabirds and their prey (i.e. key forage fish species such as Pacific sand lance). Dose-response studies have the potential to

increase our understanding of the level of oil exposure required to manifest enzymatic responses such as those observed in this study, and histopathological investigations may permit better assessments of the long-term consequences of such exposures. Advancements along these lines as well as in our understanding of how natural environmental variability influences fundamental demographic characteristics will greatly reduce our uncertainty as we seek to identify mechanisms that constrain the recovery of seabird populations impacted by oil spills.

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Table 1. Significance of difference between parameters examined pre- vs. post-spill at the oiled site, and post-spill at oiled vs. unoiled sites. When differences were detected, greater than (“>”) and less than (“<”) symbols indicate directionality relative to the column headings. “na” indicates that data were not available to make the comparison, and “-” indicates that no significant difference or trend was detected.

Parameter	pre- vs. post-spill at oiled site	post-spill at oiled vs. unoiled sites
Population growth rate (λ)	na	<[a]
% high-lipid fish in diet	>**	<***
Demersal prey fish	na	>***
availability	-	<***
Meal delivery rate (del/hr)	-	<**
Clutch size (eggs)	<**	>***
Hatching success	>*	-
Chick growth rate (g/day)	>*	<***
Peak mass of chicks (g)	-	<***
Fledging mass of chicks (g)	>***	-
Nestling survival	<***	>***
Nest predation rate (%)	>*	-
Overall productivity	na	<***
Adult body size	>***	-
Adult body mass	>***	-
Adult body condition	na	>**
CYP1A of adults	na	>[b]
Blood biomarkers of adults	na	-
CYP1A of chicks	na	-
Blood biomarkers of chicks		

* p value ≤ 0.10 (trend)
** p value ≤ 0.05
*** p value ≤ 0.01

[a] significant increase in population at unoiled site, nonsignificant trend at oiled site (see Results).
[b] significance level varied by biomarker (see Table 2).

Table 2: Comparisons of adult Pigeon Guillemot blood parameters from oiled and unoiled sites nine and ten years after the *Exxon Valdez* oil spill in Prince William Sound, Alaska. Only those parameters for which there were differences at the $p < 0.1$ level are listed; for a complete list of parameters compared between sites see Methods. Values presented are means ± standard error.

	1998			1999		
parameter	oiled (n = 9)	unoiled (n = 9)	p value	oiled (n = 13)	unoiled (n = 11)	p value
Aspartate aminotransferase (IU/l)	359 ± 44	279 ± 28	0.35	526 ± 42	413 ± 42	0.05
Bile Acid (umol/l)	12 ± 1	18 ± 2	0.07	14 ± 1	14.4 ± 1[b]	0.45
Calcium (mg/dl)	7.1 ± 0.5	8.6 ± 0.2	0.02	9.0 ± 0.5	8.8 ± 0.2	0.62
Chloride (mmol/l)	114 ± 1	115 ± 2	0.37	118 ± 1	115 ± 1 [b]	0.06
Corticosterone (ng/ml)	67 ± 9[a]	24 ± 5	0.003		no data	
Glucose (mg/dl)	433 ± 22	333 ± 23	0.004	469 ± 14	436 ± 30	0.04
Lactate dehydrogenase (ul/l)	1029 ± 168	470 ± 43	0.01	931 ± 82	627 ± 48	0.006
Phosphorus (mg/dl)	1.0 ± 0.2	2.6 ± 0.5	0.03	5.4 ± 0.8	6.8 ± 0.5	0.20
Potassium (mmol/l)	2.9 ± 0.2	2.3 ± 0.5	0.03	4.1 ± 0.2	4.2 ± 0.3[b]	0.71
Sodium (mmol/l)	155 ± 1	157 ± 0.3	0.02	158 ± 1	157 ± 1[b]	0.21
Total CO_2 (mmol/l)	23 ± 1	19 ± 1	0.01	39 ± 1	41 ± 1	0.55
Uric Acid (mg/dl)	8.9 ± 1	14 ± 2	0.06	3.4 ± 1	3.5 ± 0.5	0.10

[a] n = 6 birds
[b] n = 10 birds

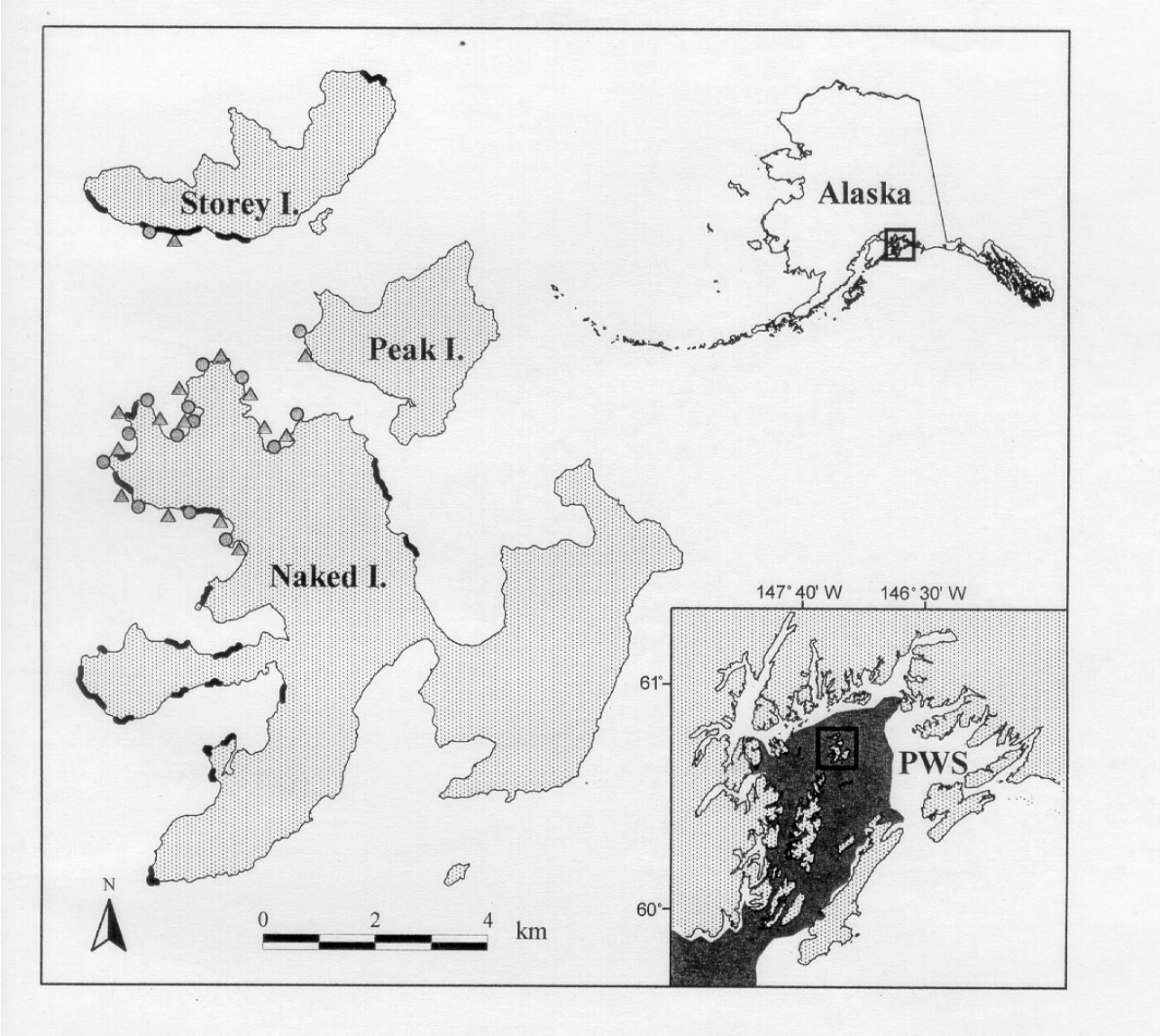

Figure 1. Map of the oiled study site. Pigeon Guillemot study colonies areas are depicted by a thick shoreline. Dive transect locations are shown as circles (1996 sites) or triangles (1997 sites). Inset map shows the location of the oiled study colonies within Prince William Sound (PWS), Alaska, and the path of oiling through PWS.

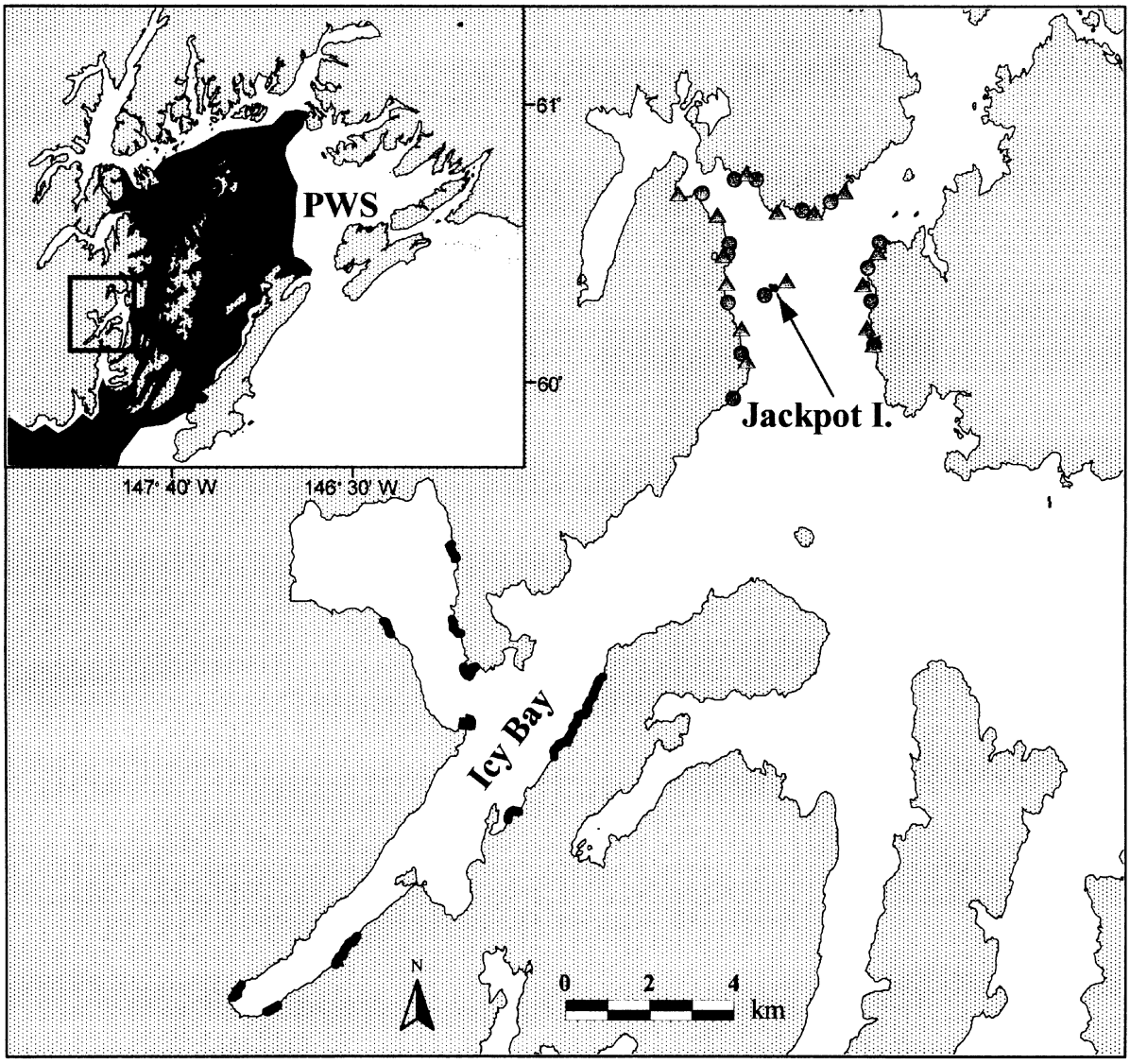

Figure 2. Map of the unoiled study site. Pigeon Guillemot study colonies areas are depicted by a thick shoreline. Dive transect locations are shown as circles (1996 sites) or triangles (1997 sites). Inset map shows the location of the unoiled study colonies within Prince William Sound (PWS), Alaska, and the path of oiling through PWS.

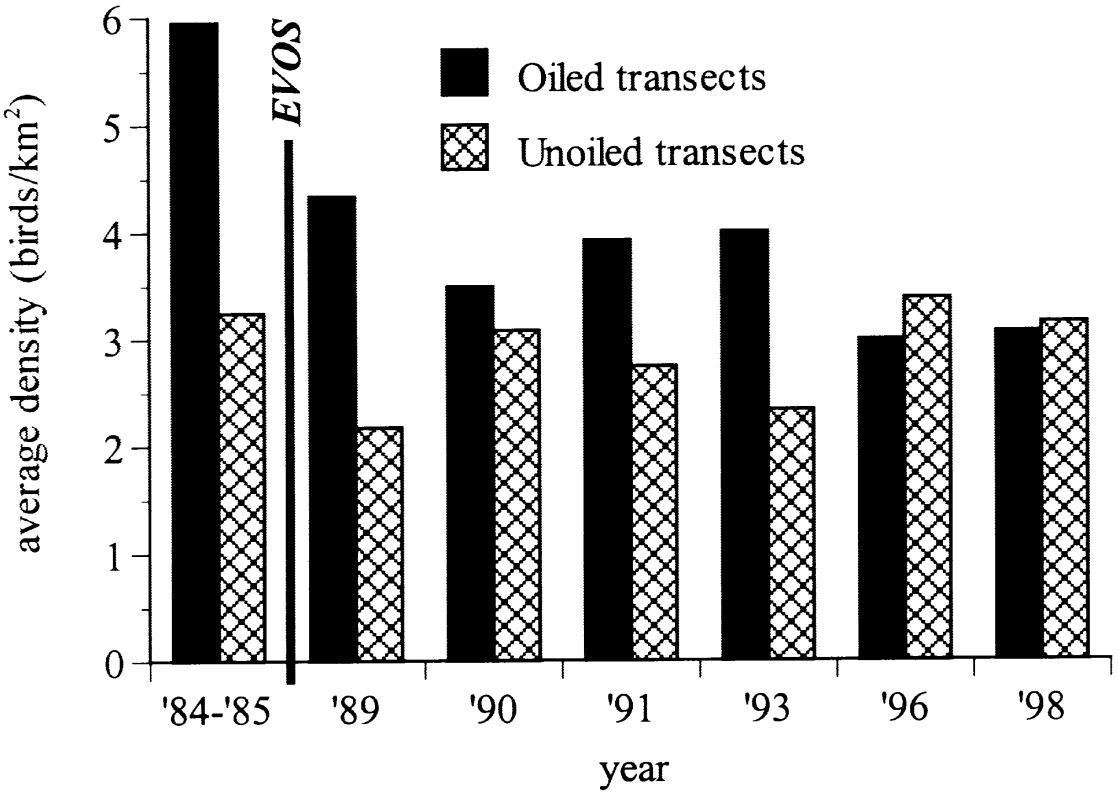

Figure 3. Densities of guillemots along oiled and unoiled shorelines before and after the *Exxon Valdez* oil spill. Densities were determined by surveys conducted during June and July. Survey transects (n = 123) were selected by stratified-random sampling to account for differences in marine habitat. Surveys were conducted from 8-m boats piloted 100 m offshore. All guillemots observed within a sampling window that extended 100 m to either side of, in front of, and above the survey vessel were counted. Intertidal rocks, beaches, and nearshore uplands were also scanned for guillemots.

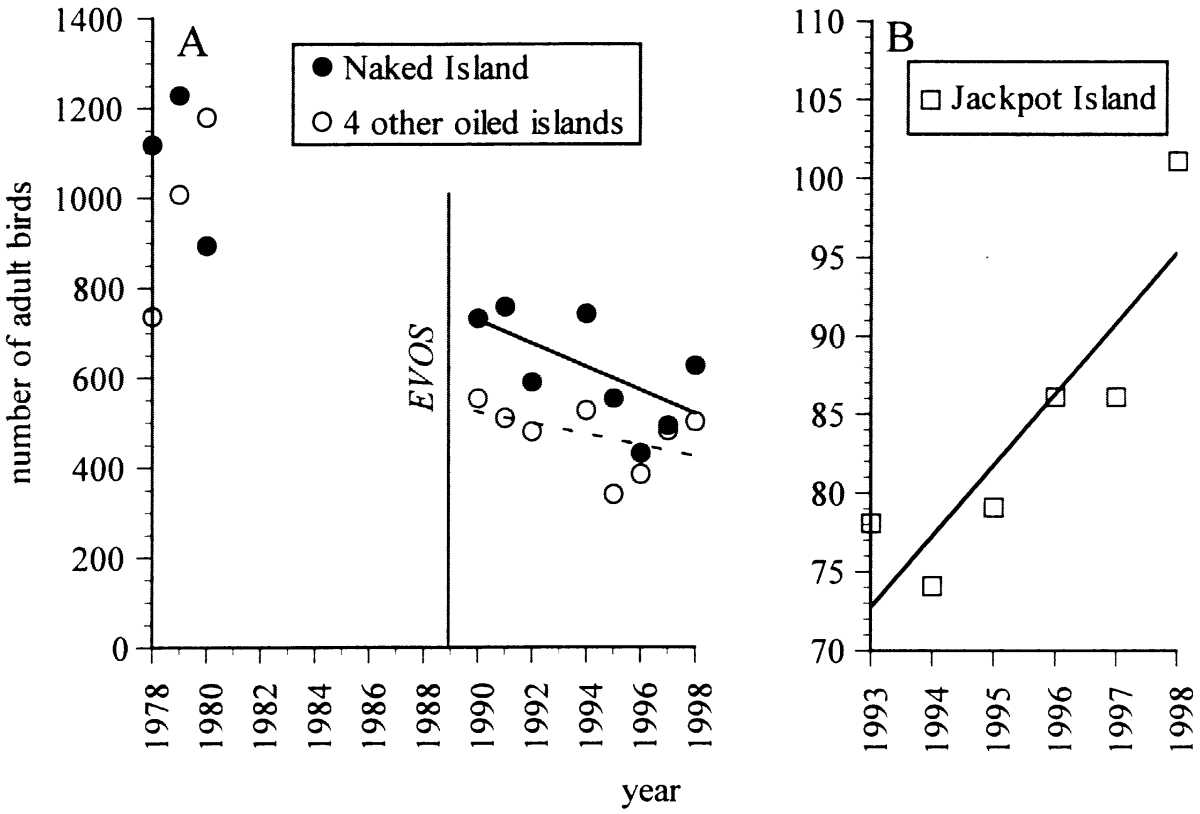

Figure 4. Population trends of Pigeon Guillemots at oiled (A) and unoiled (B) study colonies in Prince William Sound, Alaska, before and after the *Exxon Valdez* oil spill. Post-spill trends at oiled colonies are similar when considering Naked Island (y = -27.1x + 54,589, n = 8 years, r^2 = 0.42, P = 0.084, *solid regression line*), or four other oiled islands (Storey, Peak, Smith, and Little Smith) for which comparable census data were collected (y = -10.4x + 22,252, n = 8 years, r^2 = 0.17, P = 0.31, *dashed regression line*). The population of guillemots at Jackpot Island, the unoiled colony, increased significantly post-spill (y = 4.5x - 8924, n = 6 years, r^2 = 0.78, P = 0.020).

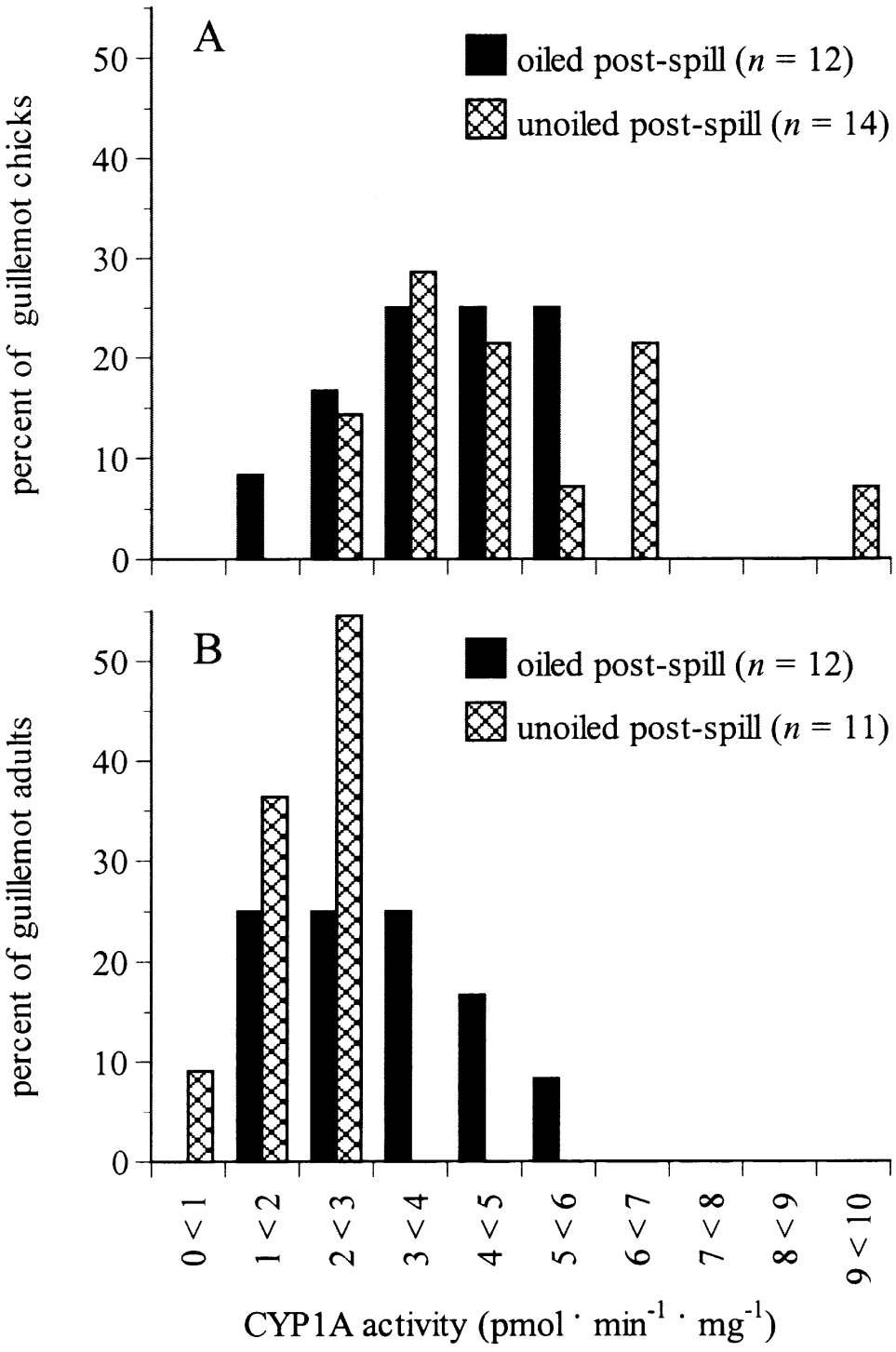

Figure 5. Frequency histogram of CYP1A for Pigeon Guillemot chicks in 1998 (A), and adults in 1999 (B), at oiled (Naked and Storey islands) and unoiled colonies (Jackpot Island and Icy Bay) in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill.

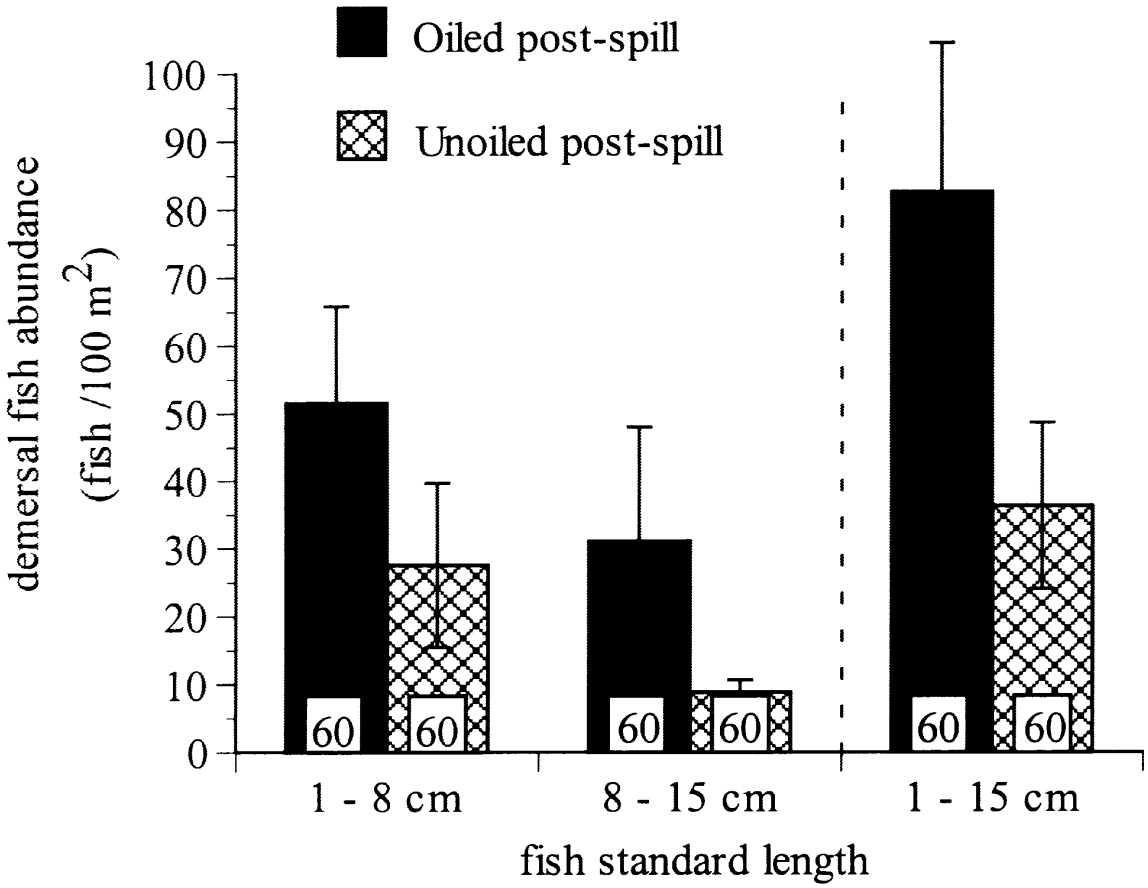

Figure 6. Demersal fish abundance at Pigeon Guillemot foraging areas near oiled (Naked, Peak and Storey islands) and unoiled colonies in Prince William Sound, Alaska, 1996 and 1997. Data from the two years were pooled because the difference in fish densities between years was not statistically significant (see Results). Mean values (± 1 SE) are presented and sample sizes are indicated at the base of the bars.

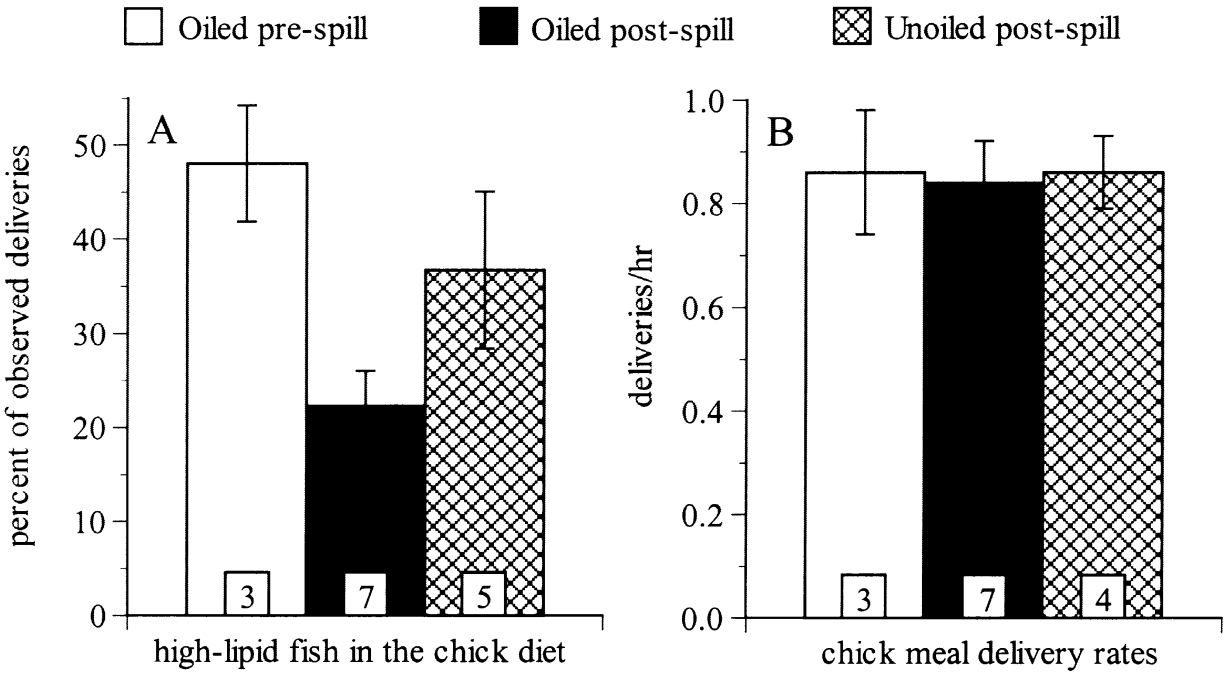

Figure 7. Percent high-lipid fish in the chick diet (A), and chick meal delivery rates (B), at oiled (Naked Island) and unoiled (Jackpot Island) colonies in Prince William Sound, Alaska, before and after the *Exxon Valdez* oil spill. Values presented are grand means (± 1 SE) of individual year means. Sample sizes are indicated at the base of the bars.

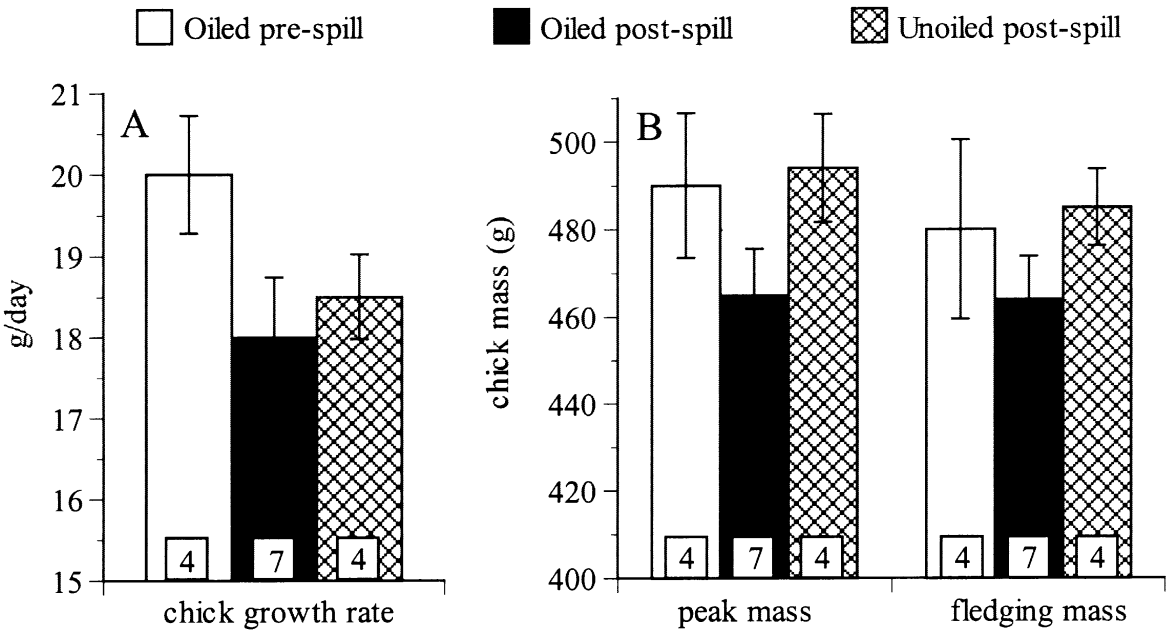

Figure 8. Chick growth rates (A), and peak and fledging masses (B), at oiled (Naked Island) and unoiled (Jackpot Island) colonies in Prince William Sound, Alaska, before and after the *Exxon Valdez* oil spill. Values presented are grand means (± 1 SE) of individual year means. Sample sizes are indicated at the base of the bars.

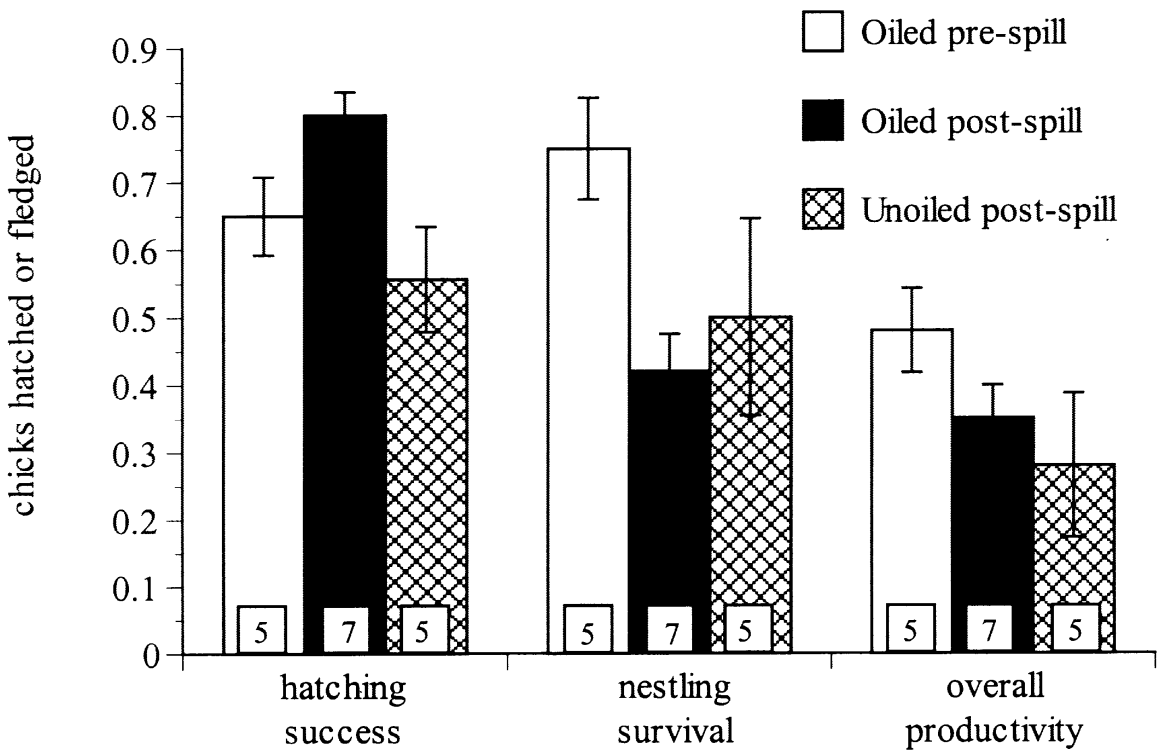

Figure 9. Reproductive success at oiled (Naked Island) and unoiled (Jackpot Island) colonies in Prince William Sound, Alaska, before and after the *Exxon Valdez* oil spill. Values presented are grand means (± 1 SE) of individual year means. Sample sizes are indicated at the base of the bars.

Appendix A1: Chick diet, chick growth and productivity parameters at Naked and Storey islands (oiled site), Prince William Sound, Alaska, before and after the *Exxon Valdez* oil spill (*EVOS*).

year	% high-lipid fish in diet	meal delivery rate (del/hr)	clutch size (eggs)	hatching success	chick growth rate (g/day)	peak mass (g)	fledging mass (g)	nestling survival	nest predation rate (%)	overall productivity
1978	NA	NA	1.5 ± 0.1	0.58 ± 0.11	19.7 ± 1.4	484 ± 10	467 ± 9	1.0 ± 0.0	0 ± 0	0.56 ± 0.1
n			13	19	15	29	29	10	32	18
1979	60 ± 2	1.1 ± 0.1	1.9 ± 0.1	0.73 ± 0.06	22.1 ± 1.0	511 ± 12	506 ± 12	0.85 ± 0.06	10 ± 6	0.61 ± 0.07
n	525	25	33	59	16	17	17	41	30	57
1980	40 ± 2	0.9 ± 0.1	1.8 ± 0.1	0.46 ± 0.07	19.0 ± 0.0	518 ± 52	518 ± 52	0.61 ± 0.12	11 ± 7	0.26 ± 0.07
n	622	30	27	46	1	2	2	18	19	43
1981	44 ± 2	0.7 ± 0.1	1.6 ± 0.1	0.73 ± 0.08	19.2 ± 1.9	445 ± 25	428 ± 29	0.61 ± .10	9 ± 6	0.44 ± 0.09
n	431	37	22	33	11	13	13	23	22	32
1984	NA	NA	1.9 ± 0.1	0.77 ± 0.15	NA	NA	NA	0.70 ± 0.15	0 ± 0	0.54 ± 0.14
n			7	13				10	7	13
					EVOS					
1989	40 ± 2	1.0 ± 0.1	1.6 ± 0.2	0.91 ± 0.09	18.1 ± 2.5	511 ± 16	507 ± 17	0.38 ± 0.18	33 ± 13	0.33 ± 0.17
n	508	21	7	11	5	10	10	8	15	9
1990	14 ± 1	1.2 ± 0.1	1.8 ± 0.1	0.78 ± 0.06	16.7 ± 1.2	442 ± 17	438 ± 17	0.44 ± 0.09	18 ± 6	0.40 ± 0.07
n	646	18	27	51	12	13	13	32	38	47
1994	12 ± 1	0.9 ± 0.2	1.7 ± 0.1	0.90 ± 0.05	15.7 ± 2.1	469 ± 12	464 ± 12	0.51 ± 0.09	39 ± 10	0.46 ± 0.08
n	927	6	23	39	10	18	17	35	23	36
1995	22 ± 1	0.6 ± 0.1	1.8 ± 0.1	0.80 ± 0.05	19.5 ± 1.4	480 ± 14	455 ± 16	0.54 ± 0.07	31 ± 8	0.43 ± 0.06
n	689	9	39	69	13	22	22	55	39	69
1996	21 ± 1	0.6 ± 0.1	1.8 ± 0.1	0.82 ± 0.04	20.9 ± 1.1	482 ± 18	455 ± 15	0.49 ± 0.06	41 ± 8	0.41 ± 0.08
n	645	18	41	74	20	15	15	61	41	41
1997	30 ± 2	0.8 ± 0.1	1.7 ± 0.1	0.79 ± 0.04	18.9 ± 0.8	440 ± 8	431 ± 9	0.47 ± 0.06	34 ± 6	0.35 ± 0.05
n	541	16	56	89	42	59	57	70	56	96
1998	17 ± 3	0.8 ± 0.1	1.6 ± 0.1	0.63 ± 0.05	15.9 ± 1.6	434 ± 15	431 ± 14	0.11 ± 0.04	82 ± 5	0.08 ± 0.03
n	149	7	74	102	18	6	6	64	66	116

Appendix A2: Chick diet, chick growth and productivity parameters at Jackpot Island (unoiled site), Prince William Sound, Alaska, 1994-1998.

year	% high-lipid fish in diet	meal delivery rate (del/hr)	clutch size (eggs)	hatching success	chick growth rate (g/day)	peak mass (g)	fledging mass (g)	nestling survival	nest predation rate (%)	overall productivity
1994	46 ± 3	1.1 ± 0.2	1.9 ± 0.1	0.80 ± 0.06	20.3 ± 1.4	520 ± 14	509 ± 11	0.86 ± 0.06	0 ± 0	0.65 ± 0.07
n	291	4	24	46	6	8	11	35	21	46
1995	41 ± 2	0.9 ± 0.1	1.9 ± 0.1	0.57 ± 0.07	17.1 ± 0.7	471 ± 12	467 ± 12	0.43 ± 0.10	22 ± 9	0.25 ± 0.06
n	628	23	29	53	16	12	12	28	23	55
1996	44 ± 6	NA	1.8 ± 0.1	0.61 ± 0.08	18.3 ± 1.3	NA	NA	0 ± 0	84 ± 6	0 ± 0
n	70		20	36	8	0	0	22	37	36
1997	4 ± 3	0.8 ± 0.1	1.7 ± 0.1	0.47 ± 0.07	18.8 ± 1.7	474 ± 12	482 ± 9	0.70 ± 0.10	6 ± 4	0.31 ± 0.06
n	484	16	30	53	16	14	13	23	33	52
1998	48 ± 4	0.8 ± 0.1	1.8 ± 0.1	0.33 ± 0.07	18.2 ± 1.0	509 ± 16	483 ± 13	0.53 ± 0.13	4 ± 4	0.18 ± 0.06
n	195	7	27	48	16	9	10	15	28	

Exxon Valdez Oil Spill
Restoration Project Final Report

Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

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Volume 2
Appendices

L. E. Holland-Bartels, Editor

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Anchorage, Alaska 99503

December 2002

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Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

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Study History: This project began with the acceptance of the 5-year study plan by the Trustee Council in March 1995. The FY 95 funds were provided to develop sampling protocols, test methodologies, and to initiate those portions of the overall study that could begin in late summer 1995. The first full field season for this study was initiated in FY 96, followed by a similar field effort in FY 97, and focused reduced effort in FY 98. Program reviews by the Chief Scientist and Trustees of work reported in this document occurred in February 1996 and 1997 and January 1998. The final report has undergone external scientific peer review conducted through the Chief Scientist's office as well as journal review as noted in the individual chapters.

Abstract: The 1989 spill of some 42 million L of crude oil into Prince William Sound, Alaska, represents not only the largest tanker spill in United States history, but the world's largest spill in northern waters. Acute effects have been studied extensively. However, efforts to quantify the spill's long-term chronic effects and develop defensible restoration measures have been plagued by varying levels of scientific uncertainty. That such uncertainty exists is not unexpected. The spill occurred in Prince William Sound's highly variable physical setting typified by its complex oceanography and fjord-like geomorphology. Additionally, uncertainty was driven by the scarcity of precise pre-spill population estimates and spotty life-history information for most species. The research reported herein in, structured in eight primary papers and 27 supporting papers (appendices), documents the state of recovery and assessments of continuing constraints to population recovery for four vertebrate predators (sea otter *Enhydra lutris*, harlequin duck *Histrionicus histrionicus*, river otter *Lontra canadensis*, and pigeon guillemot *Cepphus columba*) whose recovery status remained uncertain some 5 years after the *Exxon Valdez* oil spill. These species are used in a collective weight of evidence approach to better understand the process of coastal community recovery. Each species is examined for the strength of information it brings in health, population, and trophic metrics to support or reject the hypothesis of continuing oil effects in the nearshore system versus the alternatives that food constraints or demographic bottlenecks limit these focal species. While data for individual species contain various levels of uncertainty, scientific confidence is developed in the following picture when examined across species, metric, and hypothesis: Within the nearshore coastal environment, sporadic releases of residual oil are occurring, and benthic species, primarily invertebrates, are being exposed in a temporally and spatially patchy manner sufficient to transport oil up through the food chain. Thus, for the two invertebrate-feeders, sea otter and harlequin duck, evidence exists over several lines of investigation to suggest that local-scale populations continue to be constrained not by food availability or natural demographic processes, but by increased levels of mortality coincident with continued exposure to residual oil. Conversely, weight of evidence suggests that

only limited direct oil-related effects are being transferred through the fish trophic pathway. Sufficient evidence suggests recovery is occurring in river otter populations, while the lack of recovery in pigeon guillemot may be attributed to food limitations (both natural and indirectly related to the spill) and/or slow demographic response to initial acute mortalities. Individual lines of investigation often contained uncertainty, but the collective weight of evidence presented in this multipaper volume indicates lack of full recovery of the nearshore ecosystem from the *Exxon Valdez* oil spill nearly a decade following the event. Integrated, multispecies approaches can allow sufficient weight of evidence to develop despite inherent system variability or data limitations and, thus, facilitate both better societal understanding of such pollution events and development of appropriate restoration responses.

Key Words: Alaska, Barrow's goldeneye, biomarkers, body mass, *Cepphus columba*, clams, condition indices, cytochrome P450, demography, diet, ecosystem, emigration, *Enhydra lutris*, *Exxon Valdez* oil spill, food limitation, habitat selection, harlequin ducks, health, hematology, *Histrionicus histrionicus*, home range, hydrocarbons, immigration, intertidal, *Lontra canadensis*, masked greenlings, mortality, mussels, nearshore, pigeon guillemots, plasma biochemistry, pollution, population recovery, predator-prey interaction, prey, prey availability, prey consumption rate, prey demography, Prince William Sound, reproduction, river otters, sea otters, sea urchins, serum chemistry, sex-ratio, subtidal, surveys, survival, trophic.

Project Data: Final Restoration Report 99025, a collaborative and multiagency effort, used an integrated approach to assess recovery status of the nearshore ecosystem of Prince William Sound following the *Exxon Valdez* oil spill of 1989. As a result of this design, scientists from some 15 research organizations located in over 10 states participated and were required to openly share research results to all participants in near real-time. This distributed-organization and research-sharing requirement necessitated the development of a detailed data management plan and a process by which data could be shared and remotely accessed. Such a design was developed and documented in Holland-Bartels (1996)[1]. For the period of active study, all study data were served for project scientists by the U.S. Geological Survey's Alaska Biological Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503 (Table 1). At project completion, all study data were returned to principal investigators to be managed and archived per policy of their respective agencies. Access to these data is made by arrangement with senior authors (or agency) of the report chapters.

Citation: Holland-Bartels, L.E., editor. 2002. Mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill, volume 2 - appendices. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska.

[1]Holland-Bartels, L. 1996. Mechanisms of impact and potential recovery of nearshore vertebrate predators: Restoration Project 95025 Annual Report. Report to the *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska, USA.

Table 1. Data management summary for Nearshore Vertebrate Predator Study at study completion, 1998.

				Files present?			Compliance?	
NVP component	Files (#)	Total size (mb)	Data on file	History	Metadata	SOP	History	Metadata
Focal Species								
Sea otters	501	165.0	all:1995–98	some	some	yes	some	yes
Harlequin ducks	108	3.18	all:1995–98	yes	yes	yes	yes	yes
River otter	15	3.41	all:1996–98	yes	yes	yes	yes	yes
Pigeon guillemot	24	4.07	1996–97 (no 98)	yes	yes	yes	yes	yes
Prey Data								
Duck food	23	0.55	all:1995, 1997	yes	yes	yes	yes	yes
Intertidal clams	71	3.28	all:1995–97	yes	yes	yes	yes	yes
Mussels	137	6.99	1996 (no 97, 98)	yes	yes	yes	yes	yes
Subtidal clams	21	1.53	all:1995–97	yes	no	yes	yes	-
Subtidal fishes	51	1.25	all:1995–97	yes	yes	yes	yes	yes
Sea urchins	94	2.48	all:1996–97	yes	yes	yes	yes	yes
Other Files								
Invertebrate predators	22	1.88	all:1995–96	yes	one	yes	yes	yes
Side-scan sonar	72	4.40	all:1995	yes	yes	yes	yes	yes

Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Preadotrs Following the 1989 *Exxon Valdez* Oil Spill

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APPENDIX SYN-01

Integrating Ecosystem Studies: a Bayesian Comparison of Hypotheses[1]

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[1]Published: 1998. Pages 495–507 *in* F. Funk, J. N. Ianelli, T. J. Quinn II, and P. J. Sullivan, editors. Proceedings of the International Symposium on Fishery Stock Assessment Models for the 21st Century. Alaska Sea Grant College Program AK-SG-98-01.

*****Abstract:

Ecosystem studies are difficult to interpret because of the complexity and number of pathways that may affect a phenomenon of interest. It is not possible to study all aspects of a problem, thus subjective judgement is required to weigh what has been observed in the context of components that were not studied but may have been important. This subjective judgement is usually a poorly documented and ad hoc addendum to a statistical analysis of the data. We present a Bayesian methodology for documenting, quantifying, and incorporating these necessary subjective elements into an ecosystem study. The end product of this methodology is the probability of each of the competing hypotheses. As an example, this method is applied to an ecosystem study designed to discriminate among competing hypotheses for a low abundance of sea otters at a previously oiled site in Prince William Sound, Alaska.

*Introduction:

Ecosystem approaches are increasingly advocated as a way of improving the science and management of natural systems (Lackey *in press*). For instance, studies of the effects of anthropogenic stressors on a species can be misleading if they ignore possible indirect effects acting through predator or prey populations (Higashi and Patten 1989). Further, natural changes in these other components of the ecosystem may cause changes in the focal population, masking or exaggerating the effects of the stressor (Piatt and Anderson 1996). Many studies of the impacts of human actions on a particular species now include research on other components of the ecosystem thought to be important to the focal species.

Nonetheless, there are practical limitations to an ecosystem approach. Because of cost and logistical constraints, not all ecosystem components can be studied and therefore some indirect impacts may be missed. Experimentation or replication may not be possible, and it may thus be difficult to unambiguously assign causes to any observed differences in populations between impacted and non-impacted sites, or before versus after an impact at a single site. It is also highly likely that among the suite of studies, some will give results that are to some degree contradictory.

For these reasons, interpreting the results of an ecosystem study requires some degree of expert judgement. Synthesizing the results of numerous studies of parts of a complex problem is difficult, and it may thus be difficult for investigators to reach conclusions in a rational fashion. Further, different scientists faced with the same evidence may arrive at different conclusions. As the subjective interpretation of results tends to be an ad hoc and poorly documented process, the sources of disagreement may be difficult to uncover and resolve. This paper presents a structured method for documenting and quantifying the expert interpretation of the results of an ecosystem study.

*Proposed Methodology:

The methodology presented here is designed for testing ecosystem-level hypotheses. It integrates studies of diverse components of the ecosystem, summarizing the results as the relative evidence for each hypothesis from each study and the overall evidence for each hypothesis from the ensemble of studies. Its Bayesian features consist of incorporating and quantifying the subjective step of interpreting results, and calculating a probability that each hypothesis is true.

The method consists of the following steps:

1. Generate hypotheses
2. Summarize the experiments and their results
3. Create a table of the expected results under each hypothesis if each experiment were ideal
4. Calculate the probability of the observed result under each hypothesis using statistical considerations
5. Adjust probabilities by considering potential violations of statistical assumptions
6. Adjust probabilities to account for differences between the hypotheses tested and the hypotheses of interest
7. Summarize the evidence for each hypothesis, accounting for dependencies among experiments

Steps 3-6 deal with eliciting statements of probability from experts. Such elicitations can be problematic if experts are unfamiliar with translating their experiments into numerical probabilities (Morgan and Henrion 1990, ch. 7). Our sequence of steps is designed to overcome such problems by sequentially considering several sources of uncertainty, progressing from the most to least familiar. At each of the seven steps, in particular those where subjective judgement is required, the rationale leading to the decision should be thoroughly documented.

**Step 1. Generate hypotheses. The first step is to have the experts identify the hypotheses that are the competing explanations for the phenomenon under investigation. It is important that the hypotheses be both exhaustive and mutually exclusive. If not, the confidence assigned to some hypotheses will be overstated, as the evidence for them will in some respects be counted twice.

Often, there will be reason to believe that several of the hypothesized phenomena might act simultaneously. There are two principal ways of constructing mutually exclusive hypotheses if this is a possibility. The first is to consider a “multiple causes” hypothesis. The second is to redefine the hypotheses to allow minor effects of other factors. For instance, the two hypotheses “effect is produced by factor A” and “effect is produced by factor B” can be made mutually exclusive by redefinition as “effect is principally produced by factor A” and “effect is principally produced by factor B”.

**Step 2. Summarize the available data. In this step, the studies and their results are summarized. For clarity, it is often more useful to use a short verbal description of the results. For instance, a study of differences in prey abundance between control and treatment might be summarized as “much greater abundance found at the control site”.

**Step 3. Consider ideal studies. The third step in this process is to lay out a table with the different hypotheses as the top row and the different experiments as the left-most column (Table 1). Then, have the experts fill out this table as if each study were an ideal experiment; i.e. there were no possibility of either false positive or false negative results.

Table 1. Hypothetical results of a set of ideal experiments.

	Hyp. 1	Hyp. 2	Hyp. 3	Hyp. 4
Study A	positive	negative	negative	positive
Study B	negative	negative	positive	negative
Study C	positive	positive	positive	negative

In the hypothetical example above, Study A would distinguish between Hypotheses 1 or 4 and Hypotheses 2 or 3. In combination, the three studies would be able to determine which hypothesis was true.

**Step 4. Statistical considerations. While ideally the three studies would determine which hypothesis was true with 100% accuracy, in the real world misleading results may be obtained. One of the ways this may happen is through random sampling error. Often, almost any result is possible under any of the hypotheses. Nonetheless, the observed result will be more probable under some hypotheses than others.

The objective of this step is to calculate these relative probabilities, otherwise known as the likelihoods of each of the hypotheses (Gelman et al. 1995 ch. 1). Often, with continuously-distributed variables, the likelihood is a probability density rather than a probability per se. Likelihoods (Table 2) are usually obtained from standard statistical distributions such as the normal or binomial. The exact distribution used depends upon the assumptions made about the experimental data, such as whether each point is independent and identically distributed, whether the sampling variance is constant, etc.

Table 2. Table of likelihoods. P(Result of A|Hyp. 1) means the probability of getting the observed result of Study A if Hypothesis 1 were true.

	Hypothesis 1	Hypothesis 2
Study A	P(Result of A|Hyp. 1)	P(Result of A|Hyp. 2)
Study B	P(Result of B|Hyp. 1)	P(Result of B|Hyp. 2)

This is the first of a series of steps in which experts are asked to assign probabilities to the competing hypotheses. Some experts are unfamiliar with quantitative probability statements and scientists in particular are often uncomfortable making assertions about the relative merits of competing hypotheses without conclusive evidence. This step is important in that it introduces experts to assigning probabilities to the hypotheses, yet does so in a rigorous way using familiar statistical calculations.

**Step 5. Account for possible biases in the test or experimental results. The assumptions of statistical tests are rarely exactly met. Samples may not be completely independent, important sources of error may not be included in the statistical model (e.g., ignoring error in the measurement of the independent variable), and measurements may have some unknown biases. Historically, statistical confidence tends to overstate the certainty of scientific results (Henrion and Fischoff 1986).

In constructing the table of likelihoods of results, this overconfidence needs to be accounted for. Generally, the effect of such errors is to make the probabilities of the result under each hypothesis more similar. Based on their knowledge of the experiment, experts should determine which assumptions of the test are likely to be violated, and to what degree. These judgements are to some extent subjective, but once made the statistical literature or computer simulations can provide guidance on their likely effects. In consultation with a statistician, the experts should adjust the table of probabilities to account for such violations.

**Step 6. Account for differences between the statistical hypothesis being tested and the biological hypothesis that is actually of interest. Often, an experiment to test a hypothesis tests it only indirectly. The results may thus be ambiguous if the indirect indicator could occur in several ways, some of which are not related to the hypothesis.

For example, if the hypothesis were that some population was affected by an environmental contaminant, an investigator might test the environment for the presence of the contaminant and test individuals for signs of poor health. A positive result in either case would not necessarily implicate the contaminant; the contaminant might be present yet not be causing health effects, or poor health might be due to causes other than the contaminant.

As in step 5, the effect of a difference between the hypothesis tested and the hypothesis of interest is to even further equalize the probabilities of the observed results under each hypothesis. The appropriate amount of adjustment of the table entries depends on the probability of other (possibly unknown) alternative explanations for the test results.

Such assessments are unavoidably subjective and require the judgement of experts. Hopefully, by this point in the process the experts are comfortable with assessing the relative probability of the data under each hypothesis and how violations of assumptions may result in misleading experimental results. It is crucial that they consider alternative explanations for their data yet not be paralyzed by such possibilities. They should be willing to examine data that seems to strongly favor one hypothesis and consider whether there are other, possibly unstudied ecosystem pathways that could produce similar results and state how probable they feel such pathways are.

**Step 7. Summarize the evidence. In this step, the table of probabilities is summarized to derive the overall weight of evidence for each hypothesis provided by the ensemble of studies. If the studies are independent, then elementary statistical theory says the joint likelihood of each hypothesis is simply the multiplication of its probability under each study (Eq. 1). The overall

likelihood of each hypothesis is then simply the product of its column of probabilities (here R1, R2, and R3 signify the results of experiments 1, 2 and 3, respectively).

Likelihood of hypothesis = P(R1|hyp.) x P(R2|hyp.) x P(R3|hyp.) [1]

The different hypotheses can then be compared in terms of their relative likelihoods. This comparison is easier if the likelihoods are re-scaled so that the sum of all of the likelihoods is 1. From a Bayesian perspective, each re-scaled likelihood could then be interpreted as the probability that a hypothesis was true.

***Complication A. Dependencies among results. There are two ways that experimental results might not be independent. First, the data from two experiments may have been taken from the same random sample. Second, two experiments may measure the same ecological phenomenon two different ways. In either case, it is not appropriate to treat the results as providing independent evidence bearing on the alternative hypotheses; i.e., simply multiplying the probabilities of the two experiments together will overweight the evidence.

There are several possible methods to account for dependencies among experimental results. If experiments are highly interdependent, they should be lumped and a single probability of each hypothesis calculated for the ensemble results. If experiments are only partially dependent, the correlation of results must be accounted for. If the correlation can be calculated, probability theory provides methods for calculating a joint probability. If not, a value must be obtained from experts, although experts have been found to perform poorly at providing a numerical value for correlation coefficients (Morgan and Henrion 1990 ch. 7).

A more intuitive method for dealing with partially correlated results is to ask investigators to provide an estimate of the "effective" number of experiments. For instance, investigators may feel that dependence between two experiments is such that they jointly provide only as much evidence as 1.5 independent experiments. Then, the appropriate adjustment would be to raise each of the probabilities to the 0.75 power (e.g., Eq. 2). In general, if N experiments are correlated so that the effective number is E, probabilities for hypotheses for each experiment should be adjusted by raising them to the E/N power.

Likelihood of hypothesis = $P(R1|hyp.)^{0.75}$ x $P(R2|hyp.)^{0.75}$ [2]

***Complication B. Prior probabilities. Bayesian statistics involves multiplying the likelihoods by a set of prior weights (the prior probabilities) for the hypotheses before re-scaling to calculate the posterior probabilities. In the Bayesian approach, these prior probabilities reflect the weight accorded each hypothesis before the experiments were conducted. Assuming the probability of each hypothesis to be proportional to the joint likelihoods treats each hypothesis as being equally likely a priori, thus letting the data determine the relative probability of each hypothesis. While this is intuitively appealing, it may not be appropriate.

For instance, if the analysis were being used in a legal proceeding, it might be appropriate to give the benefit of the doubt to the defendant by assigning small prior weights to hypotheses implicating the defendant. Similarly, in investigating current scientific theory a high prior weight might be assigned to the currently accepted paradigm, so that a novel competing theory would not get much credence unless the evidence for it was overwhelming. An alternative to using prior weights is to calculate probabilities only from likelihoods, but require a very high probability that a hypothesis is true before acting on it. Whatever the prior weights, if data strongly support one hypothesis over the others the final probabilities will reflect this.

Standard Bayesian practice is to compare the evidence for competing hypotheses using Bayes factors (Kass and Raftery 1995). The Bayes factor is simply the ratio of the posterior probabilities of two competing hypotheses divided by the ratio of the prior probabilities assigned before the experiments were conducted. When the prior probabilities of the hypotheses are equal, this is simply the ratio of the posterior probabilities.

*An example -- sea otters after the Exxon Valdez oil spill:

On March 4, 1989, the supertanker Exxon Valdez spilled nearly 42 million liters of crude oil in Prince William Sound, Alaska (Spies et al. 1996). This spill is hereafter referred to with the acronym EVOS. Sea otter populations in oiled areas suffered high mortality (Loughlin et al. 1996). Other components of the ecosystem were likewise severely affected. Five years after the spill, residual oil was present in sediments and mussel beds in some areas of the spill (Spies et al. 1996). Even today, residual oil is found in some areas.

The Nearshore Vertebrate Predator (NVP) project (Holland-Bartels et al. 1996), a multi-university and agency investigation funded by the EVOS Trustee Council, is aimed at determining whether top predators in Prince William Sound are still suffering the effects of the oil spill. The question is difficult to answer unambiguously because of the complicated nature of the ecosystem and the lack of data from the period before EVOS. The NVP project studies predator populations from several points of view, and also looks at other components of the ecosystem on which these predators depend. If a population is still being affected by EVOS, the study is designed to ascertain whether the effects are due to the continuing toxic effects of oil, a slow rate of recovery from past mortality, or an indirect effect on some critical ecosystem component.

With limited resources and such an intensive approach, few populations can be studied. Sea otter abundance at Knight Island, which was oiled in 1989, is lower than at Montague Island, which was not. The NVP sea otter study has focused on these two populations, trying to find the reason for these differences in abundance. The principal hypotheses are:

1. **Direct toxicity of residual oil.** Residual oil is present and reducing the fecundity and/or survival of otters at the oiled site.
2. **Reduced forage due to oil effects.** The initial impact of oil or residual oil is reducing prey available to sea otters.
3. **Slow recovery due to demographic limitations.** Aside from the initial otter mortality from EVOS, residual oil is absent or does not affect otters or their food. However,

limitations on the maximum growth rate of the population have prevented the population from reaching capacity yet.

4. **Natural differences in capacity.** The oiled site has poorer or less abundant otter habitat.

A variety of studies have been undertaken to determine which hypothesis is the most likely. These include:

1. **Demographic comparisons**. Population abundance, age structure, and reproductive rates were compared between islands.
2. **Individual health.** Otters were captured at both locations. Individuals were weighed and measured, and blood samples taken. In particular, blood cells and serum chemistry were examined for signals of poor health, and a specific signal of exposure to oil (the enzyme P450) was tested for.
3. **Prey abundance and foraging success.** The abundance and size distribution of major prey items of sea otters were compared among islands. In addition, foraging sea otters were observed to determine relative rates of success in obtaining prey items.

Statistical hypothesis tests were performed for many of the studies but are not reported here. We chose not to calculate likelihoods based solely on statistical distributions --step 4 of our methodology -- because the limitations imposed by the design of the study tended to emphasize the considerations dealt with in steps 5 and 6. There are multiple predictions from each of the hypotheses, not all of which are distinct. Any particular study result may eliminate some hypotheses but leave several others. More likely, any particular study result would be ambiguous, as there is a small likelihood of almost any result from each hypothesis. In particular, the detection of a phenomenon does not necessarily imply that this was the cause of the difference in abundance between the two islands. For instance, oil could be present but yet not greatly affect survival. Likewise, prey abundance could differ between one site and another but be unrelated to the difference in otter abundance.

Thus, the interpretation of the results of the studies required some judgement. Our chief tool was to ask ourselves, “What is the probability we would get the result we observed from Study ___ if Hypothesis ___ was true?” We attempted to quantify our impression of the strength of each piece of evidence by filling out the table of probabilities, sequentially considering what the result would mean in an ideal world, what the statistical tests implied, how the assumptions of the tests might be violated, and what mechanisms might cause the results to be misleading.

We felt our ability to discriminate among probability levels was fairly coarse. Accordingly, we initially filled in the table of probabilities verbally, using the categories “high”, “moderate – high”, “moderate”, “low – moderate”, and “low”, which we later replaced with 0.9, 0.7, 0.5, 0.3, and 0.1, respectively (Table 3).

Table 3. First attempt at integrating studies. Top row gives hypotheses, and left column gives experiments with the results in parentheses. "M" refers to Montague Island (control), and "K" to Knight Island (oiled). The main body of the table gives the probability of obtaining each experimental result under each hypothesis. The bottom two rows summarize the result as the product of the probabilities for each hypothesis (i.e. the joint likelihood) and the probability products re-scaled to sum to 100%.

EXPERIMENT & (RESULT)	"A" DEMOGRAPHIC LIMITATION	"B" FOOD LIMITATION	"C" OIL PERSISTENCE	"D" RECOVERY HAS OCCURRED
OTTER DENSITY (K << M)	0.9	0.9	0.9	0.3
REPRO. RATES (EQUAL)	0.9	0.5	0.7	0.9
BLOOD CHEMISTRY (EQUAL)	0.9	0.7	0.3	0.9
P450 (EQUAL)	0.7	0.7	0.1	0.9
PREY ABUNDANCE (M < K)	0.9	0.1	0.1	0.1
FORAGING SUCCESS (M < K)	0.9	0.1	0.7	0.1
Joint Likelihood	**0.4133**	**0.0022**	**0.0013**	**0.0022**
Probability of Hypotheses	**98.6%**	**0.53%**	**0.32%**	**0.52%**

The result of our first analysis was to assign more than a 98% probability to the hypothesis that the population differences were due to a demographic limitation in the rate of recovery of the Knight Island population from spill mortality. All other hypotheses combined had less than a 1.5% probability of being true. We were unhappy with this result, as this high degree of confidence did not reflect our personal higher degree of uncertainty. We felt that the evidence for this hypothesis was not that strong.

In examining the reasons for this initial result, we identified three principal sources of error. First, we overstated the power of the studies to discriminate among hypotheses. For instance, we assigned a 0.90 probability of seeing greater prey abundance at the oiled site if demography was limiting recovery, but only a probability of 0.10 under any of the other hypotheses. We did not adequately address step 6 of our methodology; for instance, there would

be a fairly good chance of seeing higher prey abundance at the oiled site under several alternative hypotheses.

Second, the range of hypotheses we considered was too narrow. In retrospect, we felt there was a strong possibility that all of the hypotheses might be incorrect, and some other factor might be responsible for differences between areas. This resulted in an unrealistically high probability for the hypothesis most consistent with the data.

Third, we did not adequately account for dependencies among experimental results (step 7, complication A). While we lumped most blood chemistry measures into one result, we kept the assay for the enzyme P450 (a more direct measure of exposure to oil) as a separate experiment. Since this assay could indicate the same phenomenon, and was measured on the same sample of animals, we felt the two results were effectively equivalent to only 1.5 experiments. Similarly, measures of prey size, prey abundance, and foraging success to some extent measured the same phenomenon. In retrospect, we decided to consider them as equivalent to 2 experiments.

We therefore revised the tabled probabilities, taking what we hoped was a more realistic look at the power of the studies and adding another alternative hypothesis to those we had listed. While we were able to think of several specific alternatives, we felt the true explanation for population differences might be something we hadn't considered. Therefore, we added only one hypothesis; an "unknown causes" category. Meanwhile, the completion of analyses of blood chemistry and the enzyme P450 suggested that residual oil might be present at the oiled site, and new information became available about the size distribution of prey species (Table 4).

Table 4. Second attempt at integrating studies. Top row gives hypotheses, and left column gives experiments with the results in parentheses. "M" refers to Montague Island (control), and "K" to Knight Island (oiled). The main body of the table gives the probability of obtaining each experimental result under each hypothesis. The bottom two rows summarize the result as the product of the probabilities for each hypothesis (i.e. the joint likelihood) and the probability products re-scaled to sum to 100%.

EXPERIMENT & (RESULT)	"A" DEMOGR. LIMIT.	"B" FOOD LIMIT.	"C" OIL PERSIST.	"D" RECOVERED	"E" UNKNOWN CAUSES
OTTER DENSITY (K << M)	0.9	0.9	0.9	0.3	0.9
REPRO RATES (EQUAL)	0.9	0.5	0.7	0.9	0.9
BLOOD CBC'S & CHEMISTRY (WEAK INDICATION OF LIVER DAMAGE AT K)	0.5	0.5	0.7	0.3	0.5

P450 (M < K)	0.3	0.3	0.9	0.3	0.3
PREY ABUNDANCE (M < K)	0.9	0.1	0.5	0.3	0.5
PREY SIZE (M < K)	0.9	0.1	0.7	0.3	0.7
FORAGING SUCCESS (M < K)	0.9	0.1	0.7	0.3	0.7
Joint Likelihood	0.1581	0.0011	0.1744	0.0040	0.0764
Probability of Hypotheses	38.2%	0.3%	42.1%	1.0%	18.5%

The revised table again supports the hypothesis that the populations differ because the population in the oiled area has not had the time to recover fully from the losses due to the oil spill. However, it shows even greater support for the hypothesis that residual oil is still affecting the population. The hypothesis that some unknown factor accounts for the difference between populations is also quite probable.

Two hypotheses were eliminated from consideration, principally because of the forage abundance studies. Forage was more abundant and foraging success higher at the oiled site. These results were not at all consistent with the food limitation hypothesis, and were also unlikely if the population at the oiled site had recovered to its carrying capacity. However, it should be noted that the "unknown causes" hypothesis, which has a fairly high probability of being true, is not necessarily related to the spill. Thus it would be inappropriate to say the probability that the population is no longer suffering effects of the spill is only 0.01.

We will refine and expand this analysis as more data become available and more experts are consulted. These results are not our final interpretation, and should be viewed as a preliminary analysis. We provided this example solely to illustrate the use of the methodology.

*Discussion:

The Bayesian aspects of the proposed methodology are (1) use of subjective expert judgement in interpreting indirect tests of hypotheses, and (2) integration of experimental results and expert judgement into an overall probability for each hypothesis using Bayesian probability calculations. A large literature exists on using Bayesian methods to compare hypotheses (Kass and Raftery 1995).

Bayesian methods have been criticized from a variety of standpoints (e.g., Dennis 1996). The principal criticism is that Bayesian methods inject subjectivity into scientific analyses that should be objective. However, in extrapolating from the results of diverse studies on small aspects of a larger question, subjectivity in the form of expert judgement is unavoidable. We propose a methodology that formalizes the intuitive process experts use in interpreting the results of

ecosystem studies. This approach clearly distinguishes subjective interpretation from experimental results, and clearly shows the reasoning used.

Our methodology provides a tool for investigators to organize their thinking. The ecosystem and the results of the numerous studies may be too complex to be readily grasped in their entirety. By allowing investigators to approach the synthesis of the studies one element at a time, our method increases the tractability of the process.

The methodology also facilitates openness and discussion, since subjective components of the synthesis of the studies are documented and quantified. It clearly shows why a particular conclusion was reached, and what evidence investigators felt was ambiguous or particularly strong. Areas of disagreement among investigators are also easily identified.

Our methodology is based on principles derived from other methods widely used for eliciting probabilities from experts (summarized in Morgan and Henrion 1990, Ch. 7). Examples of such methods include the Stanford/SRI protocol (Spetlzer and Stael von Holstein 1975, Merkhofer 1987) and the Wallsten/EPA protocol (Wallsten and Whitfield 1986). We've tailored our methodology to the specific goal of summarizing the relative support for alternative hypotheses from an interrelated but necessarily incomplete set of studies.

Most methods for probability elicitation pay great attention to getting experts comfortable with the idea of translating their knowledge and judgement into probability statements, and to overcoming a tendency of experts to give probabilities that overstate the level of certainty (Tversky and Kahneman 1982, Morgan and Henrion 1990, Ch. 7). Our solution to these difficulties is to take experts through a specific sequence of probability elicitation steps. These start with specifying deterministic outcomes, then progress through familiar specifications of probability (likelihood calculations) to less familiar probability specifications (the effects of violation of statistical assumptions and of not directly testing the hypothesis of interest). This sequence gradually introduces the process of making probability statements. It also sequentially introduces more and more forms of uncertainty, continually forcing the expert to reflect on whether the degree of confidence he's previously expressed is appropriate.

Our example illustrates both the utility and limitations of the methodology. The summary table lists the hypotheses and the experimental results. Probabilities within the table explicitly document the experts' interpretation of the consistency of the results of each experiment with each hypothesis. The summary probabilities excluded two hypotheses but retained three others, one of which appears to be only half as probable as the other two.

However, the 18.5% probability assigned to the "Unknown Causes" hypothesis makes interpretation of the other probabilities somewhat ambiguous. Much of the probability assigned to this hypothesis may indicate that recovery has occurred, and the differences we found are caused by some unknown factor(s) unrelated to the spill. It is also possible that "Unknown Causes" represents effects related to the spill such as cascading ecological effects. In either case,

the results do provide guidance for further research; they suggest that continuing studies should focus on hypotheses “A”, “C”, and “E”.

The necessity for re-evaluating our initial analysis because of unrealistic results in instructive. It reinforces the experience of others who have found that numerical statements of probability given by experts tend to be overly confident (Tversky and Kahneman 1982, Henrion and Fischoff 1986). Our second try produced a result that we felt better reflected the strength of the evidence provided by the experiments.

There is a danger that allowing such reanalysis could result in investigators juggling numbers to arrive at a result that reflected their preconceptions. However, an honest reappraisal of each element in the table is not inappropriate. Most methods for probability elicitation do recommend that assessors return to an earlier phase in the process whenever questioning reveals that the probabilities elicited clearly don’t reflect the expert’s judgement (Kadane et al. 1980, Morgan and Henrion 1990, ch. 7, Laskey 1995). We found the reanalysis of the table caused us to re-examine the basis of our interpretations; rather than reinforcing our preconceptions, it tended to make us change them.

Use of our methodology will make it easier to examine the source of differences in interpretation of a study. For example, a scientist who disagreed with our conclusions might find that the basis of his difference was the weight placed on the blood chemistry results. A sensitivity analysis to alternative interpretations would be easy to perform by replacing the disputed probability with an alternative value to see if this affected the conclusions.

This method is not proposed as a substitute for good experimentation. With scarce, poor quality, and ambiguous data the conclusion reached after applying this method will be that considerable uncertainty remains. However, in such situations this methodology may identify areas of major uncertainty and suggest fruitful lines of investigation. The major benefit of this approach is the explicit documentation and quantification of the unavoidable subjective interpretation of ambiguous results that arise in many ecosystem investigations. In contrast, when strong experimental designs are available that produce clear evidence, subjective interpretation will be minimized and investigators should reach consensus.

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Biomarker Appendices

(BIO)

APPENDIX BIO-01

HEMATOLOGY AND SERUM CHEMISTRY OF SEA OTTERS IN OILED AND UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA, 1996-98[1]

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[1]In preparation for submission to _____.

Introduction

Studies on sea otters (*Enhydra lutris*) following the 1989 *Exxon Valdez* oil spill showed changes in several hematology and serum chemistry values for otters at the rehabilitation centers in 1989 (Rebar et al. 1995, Williams et al. 1995), and for otters caught in the wild in western Prince William Sound (WPWS) in 1990-92 (Rebar et al. 1996; USGS-BRD, unpublished data). Elevated serum enzymes in otters from oiled areas, relative to those from nonoiled areas, were a general finding in all years and although the degree of difference was not large, suggested the possibility of hepatocellular injury or subclinical liver disease. A further observation was an increase in eosinophils in otters from oiled areas, possibly indicating systemic hypersensitivity reactions. Combined with a lack of population increase and low survival rates in oiled areas of WPWS (Ballachey et al. 1994; Chapter 3 Part A, Chapter 3 Part B, Appendix BIO-03), these observations of abnormal blood samples generated concern that lingering effects of acute oil exposure and, possibly, continuing toxic effects of exposure to residual oil, were deleteriously affecting health and survival and limiting recovery of sea otters.

The Nearshore Vertebrate Predator (NVP) study was implemented in 1995 to assess recovery in the nearshore ecosystem, focusing on four top-level predator species inhabiting nearshore areas of Prince William Sound (PWS; Holland-Bartels et al. 1999). One NVP component was to determine whether oil exposure continued, and if so, whether it was associated with adverse toxic effects on health and survival of the predator species (Chapter 2). Sea otters were one of the predator species included in the NVP study (Chapter 3 Part A). Health of sea otters was assessed by examining hematology and serum chemistry profiles and body condition of animals captured in oiled and unoiled areas of WPWS in 1996-98. Herein we report on hematology and serum chemistry data from those animals, with the primary objective of assessing differences between otters in oiled vs. unoiled areas.

Methods

Sea otters were captured in July and August of 1996, 1997 and 1998 at Knight and Naked islands (oiled area), and at Montague Island, using either tangle nets (modified gill nets) or diver-held Wilson traps (Chapter 3 Part A). Otters were anesthetized, weighed, and morphometric measurements taken. A premolar tooth was extracted for age determination (Bodkin et al. 1997), and approximately 35 cc of blood were collected from each otter, from the jugular vein. Approximately 10-15 ml of the blood were drawn from the jugular by vacutainer, with about 3 ml being placed in an EDTA tube and refrigerated pending shipment to the clinical laboratory, and 8-10 ml into 1 or 2 glass tubes and allowed to clot for at least 30 minutes before centrifuging to separate serum. Serum was frozen in several aliquots. Two blood smears on glass slides were made from the whole blood. The rest of the blood sample, about 20-25 ml, was drawn into a 50 ml heparinized syringe for isolation of blood lymphocytes to determine levels of mRNA for the biomarker, cytochrome P4501A (CYP1A; Chapter 2, Appendix BIO-02). Whole blood in the

EDTA tube and blood smears were shipped to Quest Laboratories[2], Portland, OR, as soon as possible after collection. Only samples that arrived at the laboratory within 72 hours of collection were used in the analyses of hematology data. Serum samples were maintained in frozen storage until field work was complete, and then submitted as a batch to Quest Laboratories, Portland. Additional serum samples were shipped to the University of Alaska Fairbanks for haptoglobin analyses (Duffy et al. 1993, Chapter 5).

Stepwise model selection was conducted to determine the magnitude of the area effect on 38 hematology and serum chemistry parameters[3], and one additional measure, CYP1A. The ranks of the blood parameters were used as the responses to control for the non-normality common in the blood variables. We used a combination of forward stepwise selection and backward stepwise selection on each blood parameter starting with a model containing five main effects (sex, year, age, capture type, and area) and all two-way interactions. Variables entered and remained in the model when the p-value for the coefficient of the variable was significantly different from zero based on an F-test at the 0.05 level of significance. All terms in the final model were significantly different from zero, except for the retention of main effects if an interaction was in the model. If area was not present with the resulting model, the area term was added to determine the importance of the area effect on the response (Table 1).

An assumption in the use of covariates to help understand the influence of the area effect is that the covariates are not correlated with area. Sex, age, and capture type were found to be correlated with area in a Chi-square analysis (p-values = 0.005, 0.006, and 0.033 respectively). This imbalance is known to cause confounding and affects power to detect differences. Therefore, some of the effects of area may have been adjusted out while adjusting for the covariates. In this sense, the analysis is conservative, because some blood parameters with a marginally significant area coefficient may in fact have an area difference.

We performed a supplemental analysis of the data, using Akaike's Information Criterion (AIC; Burnham and Anderson 1998), to determine the relative magnitude of the area effect on each blood parameter relative to the four other covariates. For each blood parameter, we fit 31 models, ranging from univariate models of each covariate alone, to the fullest model with all five covariates and all two-way interactions. Models that contained three or more main effects also contained the second-order interactions. Models were weighted based on the AIC values and weights were summed from every model that contained the covariate (Burnham and Anderson

[2]Formerly Corning Clinical Laboratory (CCL), and prior to that, Physicians Medlab Laboratories (PML).

[3]Hematology parameters: WBC, RBC, HB, HCT, MCV, MCH, MCHC, RDW, PLATES, NEU#, LYM#, MON#, EOS#, BAS#. Serum chemistry parameters: GLU, BUN, CREAT, URIC, NA, K, CL, CA, P, TPRT, ALB, GLB, TRIG, CHOL, HDL, VLDL, LDL, TBIL, DBIL, GGT, AP, LDH, AST, ALT. Abbreviations given in Table 1.

1998). Covariates with high values (i.e., that covariate was present in all or most of the top AIC models) are deemed important in the explanation of the blood parameter response (Table 2).

Results

For hematology variables, no significant p-values were obtained for area in the models based on ANOVA of ranked values (Table 1). Only the p-value for WBC, at 0.066, approached significance, with higher mean levels in the unoiled area compared to the oiled (Table 3). Using AIC weights (Table 2), a similar pattern was seen for the hematology variables, with area generally being of low importance in the models. The importance value for WBC was only 0.545, which did not support consideration of an area difference based on the marginally significant p-value. Of the remaining hematology variables, only MCHC had a relatively high importance value (0.826) for area; however, the p-value for area in the MCHC ANOVA was 0.286.

A larger number of significant differences were observed between areas for the serum chemistry variables, with p-values from ANOVA models less than 0.05 for BUN, uric acid, calcium, potassium, total bilirubin, direct bilirubin, GGT, LDH, and haptoglobin (Table 1). Importance values for the area effect on these variables also were generally high (Table 2), with 0.780 for BUN and 0.832 for calcium being the lowest of the group. Importance values for area were also relatively high for albumin (0.996), creatinine (0.992) and total protein (0.809), although the p-values from the ANOVA model did not indicate area to be a significant effect for these variables.

Cytochrome P4501A (CYP1A) was significantly higher in otters from the oiled area, indicated by a p-value < 0.001 (Table 1) and an importance value of 1.000 (Table 2). No other effects in the model were found to be significant or of importance for CYP1A.

The importance values for each covariate based on AIC modeling reaffirm the traditional linear model selection process we conducted. In general, variables with high importance values were included in the final model. For the models that contained a significant area effect, the importance value for area was never below 0.78. There were four variables (ALB, CREAT, MCHC and TPRT) for which the importance value for area was higher than 0.78, but the area effect was not significant in our traditionally selected model (p-values of 0.608, 0.541, 0.285, and 0.283, respectively).

To aid in assessing potential contributions of oil toxicity to differences observed in blood parameters, we compiled selected mean values from previous blood sampling efforts in oiled and unoiled areas (Table 4). Included are data from sea otters captured in western and eastern PWS in 1992, and in western PWS and southeast Alaska in 1991. Consistent trends in means are evident for only two variables: GGT (higher in the oiled area in all three data sets, with $p < 0.001$ in 1996-98 and 1992, and $p = 0.35$ in 1991) and direct bilirubin (higher in the oiled area in all three data sets, but only significantly so in 1996-98, when the area x sex interaction was significant). Differences between the study areas in mean GGT are due not to a general elevation in otters

from the oiled area, but rather to a relatively small proportion of animals from the oiled area with higher GGT values; this pattern was evident in 1996-98 (Figure 1), and in 1991 and 1992. As well as GGT, in the 1992 and 1991 data, serum enzymes AP, AST and ALT are all elevated in the oiled areas relative to the unoiled, although differences are not all statistically significant nor large. However, in the 1996-98 data set, the mean values for AP, AST and ALT are almost identical (Table 4).

Discussion

The combination of ANOVA analyses and information values from the AIC selection procedure identified approximately 10 blood variables for which there is a statistical difference in mean values between the oiled and unoiled areas during 1996-98. However, based on examination of all variables, including those that do not differ between areas, we conclude that (1) the hematology and serum chemistry data for otters in oiled areas do not present a biological pattern which might be associated with oil toxicity, and (2) although mean values for a subset of variables do differ statistically, the absolute levels of these differences are small and do not appear to be biologically meaningful in terms of adverse indicators of health of sea otters.

Comparison of the 1996-98 data with results from 1991 and 1992 supports the first conclusion, as there is no consistent pattern in differences between oiled and unoiled areas over the longer period, with the exception of serum GGT levels. We would expect that if differences observed in the 1996-98 study were attributable to continuing oil exposure, then similar differences would be noted in the previous studies as well, presuming levels of oil contamination were greater in the earlier years. The serum enzymes, AP, AST, ALT and GGT all showed some degree of elevation in oiled areas relative to unoiled in 1991 and 1992, but only for GGT were area differences still noted in the 1996-98 samples. Elevated serum enzymes also were reported for oiled sea otters at rehabilitation centers in 1989 (Rebar et al. 1995, Williams et al. 1995), river otters in oiled areas of WPWS post-spill (Duffy et al. 1994), and mink exposed to petroleum products (Mazet et al. in press). By 1996-98, however, mean values for AP, AST, and ALT are remarkably similar in sea otters from the two areas, which suggests diminishing toxicity associated with residual oil in the environment. Although elevations in GGT persist in the 1996-98 data set, the magnitude of difference between oiled and unoiled areas has declined, relative to that seen in the 1992 data. GGT was positively correlated with AP, AST and ALT levels ($r = 0.36$, 0.61, and 0.76, respectively) in 1996-98, although mean levels of these three enzymes do not differ between areas.

The difference in 1991 and 1992 data, compared to 1996-98, may reflect the value of blood panels to detect acute injury to liver and kidney, which would have been a greater factor in the earlier post-spill years. Presumably, during that time, the proportion of sea otters in the population that had acute, sublethal exposure in 1989 (with significant exposures possibly extending into 1990-91) was relatively high. However, based on ages-at-death data, Monson et al. (Appendix BIO-03) conclude that sea otters in the oiled region had higher mortality rates postspill than prespill. Thus, by the 1996-98 study, there were probably few of these individuals

left in the population, so that blood parameters associated with organ damage had, to a large extent, normalized. The lack of difference in the later years suggests that animals with injury from acute exposure are no longer present, but does not necessarily indicate that chronic exposure, at a relatively low level, is not continuing.

Data comparisons across the different years, however, need to be viewed with caution as the specific study areas were not the same across the three data sets. Unoiled areas in 1991 and 1992 were southeast Alaska and eastern PWS, respectively. The area of WPWS that was considered oiled for purposes of otter capture and sampling in 1991 and 1992 actually encompassed both the oiled (Knight Island) and unoiled (Montague Island) study areas for the 1996-98 sampling. This design was implemented in 1996 to minimize other factors that might differ between populations which were more geographically distinct. In 1991 and 1992, however, few sea otters were actually captured in the area of northern Knight Island (the focus of captures between 1996 and 1998) as otter densities there were so low as to render capture operations very inefficient.

The extent to which the differences among study sites in 1991, 1992 and 1996-98 may be influencing our results is unknown. However, one factor known to differ between WPWS and either eastern PWS or southeast AK is length of occupation, as otters reoccupied the latter two areas more recently than they did WPWS (Jameson et al. 1982, Garshelis and Garshelis 1984), and thus likely have had more plentiful food resources available to them. The differences seen between otters in oiled and unoiled areas in the 1996-98 data may also have been influenced by factors related to otter densities and availability of prey (Chapter 3 Part A, Chapter 3 Part B). Another consideration is variation among animals associated with capture stress. We attempted to control for this by including capture method as an effect in the analyses, as otters caught in traps by divers would generally have less opportunity for physical exertion prior to sedation and sampling. However, the length of time that sea otters spent in tangle nets (which was usually unknown for an individual otter) may have varied. Once deployed, nets are checked for captured otters every 3-4 hours. If sea otters at Knight Island were removed from the nets sooner than at Montague Island, they might be expected to have lower levels of capture stress and, on the average, slightly different blood panels. The significant area difference in LDH, an enzyme that will be elevated in animals following muscle exertion (Bossart and Dierauf 1990), may reflect longer periods in nets for sea otters at Montague Island.

Significant differences were frequently noted for other covariates (age, sex, year and capture method) included in the analytical models. Effects of these covariates generally were as expected based on data from other mammalian species (Duncan and Prasse 1989) and, overall, these results tend to increase our confidence in the accuracy of the data set.

Levels of CYP1A were significantly elevated in oiled areas relative to unoiled, in the 1996-98 data (CYP1A was not assayed in the earlier studies). CYP1A is a biomarker of hydrocarbon exposure (Payne et al. 1986, Stegeman et al. 1992), and higher levels in the oiled area are indicative of ongoing contaminant exposure, most likely to residual EVOS hydrocarbons (Ballachey et al. 1999). Although CYP1A induction in sea otters from the oiled area was quite

variable (over a 300-fold difference from minimum to maximum value), little overlap was seen in CYP1A values between the two areas. A more extensive discussion of the CYP1A results is provided in Ballachey et al. (1999).

The serum enzyme GGT, which was higher in oiled areas in 1996-98 and in 1992, may be a sensitive indicator of liver damage from hydrocarbon exposure in sea otters. Differences between areas were more pronounced in 1992, with mean GGT of 29.11 that year, compared to a mean in 1996-98 of 17.78. By 1996-98, most individuals in the oiled area have "normal" GGT values, with only a subset of the animals exhibiting elevations, rather than a general population increase. A similar pattern of a relatively small proportion of animals with elevated GGT was seen in 1991 and 1992. If GGT is indeed a marker of exposure, and residual oil contamination is patchy in distribution, this might be the expected pattern. However, no relation was detected between CYP1A levels and GGT or any of the other serum enzymes. Perhaps by the 1996-98 study, absolute levels of residual EVOS oil were very low, although sufficient to maintain induction of CYP1A in the general sea otter population in the oiled area. These generally low levels of contamination may not have been sufficient to cause overall population increases in GGT. Rather, it may be that only those few animals which have happened to get greater exposure, possibly for extended periods, show increased GGT levels. Because such exposures may be sporadic (relating to patchiness in oiling of shoreline areas, individual preferences for foraging areas, and perhaps to storm events, when oil in sediments may be released), our measures of CYP1A largely do not reflect them, and thus no relation between the GGT and CYP1A is evident.

In conclusion, sea otter blood data collected during the last decade suggest that differences noted in 1991 and 1992 between otters in oiled and unoiled areas likely resulted from acute exposure with associated organ damage, but that by 1996-98, these differences have diminished in magnitude or disappeared. The majority of sea otters in oiled areas have blood values within a normal range, and only a small proportion of otters exhibit elevated serum enzymes, particularly GGT, which may be a lingering manifestation of oil toxicity. Chronic exposure, which continues through 1998 based on the biomarker CYP1A, may still be affecting recovery of the population but for the majority of animals, residual toxicity is not leading to significant organ damage or altered hematology and serum chemistry parameters in sea otters.

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Table 1. P-values from ANOVA on ranked values of blood variables, sea otter NVP data, 1996-98. Values are from final model selected by forward and backward selection. Only effects that remained in the model have p-values presented. Area was put into each model after model selection. When a significant interaction with area exists[d], the unadjusted area means are given within the levels of the interacting variable.

						Direction of Area Difference	
Variable[e]	Age[a]	Sex	Year	Capture Type[b]	Area[c]	Knight Mean	Montague Mean
ALB	d	.	d	.	0.6079		
ALT	.	.	0.0001	.	0.6265		
AP	0.0000	.	.	.	0.9898		
AST	.	.	0.0434	0.0004	0.9430		
BAS#	.	.	.	.	0.6985		
BUN	0.0000	.	.	.	0.0133	45.430	49.807
CA	d	.	d	.	d	1996: 9.120 1997: 9.144 1998: 8.570	1996: 9.025 1997: 9.329 1998: 8.951
CHOL	.	0.0028	.	.	0.9457		
CL	.	0.0002	0.0000	0.0001	0.6806		
CREAT	d	d	0.0005	d	0.5409		
CYP1A	.	.	.	.	0.0000	27.325	1.503
DBIL	.	d	0.0002	0.0010	d	F: 0.058 M: 0.061	F: 0.532 M: 0.014
EOS#	0.0032	.	0.0193	.	0.8989		
GGT	.	.	0.0000	.	0.0001	17.775	13.966
GLB	0.0007	0.0009	0.0006	.	0.1617		
GLU	.	0.0334	0.0055	.	0.1183		
HAPTOS	d	d	d	.	d	1996: 18.400 1997: 59.296	1996: 42.893 1997: 43.586
HB	0.0000	0.0000	0.0002	.	0.1353		
HCT	0.0000	0.0000	0.0000	.	0.7362		
HDL	d	d	0.0000	.	0.4131		
K	0.0014	.	0.0011	.	0.0021	4.095	4.229
LDH	.	.	.	0.0000	0.0001	334.763	461.560

Table 1 continued - P-values from ANOVA, sea otter blood data.

LDL	.	.	0.0000	0.0029	0.4414		
LYM#	.	0.0000	0.0236	.	0.4700		
MCH	0.0082	.	0.0073	.	0.5246		
MCHC	.	.	d	d	0.2854		
MCV	.	.	d	d	0.5753		
MON#	.	.	.	.	0.9155		
NA	.	0.0000	0.0000	0.0024	0.9048		
NEU#	.	0.0808	.	.	0.1302		
P	0.0000	.	0.0260	.	0.8610		
PLATES	0.0114	0.0215	.	.	0.0939		
RBC	0.0000	.	.	.	0.8918		
RDW	.	0.0001	0.0000	0.0000	0.5345		
TBIL	0.0169	d	0.0000	.	d	F: 0.298 M: 0.304	F: 0.278 M: 0.243
TPRT	.	0.0160	0.0000	.	0.2831		
TRIG	.	0.0000	.	.	0.5876		
URIC	.	0.0055	0.0000	.	0.0000	2.050	2.509
VLDL	.	0.0000	.	.	0.6955		
WBC	.	0.0000	0.0416	.	0.0656		

[a]Age in years.

[b]Two capture methods: tangle net or Wilson trap.

[c]Two areas: Knight Island (oiled) or Montague Island (unoiled).

[d]For covariates involved in an interaction, p-value for significance of covariate is not reported because it depends on the levels of the interacting variable.

[e]Abbreviations and Units: ALB - albumin, g/dl; ALT - alanine aminotransferase, IU/l; AP - alkaline phosphatase, IU/l; AST - aspartate aminotransferase, IU/l; BAS# - basophils, x 10^3/ul; BUN - blood urea nitrogen, mg/dl; CA - calcium, mg/dl; CHOL - cholesterol, mg/dl; CL - chloride, mEq/l; CREAT - creatinine, mg/dl; CYP1A, cytochrome P450 1A, molecules x 10^6 per 100 ng total RNA; DBIL - direct bilirubin, mg/dl; EOS# - eosinophils, x 10^3/ul; GGT - gamma glutamyl transferase, IU/l; GLB - globulin, g/dl; GLU - glucose, mg/dl; HAPTOS - haptoglobin, mg haptoglogin-hemoglobin complex/100 ml; HB - hemoglobin, g/dl; HCT - hematocrit, %; HDL - high density lipoproteins; K - potassium, mEq/l; LDH - lactate dehydrogenase, IU/l; LDL - low density lipoproteins; LYM# - lymphocytes, x 10^3/ul; MCH - mean corpuscular hemoglobin, pg; MCHC - mean corpuscular hemoglobin concentration, g/dl; MCV - mean corpuscular volume, fl; MON# - monocytes, x 10^3/ul; NA - sodium, mEq/l; NEU# - neutrophils, x 10^3/ul; P - phosphorus, mg/dl; PLATES - platelets, x 10^3/ul; RBC - red blood cells, x 10^6/ul; RDW - red cell width; TBIL - total bilirubin, mg/dl; TPRT - total protein, g/dl; TRIG - triglycerides, mg/dl; URIC - uric acid; VLDL - very low density lipoproteins; WBC - white blood cells, x 10^3/ul.

Table 2. Importance values for each covariate, sea otter blood data, 1996-98. Values are calculated as the sum of the AIC weight of each model containing the covariate, from a suite of 31 models run for each variable.

Variable[a]	Age	Sex	Year	Capture Type	Area
ALB	1.000	0.538	1.000	0.255	0.996
ALT	0.548	0.119	1.000	0.395	0.611
AP	1.000	0.221	0.476	0.172	0.238
AST	0.075	0.859	0.980	0.995	0.101
BAS#	0.665	0.077	0.896	0.892	0.718
BUN	1.000	0.046	0.243	0.399	0.780
CA	1.000	0.258	1.000	0.109	0.832
CHOL	0.154	0.990	0.653	0.342	0.096
CL	0.798	0.990	1.000	0.998	0.238
CREAT	0.969	1.000	1.000	0.997	0.992
CYP1A	0.098	0.093	0.299	0.147	1.000
DBIL	0.045	0.989	1.000	0.870	0.972
EOS#	0.963	0.663	0.975	0.137	0.102
GGT	0.166	0.186	1.000	0.354	0.993
GLB	0.987	0.912	0.999	0.099	0.501
GLU	0.086	0.890	0.990	0.420	0.692
HAPTOS	1.000	1.000	0.981	0.040	0.956
HB	1.000	0.996	0.989	0.012	0.072
HCT	1.000	0.998	1.000	0.208	0.015
HDL	0.562	0.606	1.000	0.056	0.350
K	0.816	0.019	0.999	0.561	0.994
LDH	0.466	0.041	0.865	0.998	0.998
LDL	0.119	0.366	1.000	0.950	0.461
LYM#	0.093	1.000	0.966	0.144	0.577
MCH	0.533	0.979	0.998	0.053	0.177
MCHC	0.195	0.087	1.000	1.000	0.826
MCV	0.080	0.976	1.000	0.988	0.059

Table 2 continued -- Importance values for covariates, sea otter blood data.

MON#	0.377	0.242	0.565	0.171	0.165
NA	0.074	0.999	1.000	0.906	0.714
NEU#	0.870	0.578	0.818	0.148	0.691
P	1.000	0.374	0.760	0.157	0.102
PLATES	0.617	0.740	0.881	0.060	0.608
RBC	1.000	0.559	0.551	0.159	0.040
RDW	0.079	0.997	1.000	1.000	0.020
TBIL	0.706	0.985	1.000	0.363	0.954
TPRT	0.242	0.638	1.000	0.356	0.809
TRIG	0.950	1.000	0.508	0.180	0.057
URIC	0.120	0.938	1.000	0.866	1.000
VLDL	0.972	1.000	0.494	0.200	0.054
WBC	0.059	1.000	0.868	0.267	0.545

[a]For abbreviations and units, refer to Table 1.

Table 3. Means (unadjusted) and standard errors of blood variables for sea otters from oiled and unoiled areas of WPWS, captured between 1996-98.

	Oiled			Unoiled		
Variable[a]	N	Mean	Std Error	N	Mean	Std Error
ALB	80	2.76	0.023	91	2.74	0.015
ALT	80	204.34	16.567	91	203.25	12.083
AP	80	132.76	5.824	91	135.85	8.112
AST	80	238.51	23.792	91	234.89	13.569
BAS#	63	39.24	7.989	52	47.46	10.403
BUN	79	45.43	1.108	88	49.81	1.142
CA	80	8.97	0.057	91	9.09	0.05
CHOL	80	139.49	2.773	91	139.16	2.012
CL	80	113.7	0.364	91	113.93	0.292
CREAT	80	0.46	0.014	90	0.44	0.012
CYP1A	71	27.32	5.131	86	1.5	0.231
DBIL	80	0.06	0.006	91	0.05	0.005
EOS#	63	1553.75	124.101	52	1627.85	113.917
GGT	80	17.78	1.226	89	13.97	0.871
GLB	80	3.78	0.055	91	3.83	0.052
GLU	80	141.89	3.766	91	139.96	4.258
HAPTOS	57	37.77	8.010	57	43.25	7.428
HB	63	19.63	0.121	52	19.56	0.131
HCT	63	58.05	0.393	52	57.82	0.575
HDL	79	89.46	2.478	91	86.27	2.711
K	80	4.1	0.035	91	4.23	0.032
LDH	80	334.76	15.125	91	461.56	23.304
LDL	76	39.68	3.274	89	40.67	2.688
LYM#	63	3349.37	177.585	52	4223.81	323.274
MCH	63	40.61	0.253	52	40.68	0.264
MCHC	63	33.85	0.171	52	33.94	0.291
MCV	63	120.16	0.943	52	120.15	1.076
MON#	63	371.9	23.789	52	373.94	29.763
NA	80	152.66	0.546	91	152.45	0.316
NEU#	63	3936.68	154.744	52	4379.17	191.948

Table 3 continued - Area means for sea otter blood variables.

P	80	4.47	0.132	91	4.49	0.149
PLATES	62	267	7.627	51	291.02	8.424
RBC	63	4.85	0.043	52	4.82	0.046
RDW	63	14.43	0.175	52	14.32	0.248
TBIL	80	0.3	0.007	89	0.27	0.008
TPRT	80	6.53	0.063	91	6.57	0.052
TRIG	80	57.91	1.917	91	66.25	2.77
URIC	80	2.05	0.068	88	2.51	0.079
VLDL	76	11.55	0.406	89	13.03	0.546
WBC	63	9.25	0.264	52	10.66	0.369

[a] For abbreviations and units, refer to Table 1.

Table 4. Comparison of mean values of selected sea otter blood variables from 1996-98 (NVP study; Knight vs. Montague Islands) with those from 1992 (western and eastern PWS) and 1991 (western PWS and SE AK)[a].

	Year								
	1996-98			1992-94			1991		
Variable[b]	Oiled (Knight) n = 63 H[c] n = 80 C	Unoiled (Montague) n = 52 H n = 91 C	P-value[d] Area	Oiled (WPWS) n = 34 H n = 35 C	Unoiled (EPWS) n = 49 H n = 53 C	P-value[d] Area	Oiled (WPWS) n = 9 H, C	Unoiled SE AK n = 8 H n = 26 C	P-value[d] Area
WBC	9.25	10.66	*0.066*	9.97	8.84	*0.013*	10.26	9.18	*0.397*
BUN	45.4	49.8	*0.013*	52.1	50.2	*0.397*	46.9	50.1	*0.508*
URIC	2.05	2.51	*0.000*	2.55	2.54	*0.938*	2.67	2.41	*0.294*
K	4.10	4.23	*0.002*	4.11	4.17	*0.411*	4.31	3.93	*0.020*
CA	8.97	9.09	e	8.85	8.73	*0.351*	8.76	8.62	*0.599*
TBIL	0.30	0.27	e	0.57	0.40	*0.080*	0.37	0.49	*0.065*
DBIL	0.06	0.05	e	0.017	0.006	*0.092*	0.011	0.008	*0.761*
GGT	17.78	13.97	*0.000*	29.11	15.68	*0.000*	16.89	14.62	*0.345*
LDH	335	461	*0.000*	483	528	*0.445*	419	374	*0.573*
AP	133	136	*0.990*	93	83	*0.086*	125	96	*0.215*
AST	239	235	*0.943*	344	302	*0.235*	264	203	*0.355*
ALT	204	203	*0.627*	322	236	*0.000*	238	181	*0.076*

[a]Rebar et al. 1995; USGS-BRD unpublished data.
[b]For abbreviations and units, refer to Table 1.
[c]H = Hematology variables (only WBC in this table); C = Chemistry variables.
[d]1996-98 - P-values from ANOVA on ranks; model selection process included covariates of age, sex, year and capture method (see Table 1). 1992 and 1991 - P-values from preliminary analysis -- t-test, two-tailed, on non-transformed data.
[e]Area involved in a significant interaction (see Table 1); because p-value depends on the levels of the interacting variable, it is not reported here.

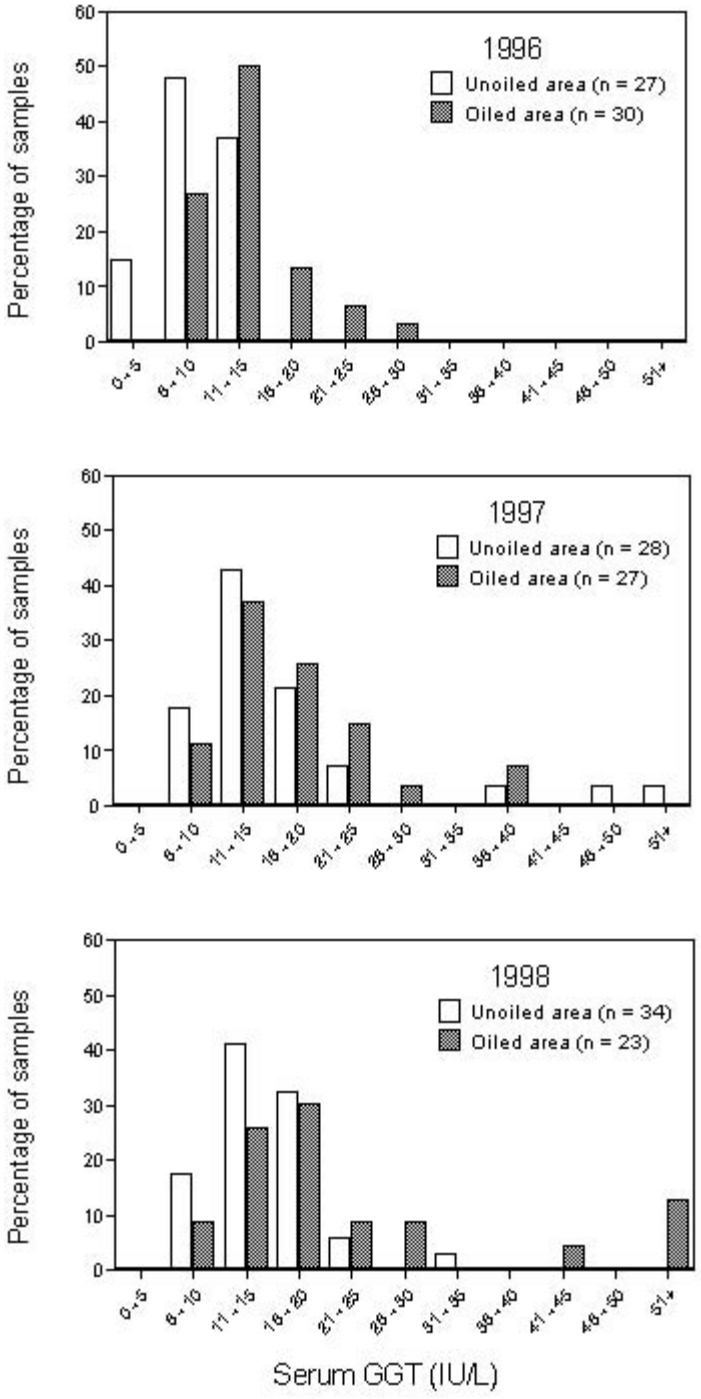

Figure 1. Distribution of serum GGT values in sea otters from oiled and unoiled areas by year.

APPENDIX BIO-02

CYP1A1 Gene Expression in Sea Otters (*Enhydra lutris*): A Quantitative Reverse Transcriptase-Polymerase Chain Reaction to Measure CYP1A mRNA in Peripheral Blood Mononuclear Cells[1]

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ABSTRACT

The nearshore ecosystem of the Prince William Sound serves as a repository for the oil spilled in 1989 from the *Exxon Valdez* tanker. Responses associated with exposure to residual oil may include transcriptional activation of mRNA for cytochrome P450 1A1 (CYP1A1). Here, we report on the isolation, cloning, and sequencing of a PCR product for sea otter (*Enhydra lutris*) CYP1A1. Additionally, we developed a quantitative RT-PCR technique to quantify the level of CYP1A1 expression in sea otter peripheral blood mononuclear cells (PBMC). Using this quantitative RT-PCR method with PBMC isolated from sea otters captured in oiled (n=20) and non-oiled (n=21) areas of the Prince William Sound, we detected a significant difference ($p < 0.001$) in the level of CYP1A1 expression between the two study areas. The mean level of expression in PBMC from sea otters (molecules/100 ng total RNA) was 1.96×10^6 for the oiled area and 0.12×10^6 for the non-oiled area. Analysis of the expression of CYP1A1 in peripheral blood mononuclear cells by RT-PCR represents a sensitive and non-lethal method for evaluating potential exposure to environmental contaminants.

INTRODUCTION

Extensive sections of shoreline in Prince William Sound, Alaska (USA), were contaminated by oil spilled from the tanker *Exxon Valdez* in 1989. Between 8 and 16% of the 10.8 million gallons of crude oil spilled by the T/V *Exxon Valdez* remains buried in marine sediments (1). Such oil is not subject to degradation by marine organisms and remains in a form that is toxic to many vertebrates (2). Moreover, microbial analyses suggest that oil in sediments along oiled shorelines is still present at higher concentrations than in unoiled sites. In 1995, studies were implemented on the effect of residual oil on nearshore vertebrate predators as a potential factor limiting their recovery from spill-related injury (3). One aspect of these studies involves the biochemical responses of individual animals to environmental contaminants.

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[1]In preparation for submission to Archives of Biochemistry and Biophysics

Members of the cytochrome P450 (CYP) family of oxidative metabolizing enzymes are important in the detoxification of environmental contaminants (4-7). Evaluations of the mRNA, protein or catalytic activity of these metabolizing enzymes have been proposed as biomarkers for exposure to a variety of contaminants. Although the CYP enzymes are involved in detoxification reactions, intermediate metabolites of these reactions are frequently more toxic than parent compounds (6,8). The central role of CYP in detoxification makes it a sensitive indicator of xenobiotic exposure compared to other biochemical parameters that are more indicative of stress and cellular damage (6,7). The substrates for CYP enzymes are widespread, ranging from physiologically occurring lipids such as steroids and prostaglandins to biologically and chemically synthesized xenobiotics. Many lipophilic organic xenobiotics such as polyaromatic hydrocarbons (PAHs) are metabolized by the CYP1 gene family (6-9). Specifically, CYPA1 is induced by PAHs (e.g., 3-methylcholanthrene) and coplanar chlorinated hydrocarbons (e.g., 2,3,7,8-tetrachlorodibenzodioxin [TCDD]). There are many reports that have attempted to correlate the level of environmental exposure to contaminants and CYP1A1 mRNA levels, enzyme activity, or CYP1A1 protein concentrations (10-14).

Basal and induced CYP1A1, at low levels, have been detected in a variety of immune tissues including cultured human lymphocytes (15), macrophages from several species (16), murine spleen (17), and human peripheral lymphocytes (18, 19). Transcriptional activation of mRNA for CYP1A1 is one of the most sensitive known responses associated with exposure to the above compounds. As part of an effort to develop biomarkers of contamination for monitoring programs and to determine whether the measurement of CYP1A1 gene induction can be useful in detecting oil exposure in sea otter populations, we have conducted a study of mRNA levels in peripheral blood mononuclear cells of sea otters.

Here we report on a quantitative reverse transcriptase polymerase chain reaction (RT-PCR) method for determining the copy number of cytochrome CYP1A1 mRNA in peripheral blood mononuclear cells of sea otters. By quantitative RT-PCR, we detected a 16-fold increase in CYP1A1 mRNA levels in peripheral blood mononuclear cells from otters in oiled areas as compared to non-oiled areas.

MATERIALS AND METHODS

Animals. Male and female sea otters were captured from oiled and non-oiled areas of western Prince William Sound. Liver was collected from sea otters that died or were euthanized in rehabilitation centers during the initial response of the oil spill. A total of 20 ml of heparinized (preservative free heparin, Sigma Chemical Co., St Louis, MO) blood was collected by venipuncture from each animal for the isolation of peripheral blood mononuclear cells. Liver samples were collected at death and maintained frozen at -70° C.

Materials. TRIzol Reagent was purchased from Life Technologies Inc., Grand Island, NY. Riboprobe® Combination System - T3/T7 RNA Polymerase, Human Genomic DNA, Phi X 174 DNA /Hae III Markers were purchased from Promega, Madison, WI. NuSieve® agarose and Metaphor® agarose were from FMC BioProducts, Rockland, ME. Molony murine leukemia

reverse transcriptase was from Gibco BRL Products, Grand Island, NY. Other reagents were of the highest grade available.

Peripheral blood mononuclear cell isolation. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by density gradient centrifugation (20). Isolated PBMC were cryopreserved and aliquots were washed and pelleted.

Isolation and Analysis of RNA. Total RNA was isolated from liver using rapid guanidinium-phenol extraction method (21), originally adapted from Chomczynski and Sacchi (22). To examine the quality of RNA extracted, electrophoresis through formaldehyde containing gels was performed as described previously (23). Aliquots of 20 mg native RNA were maintained at -70˚C.

Preparation of Internal Standard. The use of an internal standard that contains target (i.e., sea otter CYP1A1) primer sequences negates tube-to-tube variability in PCR amplification and is essential to quantifying mRNA expression by RT-PCR. We generated recombinant RNA (rcRNA) internal standards as described by Vanden Heuvel et al. (24). Using this method, a rcRNA was generated that upon amplification with sea otter CYP1A1 primers results in a product (221 bp) that is easily resolved from target product (180 bp) following agarose gel electrophoresis.

Cloning CYP1A1 from sea otter. Since sea otter CYP1A1 had not been previously described, we chose primers based on a comparison of several known CYP1A1 cDNA sequences. The CYP1A1 cDNA sequences from sheep (25), human (26), mouse (27), guinea pig (28), hamster (29), and rat (30) were aligned using MegAlign (DNAstar). From this alignment, we chose the following primers, found in a highly conserved area within the sequences listed above:

1A1FP312, 5'-CCACAGAGCTTCTCCTGGC-3';
1A1RP581, 5'-GGGTTCTTCCCCACGGTC-3';

These primers were utilized to amplify sea otter CYP1A1 from heavily oiled animals (see Figure 1). The primers were optimized for Mg, annealing temperature, pH, and number of cycles. The optimized conditions were: 4mM Mg, 54° C annealing temperature, pH 8.8, and 30 cycles.

The PCR products were cloned into T7Blue T-vector according to the manufacture's instructions (Novagen, Madison, WI). Following isolation of plasmid DNA, fluorescence dideoxynucleotide sequencing was performed at the Purdue University DNA Facility. The sequence information was used to obtain more efficient primers specific for sea otter cDNA using PrimerSelect (DNAStar, Madison, WI) as shown below.

Sea otter 1A1FP, 5'-TGGTCAATTTTCTGTTCCTAG-3';
Sea otter 1A1RP, 5'-AGGTCAGCTCAACCTTGAGA-3';

Quantitative Competitive RT-PCR. Competitive PCR was performed essentially as described by Gilliland et al. (31, 32) as modified by Vanden Heuvel et al. (33). For each sample, 8-10 aliquots of total RNA (0.1 mg) were prepared, and a dilution series of the rcRNA internal

standard was spiked into these aliquots. Reverse transcription of RNA was performed in a final volume of 20 ml containing 25 mM Tris-HCl (pH 8.3 at 25°C), 50 mM $(NH_4)_2SO_4$, b-mercaptoethanol, 0.1 mg/ml bovine serum albumin, 5 mM $MgCl_2$, 1 mM of each deoxynucleotide triphosphate, 1 unit RNase inhibitor, 2.5 units M-MLV Reverse Transcriptase (Life Technologies, Inc.), 2.5 mM oligo$(dT)_{16}$, 0.1 mg total RNA, and varying amounts of rcRNA internal standard. The samples were incubated at 45°C for 15 min., and reverse transcriptase was inactivated by heating to 99°C for 5 min. The PCR reaction mixture contained 3 mM $MgCl_2$, 2.5 units Taq polymerase, and 6 pmol of forward and reverse primers. The reactions were heated to 94°C for 3 min. and cycled 30 times through 30-s denaturing step at 94°C, a 30-s annealing step at 54°C, and a 30-s elongation step at 72°C. Following the final cycle, a 5-min. elongation step at 72°C was included.

Aliquots of the PCR reaction were electrophoresed on 2.5% NuSieve® 3:1 agarose (FMC Bio Products, Rockland, ME) gels, and PCR fragments were visualized with ethidium bromide staining. A photographic negative was prepared and densitometry was performed using a LKB Gel Scan II laser densitometer (LKB, Piscataway, NJ). Quantification of the amount of target mRNA present was determined as described by Gilliland et al. (31). Initially, a large internal standard concentration range (i.e., 100-1000 molecules/tube) was examined in order to estimate the concentration of target mRNA in each sample. Once the concentration of the CYP1A1 was determined, a more narrow range of internal standards was used to more precisely determine the levels of CYP1A1 mRNA. The actual number of molecules of CYP1A1 was determined by comparing the ratio of the volume of the internal standard to CYP1A1 mRNA. The ratio of the volume of the internal standard/CYP1A mRNA PCR products were plotted against the amount of internal standard added to individual tubes as previously described (31). Linear regression analysis was used to define the equation for the line through the data points. The amount of CYP1A1 mRNA present for individual animals was defined as the amount of rcRNA present where the volume ratio was equal to 1.

Statistical analysis. We used one-way analysis of variance with Student's test (Statview II, Abacus Concepts, Inc., Berkeley, CA) to compare the differences between the means of two groups.

RESULTS

Cloning of CYP1A1 from sea otter liver.

Since sea otter CYP1A1 had not been previously described, we initially designed primers based on a comparison of several known CYP1A1 cDNA sequences. For this purpose, cDNA was synthesized from RNA fractions purified from liver of heavily oiled sea otters. The CYP1A1 sequence was amplified with PCR forward and reverse primer sets that were optimized for Mg concentration, annealing temperature, and number of cycles. Figure 1 illustrates the ethidium bromide staining of an agarose gel, containing a 310 bp PCR product obtained with sea otter liver RNA samples. The negative control used throughout these studies consisted of PCR reactions performed with RNA samples to which no reverse transcriptase was added. The

absence of detectable signal in these controls provided evidence that the PCR products obtained in the sample lanes were the direct result of cDNA amplification.

We then utilized the pT7Blue T-Vector system for the cloning of the CYP1A1 PCR product isolated from sea otter liver. For T-vector ligation, we used small sample (e.g., 1 ml) of PCR reaction mixture amplified product. The efficiency of ligation was 72-80%. The presence of the appropriate insert was determined using direct colony PCR and colonies with CYP1A1 inserts were used for plasmid DNA isolation and sequencing. The cloned PCR product was purified using Wizard® PCR Preps DNA purification system. After purification, the PCR product showed a clear, distinct band in agarose gel. Forward and reverse primers, specific to sea otter CYP1A1 (see sequences in Materials and Methods), were developed for quantitative RT-PCR.

RT-PCR quantitation of sea otter CYP1A1 mRNA

Sea otter CYP1A1-specific PCR primers sets described above and peripheral blood mononuclear cells isolated from sea otters residing in oiled and non-oiled areas of Prince William Sound were used to develop a quantitative RT-PCR for CYP1A1 mRNA.

To achieve minimum variability in template amplification, we included an internal standard in a competitive RT-PCR reaction utilizing rcRNA templates according to Vanden Heuvel et al. (24). The internal standard was designed such that the PCR product from the mRNA (180 bp) could be easily separated from the rcRNA internal standard (221 bp). Figure 2 represents a typical relation between internal standard and CYP1A1 in competitive RT-PCR. After a series of experiments using a broad range of internal standards concentrations, we then narrowed the dilution of rcRNA internal standard (1 - 5000 molecules/tube) for more precise estimation of CYP1A1 gene expression in sea otter PBMC. Figure 3 is a representative standard curve used to determine the actual molecules of CYP1A1 in a sample. The level of expression of CYP1A1 was determined in PBMC of 21 sea otters from a non-oiled area and 20 otters from an oiled area. These data are presented in Figure 4. In the PBMC from otters in the non-oiled area, the average level of CYP1A1 mRNA was 0.12×10^6 molecules/100 ng total RNA. The average level of CYP1A1 mRNA from the otters in the oiled area was 1.96×10^6 molecules/100 ng total RNA.

DISCUSSION

Detecting and evaluating biological responses that result from environmental contamination are essential steps to determining the significance and duration of the contamination and could help in identifying potential mechanisms or toxicities involved. Biological responses resulting from exposure to contaminants can also be used as biomonitoring tools. The biological response to chemical contaminants are evaluated from the biochemical reaction of an individual animal to complex population and ecosystem interactions (6). Alterations in biochemical systems (e.g., gene expression) are typically more sensitive indicators than those at higher levels of biological organization (e.g., population densities). In this study, we evaluated the biological response of individual animals in an attempt to determine their level of exposure to residual oil.

The CYP1A1 is one of the most studied isozymes in the cytochrome P450 superfamily in regard to its role in metabolizing environmental toxicants and its use as a biomarker of exposure to a variety of environmental toxicants. Nothing is known about the CYP1 gene family in sea otters. This is the first report that the CYP1A gene is present and its respective mRNA is expressed in sea otter liver and PBMC. The use of RT-PCR has several advantages over the more conventional RNA detection procedures. First, this procedure is at least an order of magnitude more sensitive than radioimmunoassay and at least two orders of magnitude more sensitive than Northern or slot blotting for measuring induction of CYP1A1 (33). Second, the evaluation of a circulating population of cells, that can easily be obtained by venapuncture, represents a non-lethal, minimally invasive sampling technique for evaluation.

The CYP1A1 is a key participant in the metabolism of a number of xenobiotics. The reactive metabolites, if not conjugated or detoxified, can cause cell damage including immunotoxicity. The metabolites of CYP1A1 are poor substrates for conjugation and detoxifying enzymes (34). The toxicological significance of the CYP1A1 detected in lymphocytes in this study depends on whether the levels detected are sufficient for xenobiotic activation in immune tissues. The reactive metabolites so formed can induce damage within lymphocytes containing the CYP1A1. Under conditions such as an oil spill, in which unmetabolized toxins may escape the liver, CYP1A1 expression in lymphocytes could be critical to xenobiotic induction of immunosuppression. However, the susceptibility of these immune tissues to toxicant damage is likely to depend on both their activation and detoxification capabilities (35).

Analysis of the expression of P450s in circulating lymphocytes as a marker for exposure to environmental toxicants represents new approach to assess environmental stress. The molecular mechanism of the constitutive expression of CYP1A1 has to be elucidated for a better understanding of the metabolic characteristics of the sea otter. Further cDNA cloning and expression studies of sea otter P450s are required for the clarification of molecular biological properties in this species.

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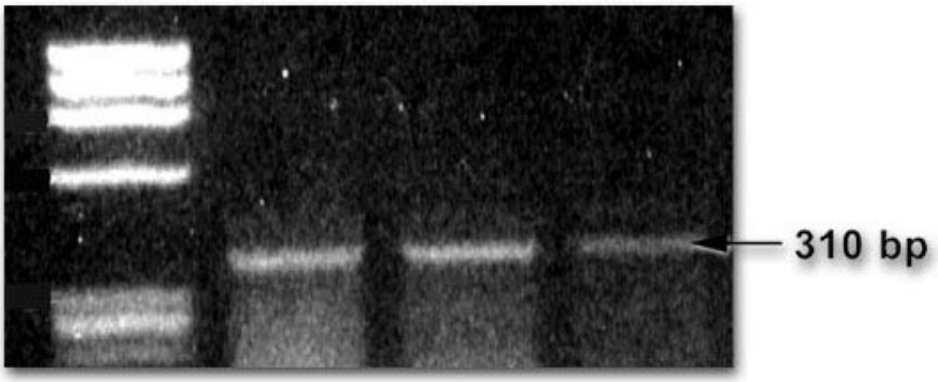

Figure 1. Sea otter (*Enhydra lutris*) CYP1A1 PCR product. Ethidium bromide-stained agarose gel containing PCR products resulting from amplification of sea otter liver CYP1A1 cDNA. Lane 1- molecular weight markers.

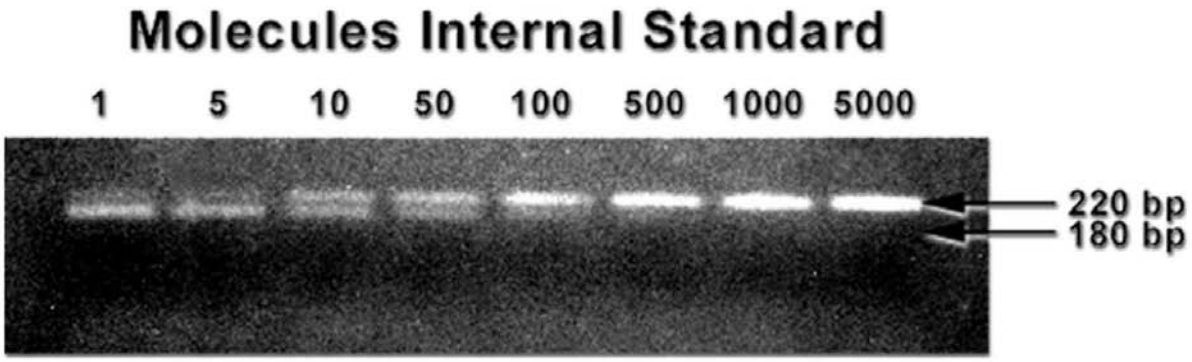

Figure 2. Ethidium bromide stained agarose gel showing quantitation of CYP1A1 mRNA expression in sea otter (*Enhydra lutris*) peripheral blood mononuclear cells. Dilution of rcRNA internal standard (1-5000 molecules/tube) were added to a constant quantity of peripheral blood mononuclear cell RNA. The PCR conditions are described in Materials and Methods.

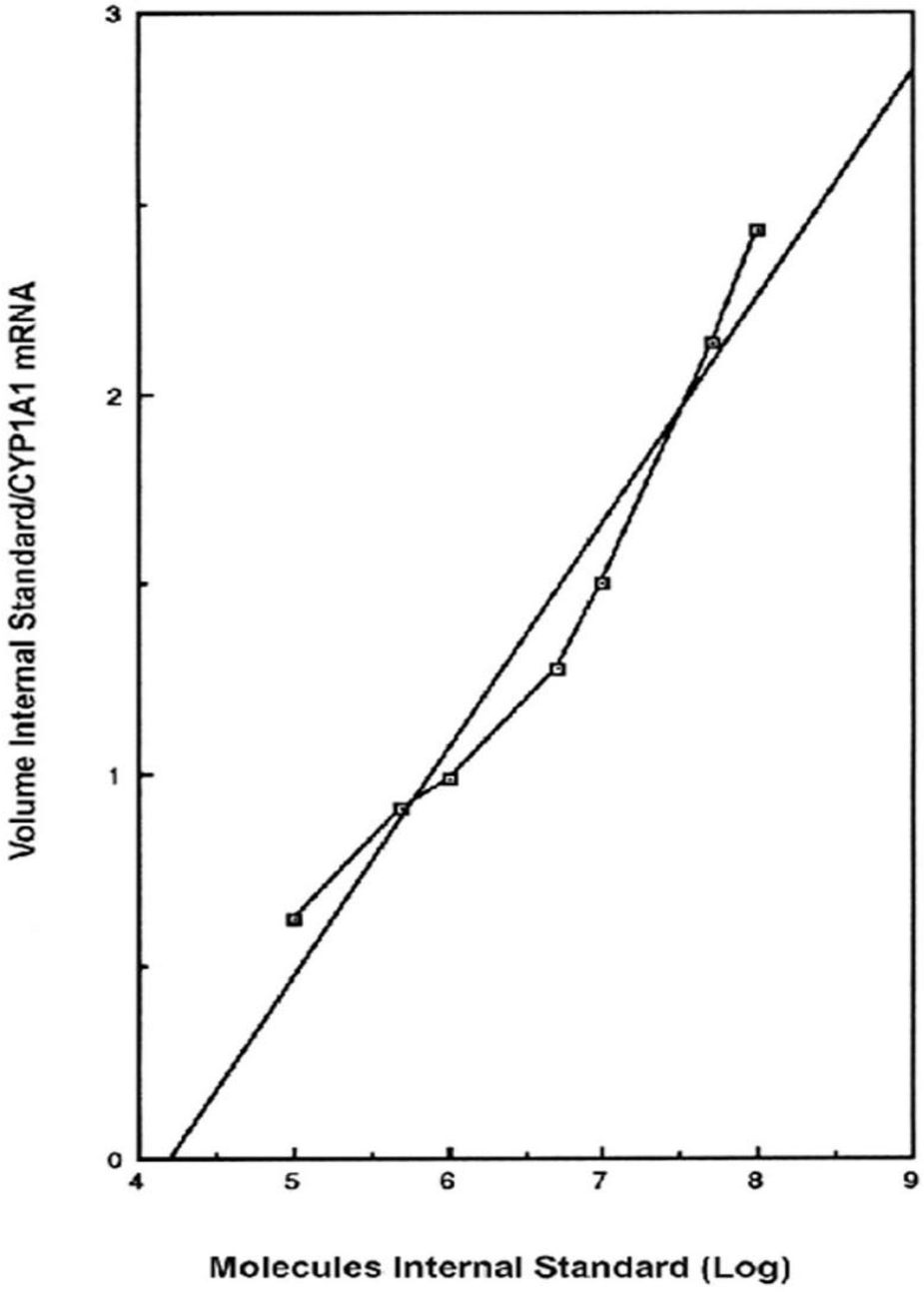

Figure 3. Quantitation of CYP1A1 mRNA expression in sea otter lymphocytes. Dilutions of rcRNA internal standard were added to a constant amount of lymphocyte RNA. The molecules of mRNA are estimated by determining where the volume of the rcRNA spot equals that of the target mRNA. The PCR conditions are described in Materials and Methods.

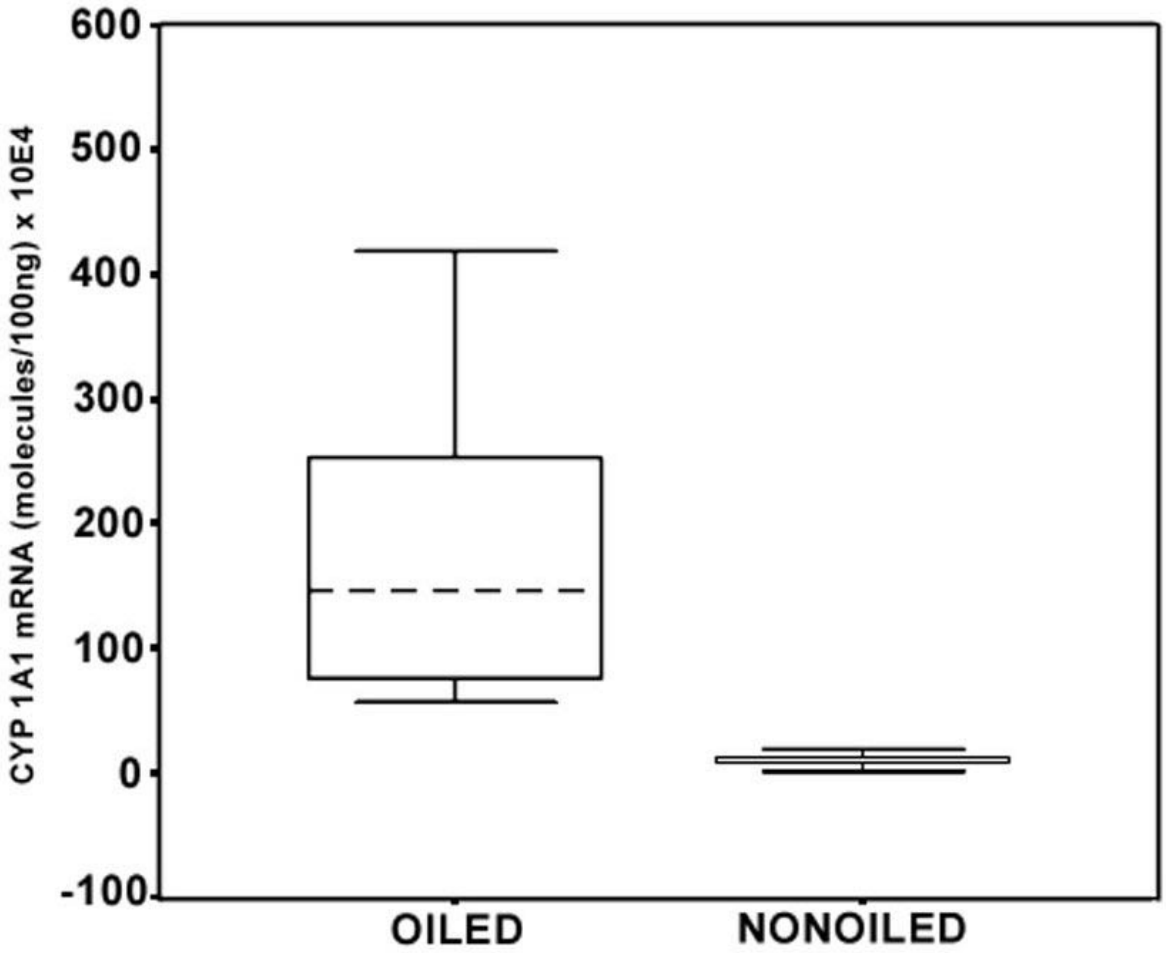

Figure 4. Box plot of CYP1A1 mRNA levels in peripheral blood mononuclear cells, isolated from sea otters (*Enhydra lutris*) in oiled and non-oiled areas, using competitive RT- PCR. The dotted line in each box represents the median, the box encompasses the 5th through 95th confidence limits and the vertical bar indicates the range of measurements.

APPENDIX BIO-03

LONG-TERM IMPACTS OF THE *EXXON VALDEZ* OIL SPILL ON SEA OTTERS, ASSESSED THROUGH AGE-DEPENDENT MORTALITY PATTERNS[1]

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ABSTRACT

We use age distributions of sea otters (*Enhydra lutris*) found dead on the beaches of western Prince William Sound, Alaska, between 1976 and 1998 in conjunction with time-varying demographic models to test for lingering effects from the 1989 *Exxon Valdez* oil spill. Our results show that sea otters in this area had decreased survival rates in the years following the spill and that the effects of the spill on annual survival increased rather than dissipated for older animals. Otters born after the 1989 spill were not as strongly affected as were those alive in March 1989, but do show continuing negative effects though 1998. Population-wide effects of the spill appear to have slowly dissipated through time, due largely to the loss of cohorts alive during the spill. Our results demonstrate that the difficult-to-detect long-term impacts of environmental disasters may still be highly significant and can be rigorously analyzed using a combination of population data, modeling techniques, and statistical analyses.

INTRODUCTION

On 24 March, 1989, the tanker vessel *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska, spilling an estimated 42 million L of Prudhoe Bay crude oil. Sea otters, a species highly susceptible to oil-related mortality (1-5), occupied the coastal waters affected by the spill. By September 1989, nearly 1,000 dead otters had been recovered in the spill area (6), and total mortality due to the spill was undoubtedly higher (7-9). While acute, short-term effects of the *Exxon Valdez* oil spill (EVOS) on sea otters are indisputable, longer-term effects on this or other species are much more difficult to document. Here, we use a combination of field data, demographic modeling, and maximum likelihood analysis to show that the sea otters of western Prince William Sound (WPWS) have incurred continuing, highly significant effects from the EVOS. Our goal is both to evaluate impacts on this particular population and to illustrate a method that can be adapted to improve assessment of many environmental impacts on populations of long-lived species.

Several lines of evidence suggest that sea otters might have faced oil-related effects long after the spill. Acute pathologies associated with oil exposure in sea otters included lung, liver and kidney damage (10, 11). Sea otters placed in aquaria after the spill had relatively poor survival rates, and at necropsy showed similar patterns of organ damage (T. Williams, pers. com.). These pathologies also resulted in abnormal hematological and serum chemistry values (12). Elevated serum enzymes associated with liver damage were documented in wild sea otters from 1989 to 1992, and again, although to a much lesser extent, in 1996-1998 (unpub. data). From 1996 to 1998 wild otters in oiled areas also had significantly higher induction of cytochrome P4501A (CYP1A), a bioindicator of exposure to aromatic hydrocarbons, than did otters from unoiled areas (13). Thus, individuals surviving initial exposure to oil but remaining in the wild are likely to have experienced initially sublethal pathologies similar to those seen in animals dying immediate after the spill.

Continued exposure to oil persisting in the environment, rather than lingering effects of acute exposure, may also account for some persistent spill effects. Following the spill, an estimated 40% of the oil (16 million L) beached in WPWS (13); by 1992 an estimated 2% of the original oil remained on beaches (14), and oil was still present in sediments on some beaches in 1997 (15). Although most remaining oil residues were deemed non-toxic by the summer of 1991 (16, 17) where oil is protected from weathering toxic components persist and may be mobilized following high energy storms (15, 18). Thus, oiled shorelines provided a reservoir for continued contamination of adjacent intertidal areas and nearshore waters.

While these facts suggest the possibility of lingering spill effects, evaluating this possibility have proven difficult and costly. At the individual level, “clinically ill” individuals are not likely to survive to be sampled, fresh carcasses for post-mortem examination are rarely found, and small sample sizes and high variability in data from live captures results in low statistical power. At the population level, pre-spill survey data from WPWS are available for comparison with post-spill numbers. However, these data are not ideally suited to a straightforward analysis of spill effects, and have proved inconclusive (7-9, 19, 20). Age-at-death data and estimated demographic rates are also available, but again variable sampling efforts and small sample sizes limit the power of simple statistical evaluations.

To overcome these problems, we use time-varying population models in combination with maximum likelihood methods to evaluate alternative hypotheses about changing

demographic rates for otters following the EVOS. We use a simple demographic model with time-varying, age-specific survival rates to predict the observed age distributions of dead otters seen each year following the spill. By modifying survival rates in the model away from pre-spill values and evaluating the fit of different modifications, we can identify the most likely ways in which the spill may have influenced the demography of the population (21).

METHODS

Study Area and Data Collection

Our primary data are the ages of sea otters found dead in WPWS both prior to and following the 1989 EVOS. From 1976 to 1985, the U.S. Fish and Wildlife Service collected sea otter carcasses each spring from Green Island, with an additional collection in 1979 from north-west Montague Island. From April through September 1989, spill response crews collected carcasses from beaches throughout oiled portions of WPWS. In addition, an unknown number of carcasses were recovered offshore within the oil-slick during early spill response efforts (9). Spring beach surveys at Green Island were resumed in 1990 and continued through 1998. In addition, in 1990 and 1991 crews monitoring spill cleanup efforts collected carcasses opportunistically on Naked, Eleanor, Ingot, Knight, Evans, Latouche, Elrington, and Perry islands and numerous smaller islands in the spill area. Monitoring of beaches in 1992 to 1995 was greatly reduced, and few carcasses were recovered other than during spring surveys at Green Island. In 1996 and 1997 opportunistic collections in the northern Knight Island area increased with implementation of a new research project in this area. Spring surveys of beaches in the larger area of oiled WPWS were conducted in 1998 by the U.S. Geological Survey, concurrent with and using similar methods as used at Green Island.

Systematic beach surveys were conducted in April or May soon after snow melt, prior to the regrowth of beach grasses which can conceal carcass remains. Beaches were walked by one or two observers, searching the strand line (the area of debris deposition from the previous winter's storms) and the upper intertidal zone. Observers recorded location, sex (if identifiable), and an age estimate (juvenile or adult) based on tooth wear and closure of skull sutures. Because many carcasses cannot be reliably sexed, we lump all data regardless of sex. The skull was collected when present, and a tooth (preferentially a pre-molar) removed for age analysis. For age estimation, several longitudinal sections of the tooth were decalcified for cementum annuli readings (22). Matson’s Laboratory (Box 308, Milltown, MT 59851) sectioned and aged all teeth.

Sea otters collected in 1989 were judged to be either pre- or post-spill deaths, based on the carcass condition at the time of recovery relative to time since the spill (23). From 1990-1992, we used only carcasses deposited during the previous winter or that spring (i.e., carcass remains had soft tissue and were located on top of previous years layer of vegetation or in intertidal zone) to avoid including pre-spill and spill-year mortalities. After 1992 all recovered carcasses were included.

Data Analysis and Modeling

We first compared the age distributions of otters collected over different time intervals and in different areas. We used two pre-spill time periods (1976-1985 and 1989-pre-spill) and three post-spill periods (1989-post-spill, 1990-1991, and 1992-1998), and two areas: Green Island (the site of systematic pre- and post-spill collections) and the rest of WPWS. To compare age distributions, we used Kolmogorov-Smirnov (K-S) two-sample tests (24). We excluded 0-yr-olds from all analyses since carcasses of the youngest animals are relatively unlikely to persist on beaches (25).

Next we constructed demographic models with survival rates varying from pre-spill estimates ("baseline rates") across both ages and years. We did not alter fecundities, as independent evidence indicates no change in otter reproductive values following the spill (26, 27). Each model was run for nine years, corresponding to the 1990-1998 post-spill years. For each simulation, we compared the predicted age distributions of otters dying in each year with those actually seen in the field, and used maximum likelihood methods to determine the most likely patterns of change. This technique provides a clear way to infer changes in demography from age-at-death data by obviating the need to make assumptions such as constant vital rates or stable age distributions (28,29).

We used a deterministic, two-sex, age-structured matrix model to simulate populations and ran the model with a large number of parameter estimates and model forms to test the robustness of our results. We initialized this model using one of three sets of baseline age- and sex-specific survival estimates from smoothed maximum likelihood analyses of ages-at-death for carcasses collected before and/or immediately after the spill (following methods in 29,30) and one set of fecundity estimates from 1989 carcass data (30,31). We started each simulation with one of two assumptions regarding the age and sex distribution of animals immediately following the spill: either the stable age distribution corresponding to the baseline demographic rates, or the distribution indicated by the presumably age and sex independent mortality patterns generated by the acute effects of the spill (29,30).

To simulate changing survival rates, we created three families of models with differing functions to modify survival rates across ages and years. These three functions are all similar, but span a range of possible forms of variable spill effects across years and ages. We first created models in which the survival rate for each age *i* and sex (male or female) in each year *j* was estimated as the baseline rate for that sex and age multiplied by a Logit function: Modeled $\text{survival}_{i,j}$ = (baseline survival_i) ($\text{Logit}_{i,j}$) where $\text{Logit}_{i,j} = (\exp(fn_{i,j})/(1+\exp(fn_{i,j}))$ and $fn_{i,j}$ = a + b*(i years since spill) + d*(age j) + e*(i years since spill)*(age j).We did not include sex as a factor in altering survivals, assuming that the survival rates of all individuals of a given age were similarly effected.

The Logit function allows quite complicated age and time-specific alterations in survival rates away from base-line estimates. However, it does not allow for survival rates *higher* than those estimated from before the oil spill, as might be predicted due to a release from density-dependent constraints (32). Therefore, we also used two other functions in our models. The first is a Modified Logit function, with each age, sex, and year-specific demographic rate equal to:

$$\text{Modeled survival}_{i,j} = (\text{Logit}_{i,j})^{(\ln(\text{baseline rate})/\ln(½))} \qquad [1]$$

Where $Logit_{i,j}$ is defined as above. This relationship allows each modeled survival rate to vary between {0,1}, both higher and lower than the baseline rate, with the modeled rate equaling the baseline when $Logit_{i,j} = 0.5$. We also used a Linear function to modify survival rates, using the $fn_{i,j}$ function described above:

$$\text{Modeled survival} = \begin{cases} (\text{baseline rate})(fn_{i,j}) & \text{if } 0 \leq fn_{i,j} \leq 1 \\ 0 & \text{if } 0 > fn_{i,j} \\ 1 & \text{if } fn_{i,j} > 1 \end{cases} \quad [2]$$

For each set of model parameter, functions, and initial conditions we found the best fit values and the confidence limits for the four parameters in fn_{ij} using each of six age-at-death data sets: otters collected prior to the spill or otters dying after 1989 and from either: Green Island (the site of the most consistent carcass collections), the rest of WPWS, or all areas. The models predict the relative number of otters dying that were of each age for each of either the sixteen pre-spill years or the nine post-spill years. For each year, this distribution was used as an expectation, and the likelihood of the observed age-distribution of carcasses, given this expectation, was calculated using multinomial probabilities (21, 29). The negative log-likelihoods from each year were then summed to yield a final estimate for each model run (a particular functional form and set of parameter values; 33). Model runs that predicted zero probability of seeing a dead otter of a given age in a year when one was in fact found were rejected outright. In calculating likelihoods, we only considered data on age 1 and older otters. Negative log-likelihoods provide the means to compare models with different parameters and functional forms (using Akaike's Information Criterion, AIC: 32) and to identify the best-fit parameter values and confidence limits on these parameters (using likelihood profiles: 33). For all comparisons, we used relative –log-likelihood values (–log-likelihoods minus constant terms); because our models did not differ in number of free parameters, differences between negative log-likelihood values are equivalent to differences in AICs (with smaller AIC values reflecting greater support for a model). To find best-fit values and confidence limits, we used downhill simplex and parabolic interpolation methods (34).

After identifying the best model forms and most likely parameter values, it is also important to ask if these models generate accurate predictions of the observed carcass age distributions. To determine the goodness of fit between the predicted and observed age distributions, we conducted one-sample K-S tests for each year of age-at-death data from 1990 to 1998 for both the linear and logistic models.

RESULTS

Age Distributions

Because Green Island shores were mostly unoiled or lightly oiled with only localized heavy oiling, we first asked if there is evidence that the demography of otters from Green Island differs from that of the rest WPWS and thus must be considered separately. For none of the time periods did age distributions differ between the two areas (K-S, $p > 0.05$ for all time periods). While we still perform some analyses for the Green Island and WPWS areas separately, these

results give no reason to suspect differences in the two areas in otter demography prior to or following the EVOS.

Next, we asked if age-at-death distributions differed across the five time periods, combining data from both Green Island and WPWS collections. Patterns in these data suggest substantial differences in demography prior to and after the spill. While the 1976-1985 and 1989 pre-spill distributions did not differ from one another (K-S, $p > 0.05$), both were significantly different from the age distributions of direct spill mortalities (post-spill 1989 carcasses) and also from the 1990-1991 distributions (Fig. 1). The 1992-1998 age distribution did not differ significantly from the pre-spill or 1990-1991 distributions, but it was different from the distribution of direct spill deaths. In general, these changes in age distributions suggest a shift in mortality patterns following the spill, with a gradual return towards the pre-spill pattern.

Modeling of Survival Changes

We first checked the reasonableness of our approach by fitting models to pre-spill carcass data. For the best fit models of all three functional forms, the confidence values for the two parameters controlling time effects on survival (b and e) bracketed zero, indicating a lack of temporal changes in survival rates in the pre-spill years (Table 1). Since no shifts in pre-spill demography are likely, this result confirms that our approach is unlikely to give spurious predictions of change. The 95 % confidence limits of the other two parameters (a and d) either encompass zero, only very small values, or are very broad, also supporting the lack of strong differences between the basic age-specific demographic rates and assumptions used in our analyses and those operating prior to the 1989 spill.

Next, we performed a total of 54 model fits, using different combinations of model assumptions and post-spill age distributions. In general the lowest negative log-likelihood (and hence AIC) values resulted from models using an initial stable age distribution, our first set of baseline demographic rates and the logistic or linear functional form. However, the striking result of all these analyses is the consistency of the effects across data-sets and model assumptions. The best-fit parameter values for each functional form predict a consistent, though complex, pattern of demographic change in the nine years following the EVOS (Table 1), regardless of carcass data (Green Island versus the rest of WPWS), initial age distribution, baseline demographic estimates, or functional form. Thus, we report detailed results only from the best-fit model in each family, estimating parameters using the combined WPWS and Green Island data-sets. While the best-fit linear and logistic models are almost equally supported by the data, the modified logistic is substantially less likely (Table 1). Using Akaike weights (35) for the best fit models of each form, the relative likelihoods are 0.40, 0.06 and 0.54 for the logistic, modified logistic and linear model forms, respectively.

The easiest way to convey the influence of the oil spill on predicted otter survivorships is as a proportion of the pre-spill survival rates for a given age in each year following the spill: values greater than one indicate higher survival following the spill, and values lower than one the converse (Fig. 2). Immediately after the spill, young animals are predicted to have suffered the greatest decrease in survivorship, but these effects dissipated rapidly with time (Fig. 2). In contrast, survival of older adults (≥10 yrs old) is initially only slightly reduced, but this effect increased with time, with poorer and poorer performance each year after the spill for a given age group. The best fit models predict that survival of prime reproductive age otters (e.g., age 5) was

reduced by as much as 50% initially and then slowly increased to values near or above pre-spill levels by 1998 (Fig. 2). The predicted effects on the oldest animals (≥15 yrs old) are likely to be somewhat inaccurate due to the small number of older carcasses found to help fit this part of the distribution. To better infer the genesis of these patterns, it is instructive to consider how an otter of a given age at the time of the spill was influenced each year as it aged (Fig. 3). These results suggest that young animals at the time of the spill (e.g. ages 0 and 1) experienced substantially higher mortality rates in the first several years following the spill, but that annual survival improved (relative to pre-spill rates) as they aged. In contrast, animals in their prime reproductive years and older (e.g. ≥5 yrs old) in 1989 have suffered strongly increasing mortality effects as time has passed. Only as these cohorts are lost from the population have demographic rates returned to normal.

While these predicted patterns of change are robust to the range of analyses explored so far, we also ran three additional analyses to gauge their strength and accuracy. First, we added environmental variability in first year survivorship, the demographic rate most likely to show substantial random variability (34; estimated from tagged otters in WPWS in 1990-1991: 36,37), and fit these stochastic simulations to post-spill carcass data (21). The best fit parameter values of these stochastic models are essentially identical to the deterministic results and showed similar confidence limits. Second, to ask if spill effects on otters born after 1989 were likely, we ran models that only modified survivorships of animals that lived through the spill. These altered models resulted in substantially worse fits for all three model functions (increases in AIC = 9.81, 15.32, 10.67 for the best fit logistic, modified logistic, and linear models respectively), directly supporting the conclusion that otters born after 1989 also have experienced spill effects. Third, we modified the basic function controlling alterations of survivorships to include quadratic terms and interactions and fit a suite of these more complicated nested models. Likelihood ratio tests suggested no justification for these more complicated models, and none yielded predictions qualitatively different from those of our simpler models. All these results confirm the robustness of our basic results.

Finally we asked if the predictions of our models accurately reflect our observed age distributions. For the Linear model (the single best model) we find no significant departure in observed carcass age distributions from those predicted until the last two years (K-S one sample tests): in these years, a surplus of older otters result in a significant deviation from the age distributions predicted by either model. For the Logistic model, three years, including the last year, show significantly different distributions; again, a surplus of older otters explained this mismatch in 1998. Overall, these results suggest that the best-fit models do a good job of accurately predicting otter age-at-death distributions, but that the model predications are worst at the end of the data collection period; as we discuss below census data of live otters suggest an explanation for this pattern.

DISCUSSION

Our results lend strong support to the hypothesis that the EVOS has had continuing impacts on the sea otter population of Prince William Sound. In particular, we found no evidence of improved performance for any age-class immediately after the 1989 spill due to a release from density-driven competition (a reasonable scenario if no lingering effects persisted). Rather otters of all ages have shown elevated mortality rates in the nine years following the spill.

These long-term effects are strongest on otters that were four to five years or older during 1989, but the modeling results also suggest that at least through 1996, animals born after the spill were also impacted by the events of 1989. Thus, while lingering effects of acute oil exposure may account for much of the longer-term spill effects, less direct impacts are also likely to have occurred, either due to maternal influences or to continued exposure to oil residues.

While the immediate loss of otters in the aftermath of the spill resulted in a decline in the local population, our results suggest that even more important long-term demographic changes have limited recovery after 1989. In our analyses, we use one population-level effect (age distributions of dead otters) as a tool to infer individual demography. However, the resulting demographic inferences can then be used to predict changes in another population attribute, total numbers. The two best-fit models suggest continuing decline of otters through 1998, while the modified logistic predicts no growth until the mid-nineties, when populations are predicted to have slowly risen (Figure 4).

Direct post-spill boat surveys indicated continued declines in sea otter numbers the first year following the spill, and no subsequent increase in population size in the spill area through at least 1991 (38). In addition, low weanling survival rates were observed in WPWS after the spill (6). Although these findings are consistent with predictions of our models, early boat surveys were not sensitive to small changes in abundance and the lack of pre-spill recruitment data limit inferences from post-spill recruitment patterns. We began more accurate aerial surveys in 1993, and found significant growth in the WPWS sea otter population, particularly since 1995 (27). At first glance, then, from the mid- to late- 1990s, censuses of the live population appear contradictory to the predictions of our two best models (although they match predictions of the Modified Logistic extremely well). However, the models rely on carcass data collected only in oil-effected portions of WPWS, including some of the habitat in WPWS currently supporting the lowest otter densities. In contrast, aerial surveys include large areas of relatively high density, unoiled sea otter habitat. In fact, much of the observed population growth over the past five years has occurred in these unoiled areas, where sea otter densities can be as much as 10 times greater than in the most heavily oiled areas. These differences, combined with our demographic results, which show poor demographic performance but more carcasses of older animals than expected, suggest that oil affected areas may continue to represent a population "sink" that benefits from immigration from healthy segments of the greater WPWS sea otter population.

Several other lines of evidence are consistent with the conclusion that mortality patterns have shown significant long-term effects of the spill and that otter movements account for much of the apparent recovery of oiled areas. Sea otter numbers in the most heavily oiled areas of northern Knight Island have shown no sign of recovery through 1999 (32,37). Lower tagged otter retention rates in this area, compared with those in a unoiled area of Montague Island, suggest sea otters at Knight Island are experienced higher mortality and/or emigration rates even though food resources and body condition of animals there should support some population growth (27,39, but see also 40). Sea otters living in the oiled area have consistently expressed higher levels of CYP1A than those captured in unoiled areas, indicating continued exposure to petroleum hydrocarbons at least through 1998 (13). Similar serological and demographic patterns for harlequin ducks (41, 42), another nearshore predator of benthic invertebrates, also support continuing spill-related effects in oiled areas of WPWS. These similarities suggest that additional species may have suffered analogous consequences to the lingering demographic spill effects we find for otters. However, while our findings document continuing demographic

effects of the EVOS, we also show that these effects have gradually dissipated with time – largely due to the death of the sea otters most impacted by the spill. This suggests that a cautious optimism is warranted concerning the gradual return of the ecological communities of Prince William Sound to pre-spill conditions.

Major anthropogenic "disasters" are usually labeled as such due to their immediate and obvious impacts. However, there is increasing recognition that long-term, large-scale effects of events such as oil spills may actually pose an even greater threat to affected populations and ecosystems. Unfortunately, accurate assessment of these impacts does not yield easily to the simplistic statistical methods usually advocated for environmental impact monitoring (e.g., 43). Here, we have used a more complex mixture of modeling, statistics, and population data to quantify and understand the effects of the EVOS, one of the best studied but also most controversial of recent marine oil disasters. Recognition that such events can have strong, long-term impacts on populations of sea otters and other near-shore species should urge greater caution in short-term assessment of environmental impacts and suggest that greater efforts are needed to understand the community-wide effects of spill events.

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Table 1. Best-fit parameter-values for different models of changing otter demography fit to age distributions of sea otters found dead before or following the *Exxon Valdez* spill. Relative negative log-likelihood values, maximum likelihood parameter estimates and one-dimensional 95 % confidence limits are given for the best model (lowest negative log likelihood) for each model family. All six best-fit models assumed an initial stable age distribution. See text for definitions of parameter effects.

Model Family	Relative –Log-Likelihood	Parameter			
		a (constant)	b (year effect)	d (age effect)	e (interaction)
Fit To Pre-Spill					
Carcasses:					
Logistic	374.55	-47.7348 (-86.4115, 4.1017)	-0.1501 (-2.7285, 0.3162)	0.02285 (0.00545, 17.5616)	0.00097 (-0.00090, 5.7620)
Modified Logistic	371.52	2.38747 (0.82337, 3.58883)	0.10258 (-0.01390, 0.24090)	-0.31686 (-0.44277, -0.17741)	0.00874 (-0.00367, 0.01995)
Linear	369.88	0.00509 (0.00509, 0.38982)	-0.00034 (-0.00034, 0.0000)	0.00018 (0.00018, 0.01785)	-0.00001 (-0.00001, 0)
Fit to Post-Spill					
Carcasses:					
Logistic	503.72	-0.8379 (-2.1982, 0.7026)	0.5133 (0.2035, 0.8375)	0.1798 (0.0812, 0.3179)	-0.0576 (-0.0922, -0.0269)
Modified Logistic	507.55	-1.1747 (-2.2327, 0.2638)	0.5225 (0.3141, 0.7037)	0.06915 (-0.0570, 0.1842)	-0.0706 (-0.0980, -0.0436)
Logistic Logistic					
Linear	503.12	0.2536 (-0.0033, 0.3332)	0.1062 (0.0612, 0.1150)	0.03455 (0.0179, 0.0495)	-0.0107 (-0.0135, -0.0064)

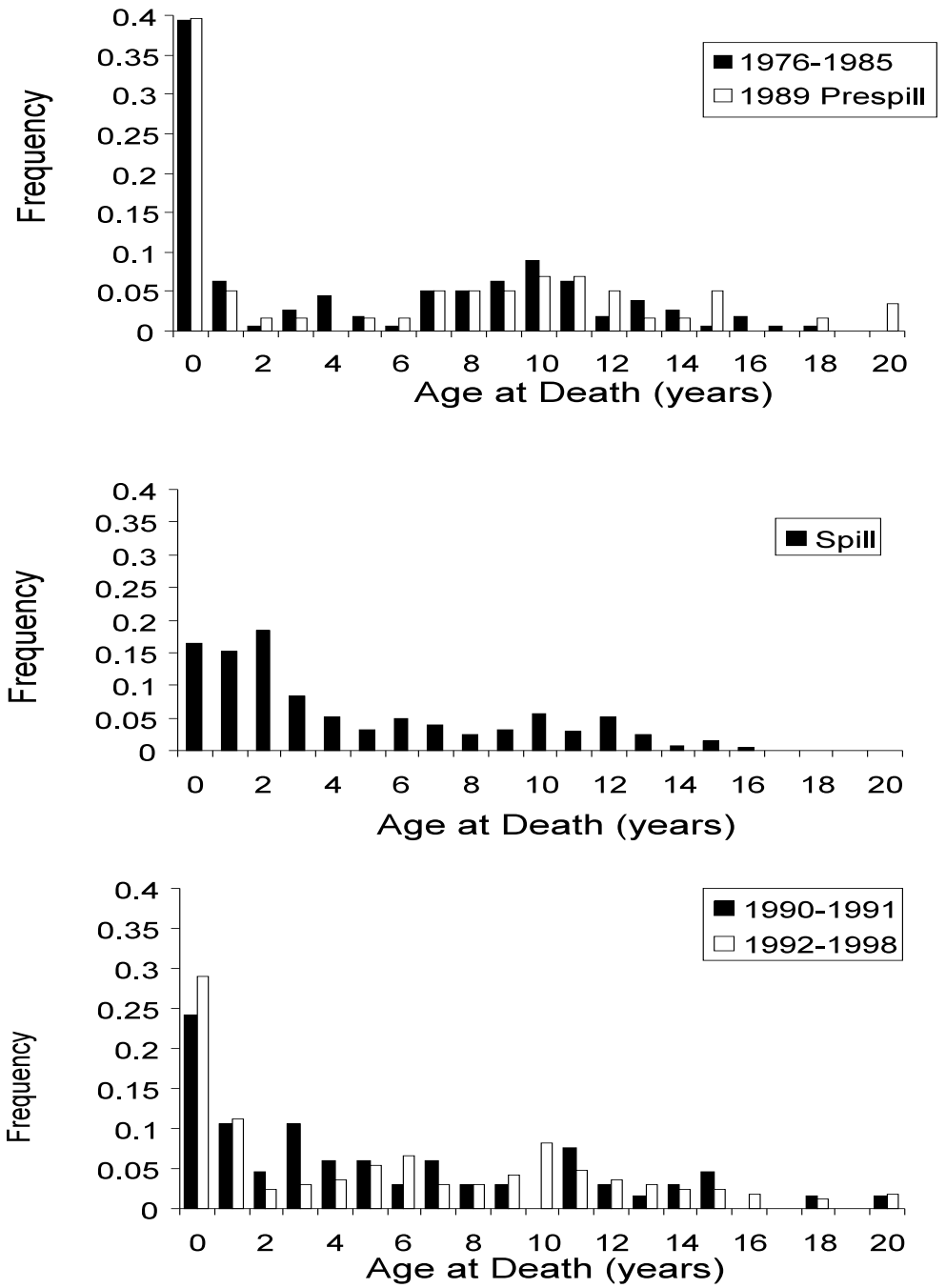

Figure 1. Age distributions of sea otters found dead in western Prince William Sound during five time-periods.

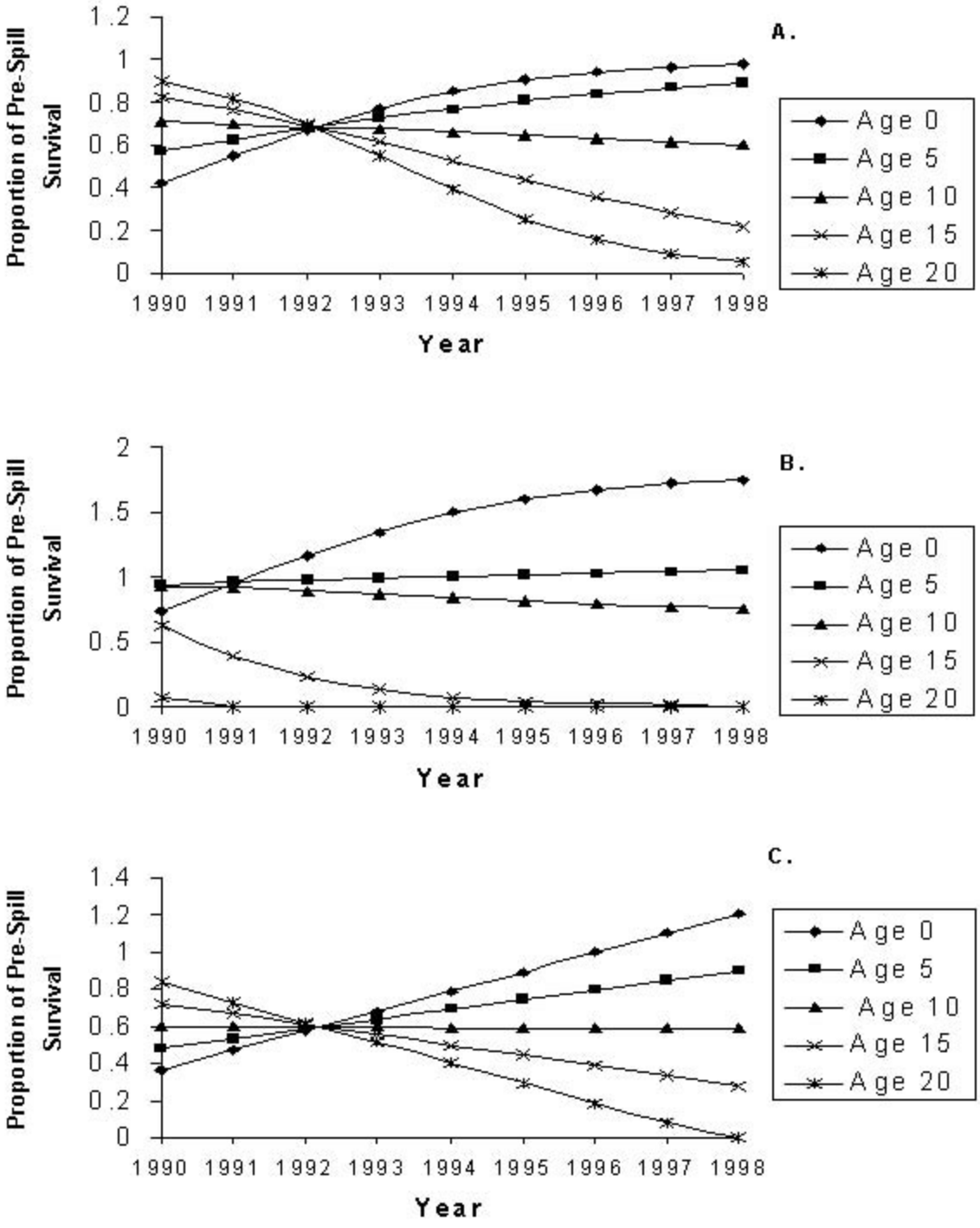

Figure 2. Estimated post-spill effects on age-specific survival rates. The best fit estimates of survival rates are shown as proportions of pre-spill baseline) rates for five representative ages. A. Best-fit results for logistic model; B. Best-fit results for modified logistic model; C. Best-fit results for linear model.

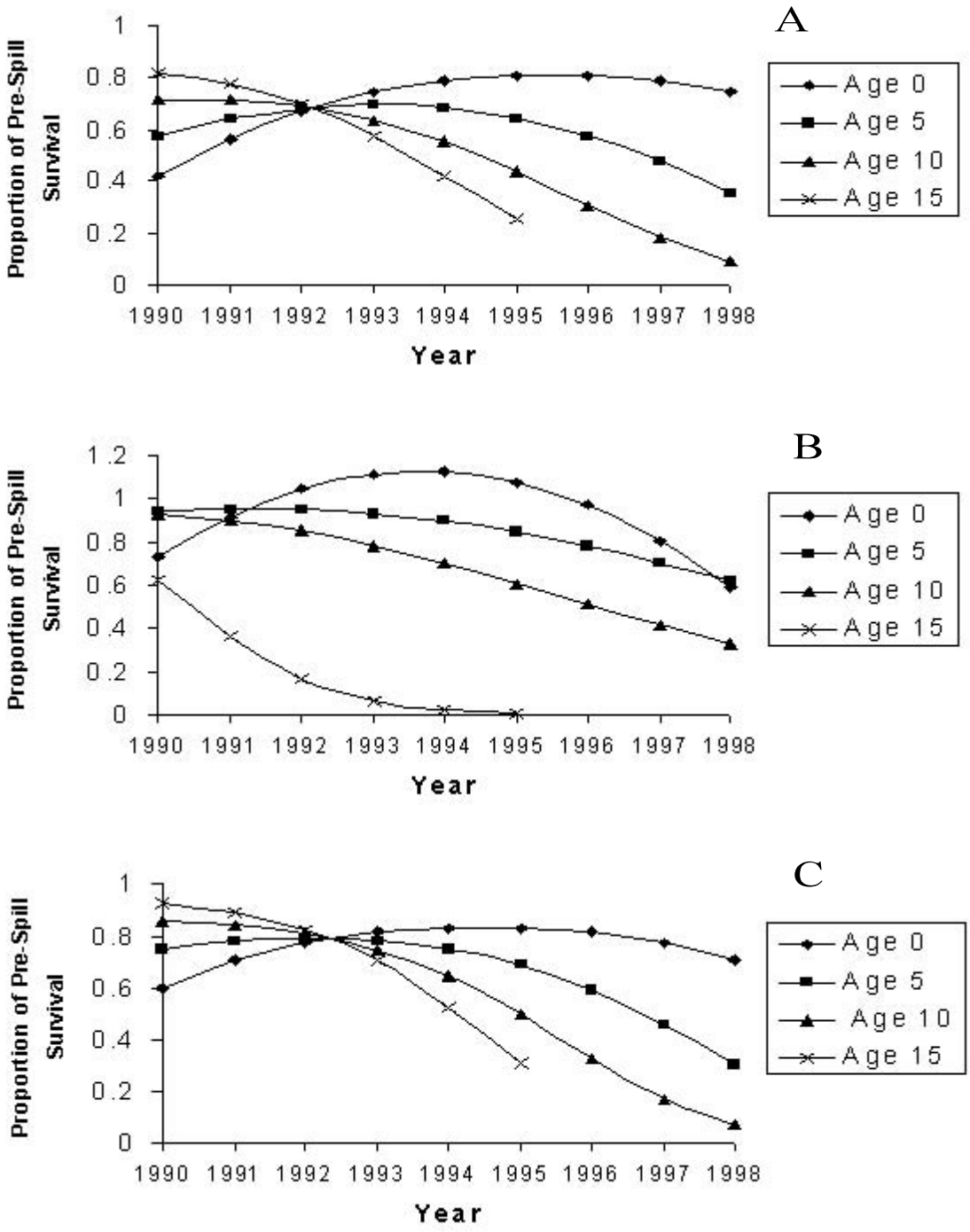

Figure 3. Changing post spill effects for cohorts of otters. Each line represents annual survivals experienced each year for an aging group of otters that were either 0,5,10 or 15 years old at the time of the 1989 spill, expressed as a proportion of pre-spill survival rates. A. Best-fit results for logistic model; B. Best-fit results for modified logistic model; C. Best-fit results for linear model.

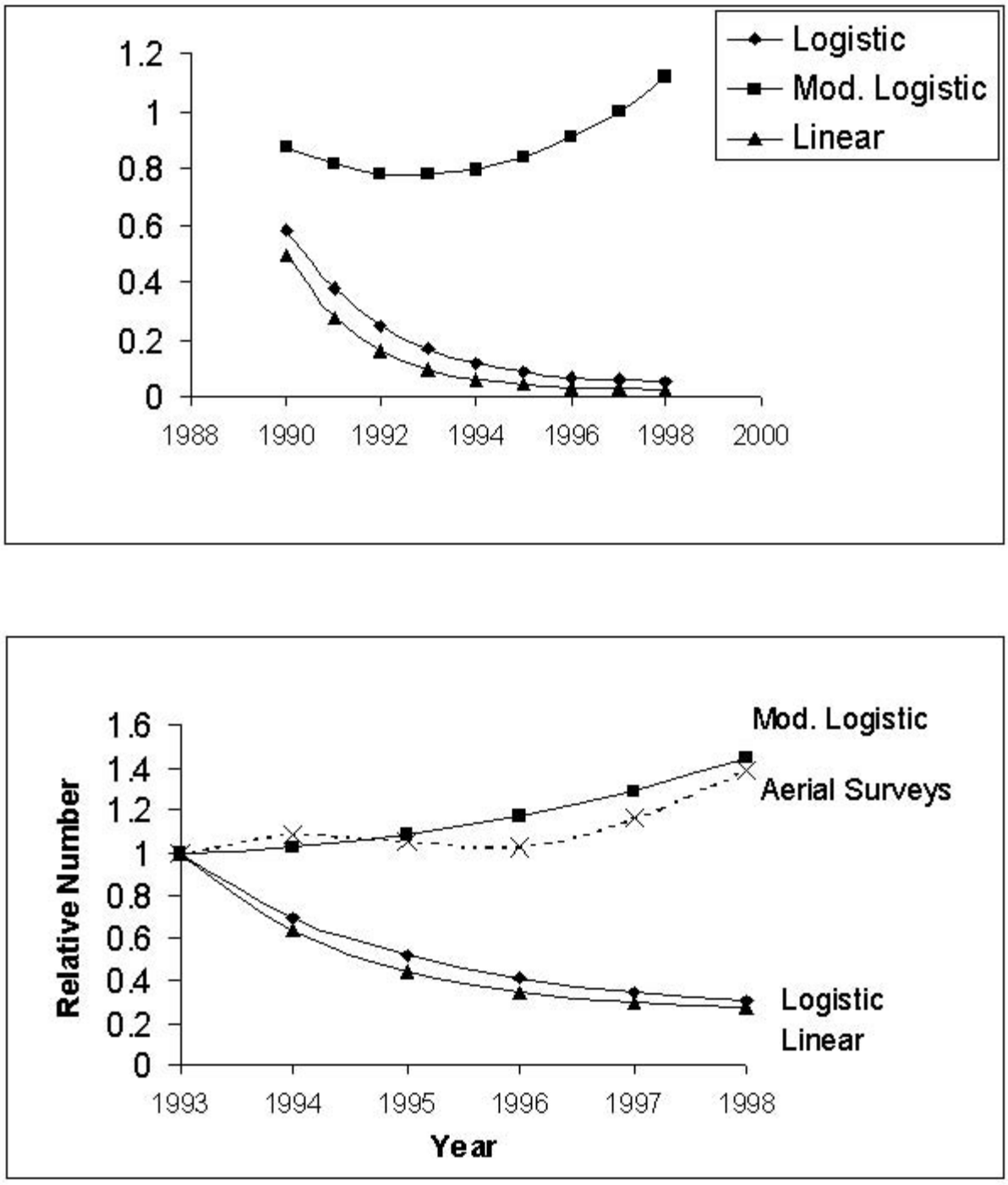

Figure 4. Predicted proportional changes in population size predicted from the three best-fit demographic models (Table 1) and from aerial surveys of WPWS (27).

Sea Otter (*Enhydra lutris*) Appendices

(SO)

APPENDIX SO-01

An aerial survey method to estimate sea otter abundance[1]

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ABSTRACT: Sea otters (*Enhydra lutris*) occur in shallow coastal habitats and can be highly visible on the sea surface. They generally rest in groups and their detection depends on factors that include sea conditions, viewing platform, observer technique and skill, distance, habitat and group size. While visible on the surface, they are difficult to see while diving and may dive in response to an approaching survey platform. We developed and tested an aerial survey method that uses intensive searches within portions of strip transects to adjust for availability and sightability biases. Correction factors are estimated independently for each survey and observer. In tests of our method using shore-based observers, we estimated detection probabilities of 0.52-0.72 in standard strip-transects and 0.96 in intensive searches. We used the survey method in Prince William Sound, Alaska to estimate a sea otter population size of 9,092 (SE = 1422). The new method represents an improvement over various aspects of previous methods, but additional development and testing will be required prior to its broad application.

Keywords: abundance, detection, distribution, disturbance, *Enhydra lutris,* Prince William Sound

1 INTRODUCTION

Conservation and management of sea otters (*Enhydra lutris*) often requires estimates of population abundance. While indices of abundance may be adequate for some purposes, accurate estimates are necessary in some situations (e.g., following an oil spill or managing harvests). Unbiased estimates of abundance are difficult to obtain, largely due to diving behavior which makes sea otters, as well as other marine mammals, undetectable. Estimating that proportion of animals not detected will reduce bias in population estimates.

Several characteristics of sea otters facilitate their detection, compared to most marine mammals. First, sea otters are relatively shallow divers, feeding almost exclusively on benthic prey, resulting in well defined spatial boundaries to their distribution. Foraging depths to 25 m and 40 m have been reported (Wild & Ames 1974, Reidman & Estes 1990) with maximum dive depths estimated from 54 to 100 m (Kenyon 1969, Newby 1975). Although Schneider (1976) observed sea otters as far as 40 km offshore in Bristol Bay, Alaska, he also found that > 90% of his sightings were between the shoreline and the 40 m depth contour. Along coastlines with narrow bathymetric contours, the

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seaward limit to sea otter distribution may be < 1 km from the shore, allowing shore-based surveys, for which detection probabilities have been estimated (Estes & Jameson 1988).

Dive duration, another factor in marine mammal detection, is relatively short for sea otters, resulting in frequent periods at the surface. Dive times averaged from 25-155 sec among 32 individual sea otters in California (Ralls et al. 1988) and dive times of 10 sea otters in southeast Alaska averaged 62-173 sec (JLB unpub. data). Foraging generally occurs alone (Estes & Jameson 1988) and is distinctly crepuscular, with resting peaks near midday (Kenyon 1969, Estes et al. 1986).

Sea otters often rest in groups that may be more visible than single animals. The sexes are largely segregated with most habitat occupied by adult females and fewer territorial males. Non-territorial males occupy a relatively small portion of habitat (Kenyon 1969, Riedman & Estes 1990). Female group sizes are usually between 1-12 while male aggregations may reach many hundreds of individuals (Riedman & Estes 1990). Where canopy forming kelp beds occur, these habitats tend to be preferred resting areas, possibly resulting in larger, more easily detected groups. However, due to reduced contrast between otters and kelp, detection may be lower in kelp forests.

Although feeding and resting habits can facilitate detection, environmental and observational factors may compromise detection. Their dark pelage can provide good visual contrast with a generally homogenous background, but environmental factors such as sea state, glare, wind speed, and precipitation may make sea otters difficult to see. Detection can also vary with distance, survey platform, number of observers, observer skill, disturbance and search intensity.

Sea otters have previously been counted from shore (Estes & Jameson 1988), small and large vessels (Jameson et al. 1982, Pitcher 1989, Estes 1990) and fixed (Ebert 1968) or rotary wing (Drummer et al. 1990, DeGange et al. 1995) aircraft, or a combination of two or more methods (Estes & Jameson 1983, Geibel & Miller 1984, Jameson et al. 1986). With few exceptions (Estes & Jameson 1988, and Jameson et al. 1986) survey methodologies have not been standardized by search intensity, altitude (for aircraft), number of observers, or environmental conditions, and the proportion of animals detected has not been estimated, biasing results to an unknown extent.

Shore-based surveys have provided the most accurate estimates of nearshore sea otter abundance. Estes & Jameson (1988) estimated an overall probability for sighting sea otters of 94.5% in standardized shore side counts. Theirs was the first study to rigorously evaluate the effect of activity, group size and distance from observer on sea otter detection, and provides a baseline against which other methods can be evaluated. However, because sea otters can occur too far offshore to count from shore and access along most coastlines is limited, shore counts are applicable only to a portion of sea otter habitat.

Aerial surveys are applicable over a broad range of areas. Line transect and strip transect methods are widely used in aerial surveys to estimate population densities (Eberhardt 1978, Burnham et al. 1980, Buckland et al. 1993). The assumption that all animals on the line or strip transects are seen cannot be made with diving mammals, such as the sea otter, and requires estimating detection to reduce bias.

Because of the need for unbiased estimates of sea otter abundance we developed a new aerial survey method that uses intensive searches within strip transects to adjust for availability and sightability biases (detection bias). We report initial test results to determine relations between (1) distance and detection, (2) search intensity and detection (using shore-based observers) and (3) methods of sample selection. We then provide an example of the results of a survey of Prince William Sound, Alaska, incorporating our method and discuss remaining problems.

2 METHODS

2.1 *Preliminary line-transect surveys*

In 1991 we surveyed a series of randomly located line-transects (Buckland et al. 1993) in Western Prince William Sound to test the detection distance of sea otters from the air. In this (and other surveys unless otherwise specified) we flew a Piper PA-18 aircraft at 27 m/s (60 mph) and an altitude of 91 m and the pilot did not aid in observations. The observer recorded the number of individuals and the perpendicular distance to each group of otters detected from one side of the aircraft only. Distances were recorded in 50 m categories based on calibrated wing strut marks.

2.2 *Shore-based evaluation of detection*

In 1991 we used shore-based observers to evaluate effects of altitude, search pattern and search effort on the detection of sea otters in aerial strip-transect surveys and intensive searches within strips. Shore-based observation techniques followed Estes & Jameson (1988). All trials were conducted on areas (search units) without canopy forming kelps large enough to contain a full search pattern, allowing unrestricted observation from an adjacent vantage point on shore, and containing one or more otters immediately prior to arrival of the aircraft.

Shore crews traveled to survey units by skiff, minimizing disturbance to sea otters. Shore crews defined unit boundaries, established an orientation for the aerial search pattern with flagging visible to the pilot and determined the position and activity of each otter within the unit. Shore crews recorded the location, group size, number of pups and activity (swimming, resting or diving) and initiated the trial by radio call to the pilot.

We first tested the effect of altitude (46, 91 and 137 m above sea level) on detection, in sets of three trials. Tested altitudes were randomized in each trial set. All altitude evaluation trials were conducted using a 750 m circle intensive search pattern. We flew along the circumference of a 750 m diameter circle while the aerial observer viewed the circumscribed area. The pilot used a stopwatch, airspeed and minute of turn to define the 750 m diameter circle (128 seconds to complete, 32 seconds through each quadrant). The aerial observer recorded the time, location, group size, number of pups and activity of each sea otter or group of sea otters observed. Groups were defined by a distance $\leq$ one otter length (about 1.5 m) between successive otters. Circling was continued until 5 min had passed without any additional otters being observed.

We next tested the effect of search pattern and search effort on detection using three different intensive search patterns in conjunction with a strip count. Each pattern trial began with a strip count in which the plane flew along one edge of a strip transect while the aerial observer recorded the location, group size, number of pups and activity of each sea otter or group of sea otters observed in the strip. Width of the strip was determined by the aerial observer using distance indicators marked on the wing struts and was either 400 m or 750 m, depending on the subsequent search pattern. The length of the strip was either 400, 750 or 800 m, depending on the search pattern. Immediately following the strip count, the plane began one of three search patterns over the strip that had just been counted. The aircraft was piloted along the circumference of either a 400 or 750 m diameter circle, or a 400 x 800 m oval while the aerial observer viewed the circumscribed area. Selection of the search pattern was made by the shore crew while attempting to obtain an equal number of trials for each pattern. The pilot used techniques analogous to those developed for the 750 m circle to maintain each of the other two search patterns. The aerial observer recorded the circle or oval number, location, group size, number of pups and activity of each additional sea otter

or group of sea otters observed during each pass of the intensive search area. Intensive search patterns were continued until minutes had elapsed without any additional otters being observed. Both air and shore crews recorded the location and behavior of all otters observed outside the boundaries of the unit, changes in sea otter activity over time, and the time the aircraft entered and departed the unit.

At the end of each day, shore and aerial crews compared the mapped locations of all observed otters (for all shore-based trials). For the otters present in each trial we determined the number of otters observed by both crews (b_i), and the number observed only by the shore crew (g_i), in the observation circle or strip. The number of otters in the circle or strip before any response to the approaching aircraft was determined based on shore crew observations prior to the arrival of the aircraft.

Sea otter detection probabilities for the aerial observer were estimated as:

$$\hat{P}_d = \frac{\sum_{i=1}^{r} b_i}{\sum_{i=1}^{r} (b_i + g_i)} , \qquad (1)$$

where r was the number of trials. Detection was also estimated separately for each trial and Kruskal-Wallis tests were used to evaluate differences in detection probabilities between altitudes and patterns. Fisher's exact test for contingency tables was used to evaluate the effect of altitude and pattern on the proportion of trials in which all otters were detected from the air and the proportion of trials in which otters exhibited disturbance behavior, determined by the shore crew. All statistical tests were conducted at the 0.05 significance level.

2.3 *Systematic vs. group initiated intensive search units*

Our shore-based tests suggested we could develop correction factors for adjusting aerial strip-transect surveys, by conducting intensive searches over portions of the strip transects. We refer to the portions of strips on which intensive searches are conducted as intensive search units (ISUs). Correction factors are based on comparing numbers of otters detected during standard strip-transects to numbers detected on the ISU. Precision of estimated correction factors depend on the number of ISUs in which otters are observed. ISUs could be located systematically, but only ISUs with otters could be used to estimate detection. If the probability of detecting each group of otters is independent, then detection probabilities could be estimated for ISUs initiated upon detection of a group, with the estimate only based upon any additional groups present in the ISU. Group initiated ISUs would be usable only if they contained additional groups. Because otter groups tend to occur in clusters, using detection of otters to locate ISUs could result in a higher proportion of usable ISUs. We investigated the relative merits of these 2 approaches for locating ISUs by comparing the proportion of usable ISUs and the estimated strip detection probabilities from samples of systematic and group-initiated ISUs obtained in 1993 and 1996.

Systematic ISUs were located at 2 min intervals along 400 m wide strip transects in Prince William Sound. Group-initiated ISUs were located at each detected otter group separated by more than 800 meters (30 sec) along 400 m wide strip transects, also in Prince William Sound. Observer 1 obtained samples in 1993 and 1996. Observer 2 obtained samples in 1993 only. For each ISU, observers recorded the activity and number of otters with standard strip-transect methodology and the number observed during the ISU. Detection probabilities were estimated based on all detected

otters in each systematically located ISU that contained otters. For group-initiated ISUs, detection probabilities were based on all otters except the initial group in ISUs that contained additional groups. Detection probabilities were estimated according to equation (1). Analyses for effect of observer, year, and type of ISU (systematic vs group initiated) on detection probabilities pooled 1993 and 1996 data and included 150 ISUs. Estimated strip detection was equal to 0 or 1.0 on 73% (109/150) of the ISUs. We categorized detection probabilities as either ≥ 0.5, or < 0.5 for each ISU and used logistic regression to examine differences due to observer, year, and ISU type. We treated observer by year combinations as blocks. If the block effect was significant, we used contrasts to test for observer effects within year (1993) and year effects within observer. Significance tests were based on Wald statistics (Agresti 1990) at $\alpha = 0.05$.

2.4 *1992 distribution survey*

In 1992 we implemented a pilot survey in Western Prince William Sound to determine the spatial distribution of sea otters relative to bathymetric zones. We used this information to define and allocate sampling effort among strata in future surveys. Design for the distributional survey was a series of parallel strip transects, 400 m wide and 1.2 km apart. Each transect was identified by its intersection with the shoreline and an offshore boundary based on shoreline physiography (bays and inlets < 6 km wide were included in the study area regardless of depth), and the 100 m depth contour or a distance of 2 km from the shore, whichever was greater. A GPS in the aircraft was used to locate the endpoints and navigate along each transect. The study area contained 2,404 km^2. Locations and size of each otter group were recorded on a transect map.

2.5 *1994 Prince William Sound survey*

In 1994 we implemented a full survey of Prince William Sound (Figure 1) using group-initiated ISUs to adjust for detection. The survey area was stratified into areas of expected high and low sea otter density based on results of the distributional survey. Sampling effort was allocated to strata in proportion to expected otter densities with approximately 0.18 of the high density stratum sampled and 0.03 of the low density stratum sampled. We flew a Bellanca Scout (a plane similar to the PA-18 used in earlier trials) along systematically located, parallel strip-transects, 400 m wide and 2 km apart (every 5th strip) in the high stratum and 8 km apart (every 20th strip) in the low (Figure 1). The observer searched the 400 m strip between the float and the strut marks, scanning as far forward as conditions allowed. We noted wind, seas, cloud cover, and glare for each transect.

We initiated ISUs at the first sea otter group observed within each 15 minute period of an hour (0-15, 15-30 ...) in the high density stratum and by each group sighted in the low density stratum. Successive ISUs were no closer than 800 m (30 sec) to avoid affecting otters in other ISUs. Intensive searches consisted of 5 concentric circles over the 400 m strip. Circling began at a point indicated by a perpendicular line from the transect edge to the initiating group. The pilot used a stopwatch to time the minimum 30 second spacing between consecutive ISUs and to navigate the circumference of each circle. ISU locations were drawn on the transect map and group size and activity recorded for otters in each ISU. For each group, we recorded the number of otters observed on the strip count and the number observed during the intensive search. Otters that were observed to swim into an ISU post factum were not included. Groups initiating ISUs were not used to calculate detection probabilities.

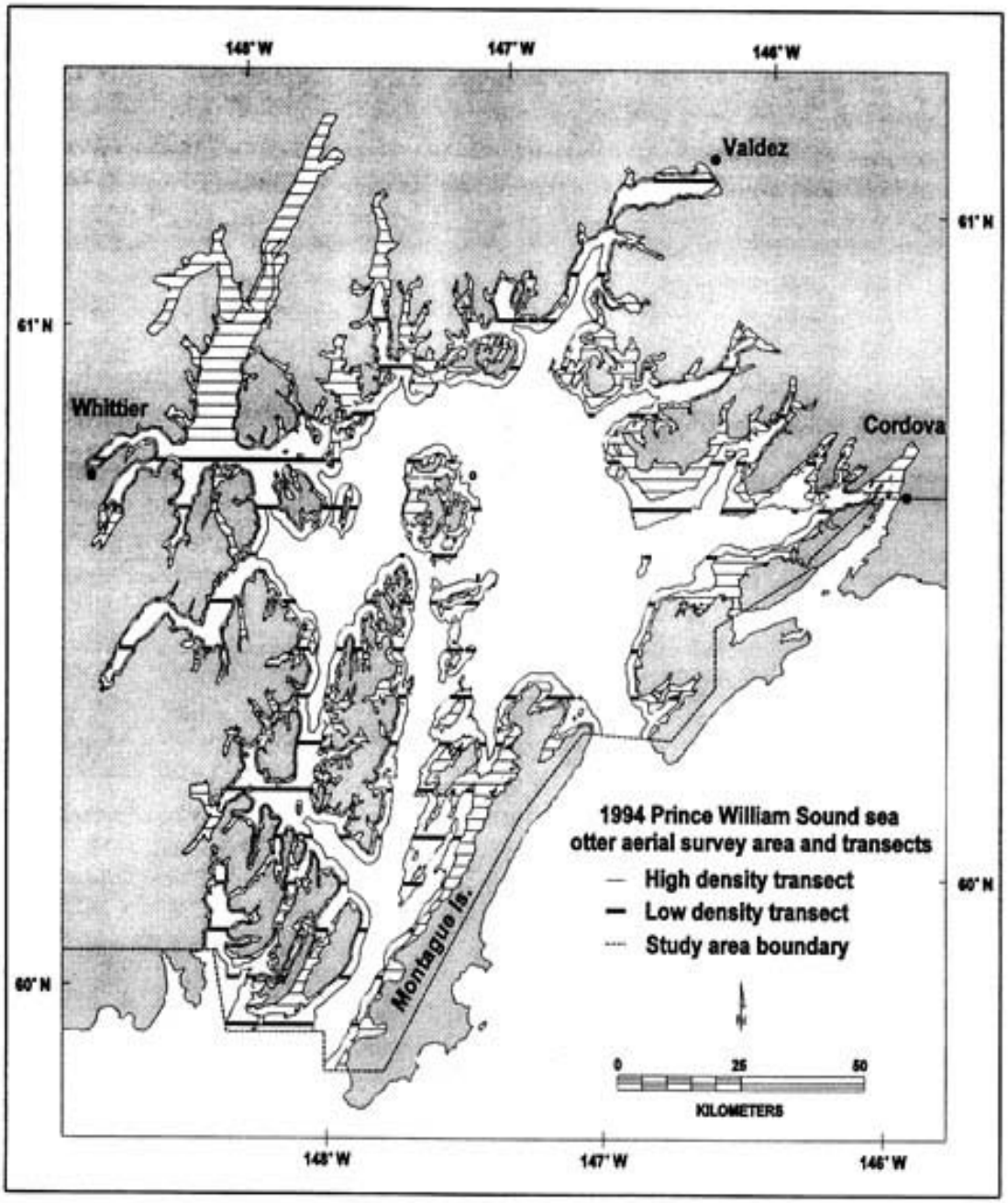

Figure 1. 1994 survey area in Prince William Sound. High and low density transects are 2 and 8 km apart, respectively.

The intensive search method of estimating detection developed here was expected to only be useful for relatively small groups of otters. We assumed groups of 30 or more otters within a 400 m strip would be detected with certainty. Thus, we conceptually divided the population into two portions that are sampled simultaneously and derived separate estimates for the portion that occurred in groups of 30 or less (small groups) and the portion that occurred in groups of more than 30 (large groups). Complete counts, aided by photography (35 mm, 70-210 mm lens), were made of all large

groups detected. These counts were expanded directly based on the proportion of the total area sampled, without any adjustment for detection (i.e., detection was assumed to be 1.0 for this portion of the population). The estimate for the portion of the population occurring in small groups was also expanded based on the portion of the total area sampled but was then adjusted based on the estimated detection of otters in these groups. The overall estimate of the population size was obtained by summing the estimates for these two components of the population.

Two observers were used in 1994, requiring a separate estimate of small group detection for each observer. Each estimate was based only on intensive searches conducted by that observer. For notational convenience, we consider each portion of a stratum surveyed by a different observer to be a separate stratum. The unadjusted population size for stratum j was estimated as:

$$\hat{Y}_{(un)j} = \frac{\sum_{i=1}^{n_j} y_{ij}}{\sum_{i=1}^{n_j} a_{ij}} A_j \qquad (2)$$

$$var(\hat{Y}_{(un)j}) = \frac{A_j^2 (1-f_j) n_j}{\left(\sum_{i=1}^{n_j} a_{ij}\right)^2 (n_j - 1)} \sum_{i=1}^{n_j} \left(y_{ij} - \frac{a_{ij} \sum_{i=1}^{n_j} y_{ij}}{\sum_{i=1}^{n_j} a_{ij}} \right)^2$$

where

n_j = number of surveyed transects in stratum j,
y_{ij} = number of otters detected in strip count on transect i in stratum j, i=1,⋯,n_j,
a_{ij} = area of transect i in stratum j, and
f_j = the sampling fraction, approximated by

$$f_j = \frac{1}{A_j} \sum_{i=1}^{n_j} a_{ij} \; . \qquad (3)$$

the correction factor for observer k was estimated as:

$$\hat{p}_k = \frac{\sum_{i=1}^{t_k} c_i}{\sum_{i=1}^{t_k} s_i}$$

$$var(\hat{p}_k) = \frac{t_k \sum_{i=1}^{t_k} (c_i - \hat{p}_k s_i)^2}{(t_k - 1)\left(\sum_{i=1}^{t_k} s_i\right)^2} \qquad (4)$$

where

s_i = number of otters detected in strip count of ISU i, i=1,…,t_k, and
c_i = total number of otters detected after intensive search of ISU i.

The adjusted population size for stratum j (surveyed by observer k) was estimated as:

$$\hat{Y}_j = \hat{p}_k \hat{Y}_{(un)j}$$

$$var(\hat{Y}_j) = \hat{Y}_{(un)j}^2 var(\hat{p}_j) + \hat{p}_j^2 var(\hat{Y}_{(un)j}) - var(\hat{p}_j) var(\hat{Y}_{(un)j}) . \qquad (5)$$

For the portion of the population in large groups, population size estimates for each stratum were obtained as in (2) with no adjustment for detection. The overall estimates of population size and variance for each stratum were then obtained by summing the respective estimates for otters in small and large groups. Combined estimates of population size and variance for groups of strata were obtained by summing the respective overall stratum estimates. A more detailed protocol for the survey method, including analytical programing is available from the authors.

3 RESULTS

3.1 *Preliminary line-transect surveys*

Preliminary line-transect surveys indicated a region below the plane that was obscured by the aircraft and not visible, and the flight path would have to be offset (64 m horizontal offset at 91 m altitude) from the transect. The detection function appeared to increase with distance from the plane up to a maximum at about 250 m and then decreased with distance beyond that (Figure 2). Few otters were detected at distances beyond 450 m. There was no evidence of an effect of group size on detection, but few groups with more than 2 sea otters were detected in these preliminary surveys (Figure 2).

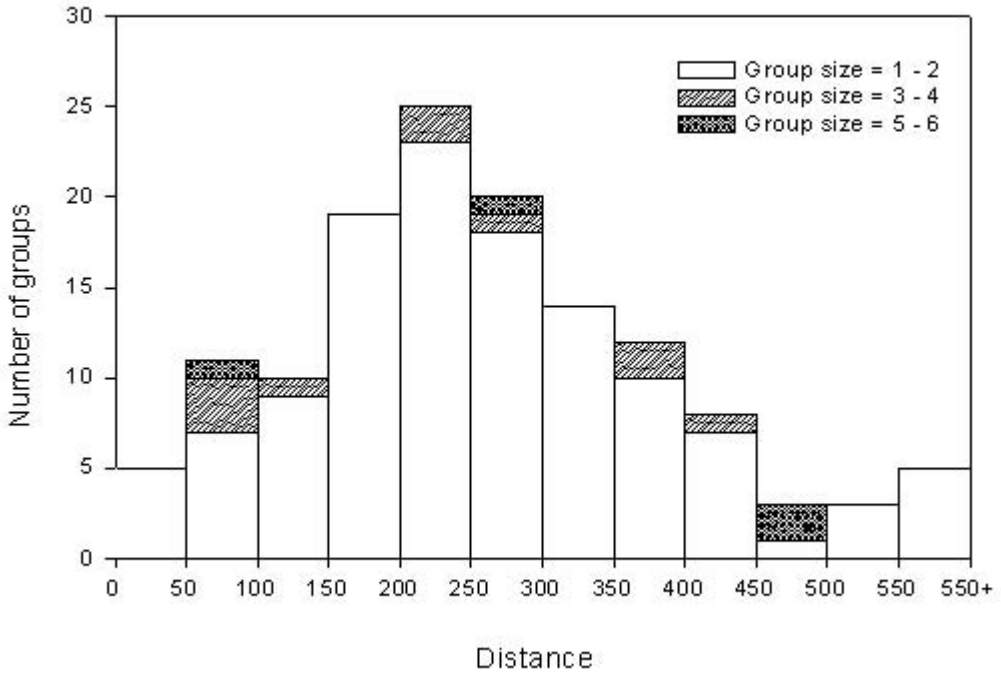

Figure 2. Relation between distance estimated from air and frequency of sea otter sightings using wing strut calibrations in 50 m increments.

3.2 *Shore-based evaluation of detection*

We conducted 98 trials, observing 329 groups of sea otters (741 individuals), in our tests of altitude and search pattern. Intensive searches resulted in detection estimates ≥ 0.90 for all altitudes and patterns investigated (Tables 1 and 2). We detected all otters in over half of the samples (Tables 1 and 2). The type of avoidance behavior observed in boat surveys (Udevitz et al. 1995), in which otters leave the search area before the survey platform arrives, was not observed in response to the aircraft. On 0.08-0.26 of the trials it was apparent that otters were disturbed by the aircraft (Tables 1 and 2), and began diving, swimming out of the area, or swimming erratically within the search area. However, due to the approach speed of the aircraft, otters were unable to leave the survey area before the aircraft arrival.

Detection probability did not differ among trials conducted at 46, 91, or 137 m altitude ($P = 0.72$, Table 1). We would expect detection to decrease at altitudes much greater than those we considered. In general, safety is expected to increase with altitude and we considered 46 m as the minimum altitude safe for this type of survey work. However, at 46 m, disturbance to sea otters within the survey area occurred on 0.23 of our trials, compared to 0.08 at 91 and 137 m altitude (difference not significant, $P = 0.84$, Table 1). We selected an altitude of 91 m for conducting subsequent work because it provides a margin of safety and minimized disturbance without decreasing detection.

Table 1. Detection probabilities (estimated by comparing air to shore observations) at three altitudes in a 750 m diameter search pattern continued for 5 minutes following the last otter sighting.

	Altitude		
	46 m	91 m	137 m
Number of trials	13	12	12
Number of groups	58	43	44
Number of otters	133	104	106
Detection probability	0.92	0.91	0.90
Detection = 1.0[a]	0.62	0.50	0.50
Disturbance [b]	0.23	0.08	0.08

[a] Proportion of samples in which all otters were detected.
[b] Proportion of samples when disturbance by aircraft was detected by shore.

Table 2. Detection probabilities (estimated by comparing air to shore observations) for three search patterns at 91 m continued for 5 minutes following the last otter sighting.

	Search pattern		
	400 m Circle	750 m Circle	800 m Oval
Number of trials	20	19	22
Number of groups	58	40	86
Number of otters	113	72	213
Detection Probability	0.96	0.93	0.90
Detection = 1[a]	0.80	0.79	0.68
Disturbance [b]	0.15	0.26	0.19

[a] Proportion of samples in which all otters were detected.
[b] Proportion of samples when disturbance by aircraft was detected by shore.

We found no differences among the three intensive search patterns evaluated (P=0.64, Table 2). However, with the 400 m circle, the entire ISU remained within the observer's view at all times, making it easier to keep track of which otters and groups had already been detected. With the other two search patterns, the portion of the ISU furthest from the plane was always out of view (although all portions of the ISU were eventually seen each time the plane circled around). Detection probability estimates for initial strip-transect counts ranged from 0.52 to 0.72 (Figure 3). Detection probabilities increased sharply with the first 3 circles or ovals after the strip count (range 0.88 - 0.93) and continued to increase slightly for the next 3 to 4 circles or ovals (Figure 3). No new otters were ever detected after the 7th circle or oval. In the absence of strong differences in detection probabilities, selection of a search pattern could be based on the probability of encountering otters in each search. This probability likely decreases with decreasing the size of the search pattern, thus increasing the number of ISUs necessary to obtain a detection probability estimate with a given level of precision. However, because of decreasing detection probabilities with larger distances from observers (Figure 2) and the need to keep track of otters within ISUs, the 400 m diameter ISU and the corresponding 400 m strip width were selected for use in future work.

The data suggested the most efficient search was three circles or ovals after an initial strip count (Figure 3). Even with intensive searches, however, not all of the otters were detected. Population size estimates based on correction factors derived from these types of intensive counts can be expected to be negatively biased on the order of 0.05-0.10 (Tables 1 and 2).

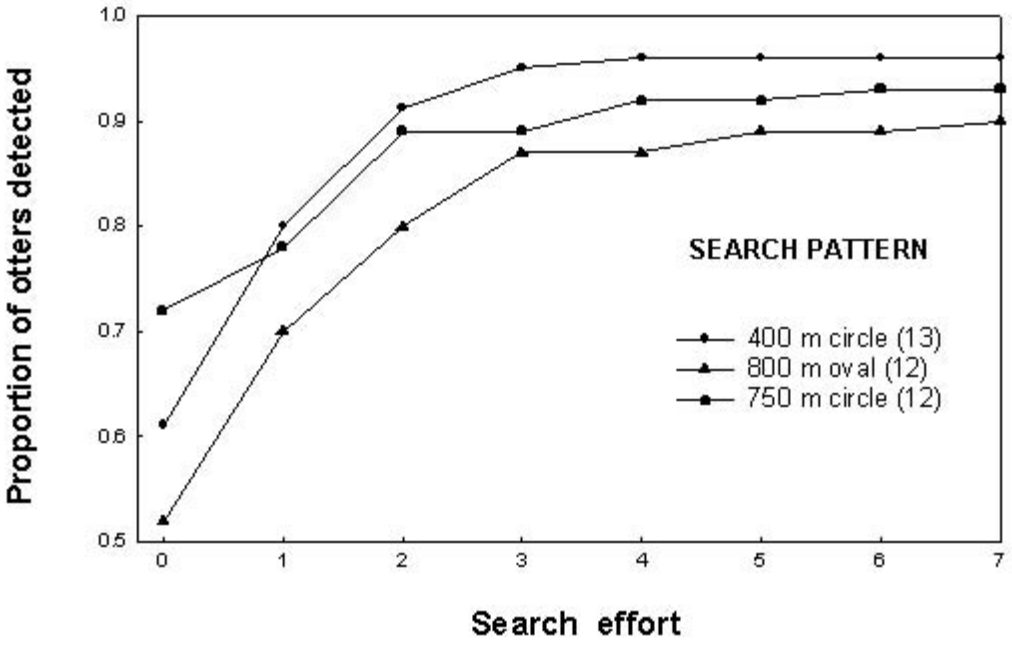

Figure 3. Relation between search effort (number of circles in intensive search) and detection probability for three search patterns.

3.3 *Location of intensive search units*

We estimated detection probabilities from 39 systematically located ISUs and 111 group initiated ISUs. Systematically located ISUs could only be used for estimating detection probabilities if they contained at least one otter; 23% (39/170) met this criteria. Group initiated ISUs could be used for estimating detection probabilities only if they contained more than one group of sea otters, 51% (111/219) met this criteria. Differences in detection probabilities due to type of ISU (P = 0.59) or year (P = 0.65) were not significant, but detection probabilities were significantly different between observers in 1993 (P < 0.01, Table 3).

Table 3. Comparison of detection probabilities obtained from systematically located and group initiated intensive search units (ISUs), by observer and year.

Observer[a]	Year	Type of ISU	Number of ISUs	Ratio estimate of detection probability
2	1993	Group initiated	29	0.18
		Systematic	13	0.41
1	1993	Group initiated	12	0.54
		Systematic	6	0.78
1	1996	Group initiated	70	0.76
		Systematic	20	0.77

[a] Difference between observers significant (P<0.01).

3.4 *1992 distribution survey*

In the 1992 survey, a single observer surveyed 1,936 km of transects (744.4 km^2) in Western Prince William Sound. We found more than 80% of the sea otters in the two near shore bathymetric zones that made up < 35% of the area surveyed (Figure 4). Based on this, we partitioned sea otter habitat in Prince William Sound into two strata. The high density stratum extended 400 m seaward from shore or to the 40 m depth contour, whichever was further from shore. The low density stratum extended from the seaward high density boundary to an offshore boundary based on shoreline physiography, and the 100 m depth contour or a distance of 2 km from shore, whichever was greater. Bays and inlets < 6 km wide were always in the high density stratum.

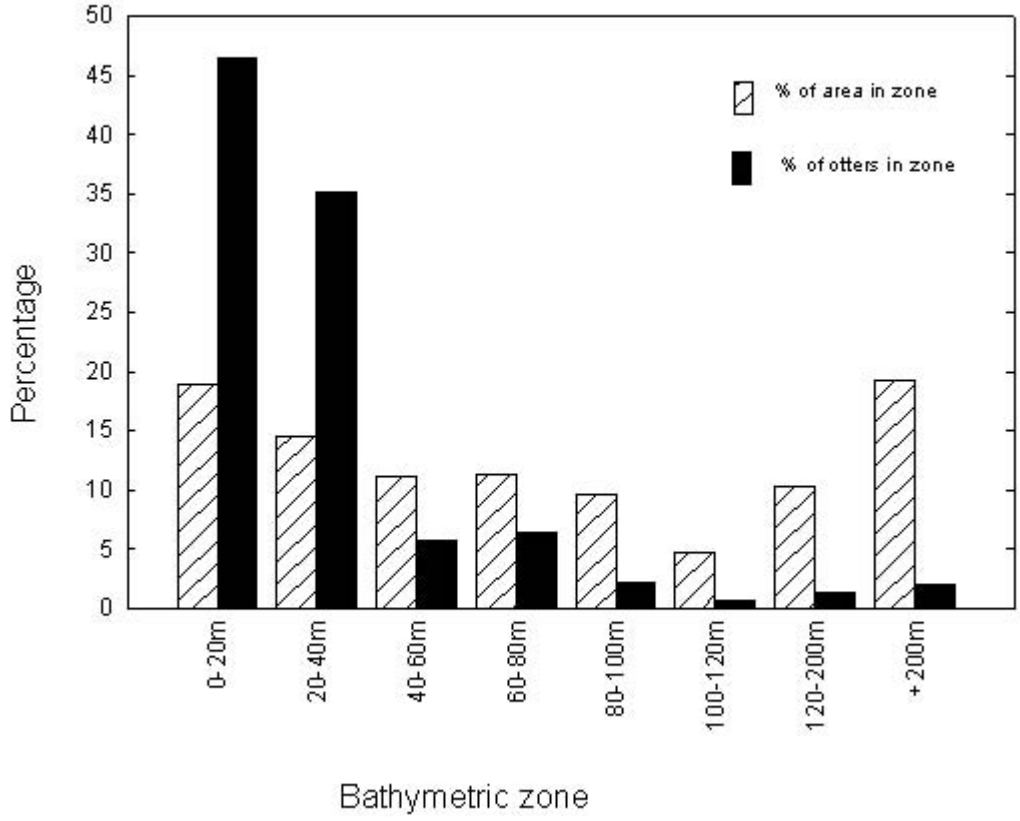

Figure 4. Proportional distribution of sea otters observed in 1992 experimental survey in Western Prince William Sound relative to bathymetric contour intervals.

3.5 *1994 Prince William Sound survey*

The full survey of Prince William Sound was conducted in August 1994 and consisted of 7,120 km^2 (Figure 1). We surveyed 820 km of high stratum transects and 123 km of low stratum transects (Table 4). We counted 888 otters in small groups (size $\leq$ 30). Ninety-seven ISUs with > one group of otters were searched, resulting in individual observer correction factors of 1.39 and 1.92 (Table 4). In addition to the small groups, 131 otters were detected in large groups (size >30). This expanded to a population size estimate of 716 (SE=443) otters occurring in large groups. Combining the

adjusted stratum estimates for small groups (Table 4) and the estimate for large groups gave an estimate of 9,092 sea otters (SE = 1,422) in Prince William Sound. Flight time required to complete the survey was 70 hrs, including transit.

Table 4. Otter counts, unadjusted population size estimates, correction factors and adjusted population size estimates in the 1994 sea otter survey, Prince William Sound, Alaska.

Counts and Unadjusted Estimates

Observer	Stratum	Count[a]	Area[b]	Unadjusted estimate	SE
1	High	221	223	1,209	162
	Low	16	87	532	270
2	High	649	285	3,548	395
	Low	2	43	67	47
	Complete	131	[c]	716	443
	Total	1,042		6,072	674

Correction Factors

Observer	# ISUs	Factor	SE
1	42	1.92	0.20
2	55	1.39	0.08

	Combined Adjusted Estimate	SE
Total	9,092	1,422

[a] Number of otters observed on transects.
[b] Area of surveyed transects (km^2).
[c] Area sampled was the same as high density strata for observer 2. Large groups not observed in other strata, or by observer 1.

4 DISCUSSION

Previous researchers have recognized that some proportion of sea otters in the area surveyed, regardless of the method employed, is not observed, due to diving behavior and sighting error (Geibel & Miller 1984, Estes & Jameson 1988, Udevitz et al. 1995). The result is a bias in the estimate of abundance. This bias can be reduced by estimating the proportion of animals not observed (detection), and using the reciprocal of this proportion as a correction factor. Correction factors may be affected by many variables, including observers, habitat, and survey conditions. Thus, any survey method should incorporate techniques for estimating a correction factor specific for the observers and conditions associated with each application of the method.

We found that detection probabilities in aerial strip counts were low (0.52-0.72), but that intensive searches over selected portions of the strip could provide correction factors to compensate for most of the detection bias. Research conducted in 1993 and 1996 indicated that for a given number of ISUs, the number of usable ISUs could be approximately doubled by initiating searches only when a group was detected. Detection probability estimates based on group initiated ISUs will not be more biased than estimates based on systematically located ISUs if the initiating group is not included in the estimate and if the detection of groups is independent. The assumption of

independence of group detection is common in line transect theory (e.g., Burnham et al. 1980, Quang & Lanctot 1991, Buckland et al. 1993). Our inability to find a difference in estimated detection probabilities from the two methods for locating ISUs is consistent with this assumption. The potential for relations between size and detection of animal groups is well known (Buckland et al. 1993) and the relation has been demonstrated for sea otters in certain cases (Estes & Jameson 1988, Drummer et al. 1990). Our line transect data did not indicate any effect of group size on detection, but the range of observed group sizes (Figure 2) was small. Other studies have found that group size effects were not evident for sea otters when there was little variation in group size or observation distances were relatively short (Drummer et al. 1990, Udevitz et al. 1995). Buckland et al. (1993) suggested that group size effects can usually be eliminated by truncating observation distances. We only apply the ISU technique for estimating detection probabilities of groups with less than 30 individuals and observation distances are truncated at 400 m. Thus, it is unlikely that there would be any strong group size effects on detection in these surveys. In any case, if detection of groups are independent, the size of the initially detected group (or its detection probability) will not affect the estimated detection probabilities in group-initiated ISUs.

Results of the 1994 survey indicate that differences in detection between observers may be large. Difference in detection between observers will not increase the bias of the adjusted population estimate as long as the correction factor for each observer is estimated separately. This can be done, provided each observer can achieve an acceptable level of detection (we suggest >0.90) in the ISUs. The precision of the estimated correction factors will depend on the number of usable ISUs for each observer. To achieve an acceptable level of precision for the adjusted population size estimates, it will be necessary to obtain a sufficient number of usable ISUs for each observer.

It is likely that our ability to detect otters varies with factors we have not tested, including canopy forming kelp beds, and this should be evaluated. Because detection may vary among observers, testing of observers to determine individual relations between search effort and detection for ISUs should be done. Additionally, we conducted shore-based observer trials and surveys only under environmental conditions of calm to light winds, good visibility, and calm seas (Beaufort scale 0-2, rarely 3). Detection probabilities should be tested before applying this method under different environmental conditions.

Further efforts to improve precision and efficiency should include training to increase precision in detection probabilities and assure that all observers detect at least 90% of the otters within ISUs. Precision may be improved by analyzing separately the two components of detection: (1) the probability of detecting a group, and (2) the proportion of the otters detected in a group, given that the group is detected. This separation would allow the use of all ISUs in estimating the second component of the detection probability. Greater overall sampling effort would also increase precision of the population estimate. The precision of the estimates obtained with this survey method is limited by the number of usable ISUs an observer can accumulate during a survey. In areas less than a few hundred km^2 it may be impossible to acquire a sample of ISUs necessary to achieve desired levels of precision, particularly if sea otter densities are low. In such cases, it may be possible to increase precision to an acceptable level by conducting replicate surveys.

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APPENDIX SO-02

CHEMICAL RESTRAINT OF NORTHERN SEA OTTERS: RESULTS OF PAST FIELD STUDIES[1]

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Abstract: Between 1987 and 1997 we chemically immobilized 598 wild sea otters (*Enhydra lutris*) in Alaska for the collection of biological samples or surgical instrumentation. We experienced only one drug-related fatality in this time. Fentanyl in combination with diazepam produced consistent, smooth inductions with minimal need for supplemental anesthetics during procedures lasting 30 to 40 minutes. Reversal with naltrexone or naloxone was rapid and complete, although we observed narcotic recycling in sea otters reversed with naloxone. For surgical procedures, we recommend a fentanyl target dose rate of 0.33 mg/kg of body mass and diazepam at 0.11 mg/kg. For non-surgical sample collection procedures, we recommend fentanyl at 0.22 mg/kg and diazepam at 0.07 mg/kg. We advise use of naltrexone for reversal at a dose equaling twice the total fentanyl administered during processing.

Key words: Anesthesia, azaperone, diazepam, fentanyl, naloxone, naltrexone, sea otter.

INTRODUCTION

Researchers have captured several thousand sea otters throughout their range since the 1950s for translocation, tagging, and collection of biological samples. Capture methods have been well described,[1] and include modified gill nets (also called "tangle nets"), dip nets and diver-operated Wilson traps. Tagging and surgical procedures for the implantation of radiotelemetry transmitters have changed little since they were developed.[4,14] However, chemical immobilization protocols have changed with time. Researchers administered "anti-stress" drugs during translocation projects as early as 1959.[1] Full chemical immobilization protocols were developed for instrumentation and veterinary care.[7,11,13,15] We found that published protocols, although appropriate for clinical settings, gave dosages lower than those which we found necessary for wild, healthy sea otters. Here we describe drug combinations used in Alaska from 1987-97, and recommend dosages for routine biological sampling and surgical instrumentation of wild sea otters.

METHODS

Study area

We captured sea otters along the north Pacific Rim from Vancouver Island, British Columbia to Attu Island, Alaska at the western end of the Aleutian Island chain. Most captures occurred within Prince William Sound in south-central Alaska or at Amchitka Island in the Aleutian chain. We captured otters for the collection of biological samples and, in some cases, for surgical instrumentation.[2,3,9,10]

Capture and immobilization

We employed several capture techniques including tangle nets, dip nets and Wilson traps.[1] We visually estimated total mass of sea otters to calculate the induction dose, which was administered by intramuscular (IM) injection to the hind limb with a hand syringe. Two drug combinations were used: fentanyl citrate (RBI, Natick, Maine, USA) combined with azaperone (Stesnil®, Pitman-Moore, Inc., Washington Crossing, New Jersey, USA), and fentanyl combined with diazepam (Steris Laboratories Inc., Phoenix, Arizona, USA).

Until 1990, we used an equal ratio of fentanyl and azaperone for initial injections. After 1990, azaperone was no longer readily available and we switched to a 3:1 ratio of fentanyl to diazepam for initial injections. We administered Supplemental IM injections of fentanyl as required to maintain an adequate level of anesthesia for the procedures being performed. We also gave supplemental IM or intravenous (IV)

injections of diazepam (0.5-1.5 mg) as needed to control convulsive seizures.

Anesthetized otters were weighed, and actual drug dose calculated. We measured induction time opportunistically (minutes from injection until fully anesthetized) in a subset of 101 animals and "time to first procedure" (TFP) in all animals. Rectal temperature was monitored throughout the period of sedation and handling. We recorded the times of all drug injections, body temperature readings, and tremors or convulsions.

After processing, the otters were reversed with naloxone (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) or naltrexone (Trexonil®, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) at a dose of 1.5-2X the total fentanyl dose administered. Use of naloxone was discontinued in 1992, when naltrexone became available. The reversal agent was given either half IV and half IM, or all IM. The naloxone or naltrexone was drawn up before administering the fentanyl so that it would be immediately ready for use if needed.

Before 1990, we reversed animals with IV and IM injections of naloxone (equal dose by each route) at the water's edge and released them to the water as soon as a conscious "head up" response was observed, generally within 30 seconds of injection. However, naloxone has a shorter half life than fentanyl, and the narcotic can "recycle" if the naloxone wears off before the fentanyl is completely metabolized. Between 1990 and 1992, because of concerns about possible narcotic recycling, we released the otters to floating net pens and observed them for one to two hours after reversal. Because signs of narcotic recycling were observed frequently, we initiated the practice of giving supplemental naloxone at a dose equaling half the initial reversal dose just prior to release from the net pen. After 1992 we accomplished reversal with naltrexone, and because of its longer half-life we found holding and subsequent supplemental doses unnecessary. After this time we gave only IM injections of naltrexone, holding the otter within a capture box until fully alert (usually 1 to 3 minutes), and then released it to the water.

Data analysis

We used logistic regression and the Wald Chi-square statistic to examine four response variables (coded as 1=yes, 2=no): 1) supplemental fentanyl required, 2) tremors or convulsions observed, 3) narcotic recycling observed (only for otters held ≥1 hour post-reversal), and 4) hyper thermic problems observed (defined as >40 ºC). Full models included drug dose rates and drug type (sedative - azaperone vs. diazepam, or reversal - naloxone vs. naltrexone). Obviously, doses rates among drugs administered in combination are highly correlated and thus we used only one drug type dose rate at a

time in each analysis (i.e., one model may include fentanyl dose and sedative type and a second model may include sedative type and sedative dose but no model included both fentanyl dose and sedative dose). We included a drug type*dose interaction term when both were in the model and repeated the analysis separately for each drug type if the interaction was significant. We included body temperature, and capture type (surgical vs. blood sampling only) as covariates in the full model for response variables 1 and 2. Covariates for response variable 3 included handling time and post reversal holding. We included handling time as a covariate for response variable 4 along with capture type and body temperature. We used indicator variables coded as 1 or 0 to represent sedative type, reversal type and capture type in the model. We reduced models using stepwise selection and chose the best fit model based on AIC values (i.e., the best fit model had the lowest AIC value). We present the results of the full and best fit model. We used linear regression analysis to examine the relationship between drug doses and induction times, and Fisher's exact test to compare naloxone and naltrexone recycling rates. We used SAS (version 6.12, SAS Institute Inc. Cary, NC) statistical software for all analyzes. Differences were considered significant at $\alpha \leq 0.05$.

RESULTS

From 1987-97, 265 sea otters were anesthetized for sampling and surgical instrumentation, and 303 sea otters were anesthetized for biological sampling only (Table 1). An additional 30 sea otters were anesthetized for semen collection via electro-ejaculation and required anesthesia similar to surgical levels; these were included in the "surgical" category for analysis. Sea otters handled during rescue efforts at the time of the 1989 *Exxon Valdez* oil spill[11] were not included in this study.

During these studies we had six capture-related mortalities giving an overall loss rate of 1%. Only one death (0.17%) was drug-related, and this involved an animal compromised by injury prior to immobilization.

Drug protocol

Initial surgical drug dosages ranged from 0.16-0.60 mg/kg for fentanyl, 0.16-0.33 mg/kg for azaperone, and 0.07-0.23 mg/kg for diazepam. Initial biological sampling dosages ranged from 0.09-0.38 mg/kg for fentanyl, 0.10-0.44 mg/kg for azaperone, and 0.04-0.17 mg/kg for diazepam. We present mean dose rates actually administered in Table 1. Supplemental fentanyl was required in 45 of 295 (15%) animals undergoing surgical procedures, and 29 of 303 (10%) of animals during sampling procedures. One female and one male never became

adequately immobilized despite several injections totaling approximately 2½ times the estimated required dose, and were reversed and released without processing. Additional diazepam was required during only 15 immobilizations (fentanyl-diazepam combination) for control of convulsive seizures and tremors.

Mean induction time was 9 minutes (SD = 4 min.), and mean TFP was 15 minutes (SD = 4.5 min.) for the subset of 101 otters where both times were recorded. The difference of 6 minutes represents the average time to weigh, measure and secure the otter for processing. For all other anesthetizations, mean TFP was essentially the same ($\bar{x}$ = 14.5 min., SD = 5 min., N = 458) indicating it could be used as an index of induction time. Induction times and TFP were not dose-responsive to any of the anesthetic agents ($R^2 \leq 0.03$ for all drugs). Induction times were similar for azaperone anesthetizations($\bar{x}$ = 9.2 min., SD = 5.1 min.) and diazepam anesthetizations($\bar{x}$ = 8.9 min., SD = 4.0 min.; $t_{(98)}$=0.23, P=0.8).

As would be expected, low initial fentanyl dose lead to a higher probability that supplemental fentanyl would be required, and we required more narcotic for surgical procedures (Table 2, Fig. 1). However, we also required higher doses of fentanyl when used in combination with azaperone as compared with fentanyl-diazepam anaesthetizations (Table 2, Fig. 1).

We observed tremors or convulsions during 71 of 598 (12%) anesthetizations. The probability of tremors or convulsions was significantly less when using diazepam (Table 3), with 47% of otters immobilized with the fentanyl-azaperone combination experiencing tremors or convulsions vs. only 8% for those immobilized with fentanyl-diazepam. Tremors were not related to sedative dose rate or body temperature (Table 3).

Three of 33 otters (9%) reversed with naloxone and held from 30-60 minutes had already shown signs of recycling. An additional 41 of 186 otters (22%) held at least 1 hour after reversal showed signs of narcotic recycling. Recycling was not related to fentanyl dose, but a significant interaction between sedative type and sedative dose was found (Table 4). Thus azaperone and diazepam anaesthetizations were analyzed separately. Twenty of 38 (53%) otters recycled when sedated with azaperone, and the probability of recycling increased with the amount of naloxone or azaperone administered (Table 4; Fig. 2). In contrast, only 20 of 169 (12%) of otters recycled with diazepam, and showed no relationship with diazepam or naloxone dose (Table 4). This suggests azaperone was the primary cause of the dose response seen between recycling rate and azaperone or naloxone dose rate. First signs of recycling usually occurred from one to two hours after narcotic reversal (mean = 80 min., range 8-152 min.).

We monitored 26 otters after reversal with naltrexone, including 14 held between 30-60 minutes and 12 observed for

over an hour (maximum = 3.3 hrs). None showed any signs of renarcotization. Although power is low due to small sample size, the result approached statistical significance when compared with the renarcotization rate of naloxone reversals (*Fisher's exact 1-tailed*, P=0.056 for otters held >1 hr.).

Mean initial body temperature for all captures was 37.5° C (SD = 0.9). During handling, body temperature generally increased with mean changes of +1.2° C and +1.6° C for blood sampling and surgical captures respectively. Elevated temperatures did occur occasionally even with close monitoring and efforts to keep the otters cool. Body temperature of 21 otters surpassed 40° C, at which point they were reversed immediately. We found no relationship between hyperthermia and drug doses or capture type, but the probability of hyperthermia increased with handling time and initial body temperature (Table 5; Fig.3).

DISCUSSION

The most recently published drug protocol for sea otters[11] was developed from experience handling sea otters captured for rehabilitation during the 1989 *Exxon Valdez* oil spill. In the early days after the spill, many otters were severely compromised by exposure to oil and anesthesia was considered risky. Immobilizing these animals, when necessary, was accomplished with low doses of weak narcotics such as meperidine hydrochloride in combination with diazepam. The general health and vigor of animals coming into the rehabilitation facilities increased with time, and more potent drugs were required. Fentanyl, in combination with diazepam (supplies of azaperone were limited), was most commonly used at initial dosages of about 0.1 mg/kg for both fentanyl and diazepam. However, due to prolonged procedures, supplemental doses up to a total of 0.8 mg/kg of fentanyl and 0.2 mg/kg of diazepam were sometimes required.[11] The combination of fentanyl, azaperone and diazepam was also used, and the final recommendation of Sawyer and Williams[11] for the immobilization of sea otters up to 2.5 hours included 0.1 mg/kg of fentanyl, and 0.5 mg/kg of azaperone in combination with 0.1 - 0.5 mg/kg of diazepam. As an alternative to azaperone, they recommend acepromazine at a dose of 0.05 mg/kg.

The protocol recommended by Sawyer and Williams[11] worked well in the clinical setting for sea otters needing to be cleaned, as washing and related handling sometimes continued for several hours. The use of the longer lasting, nonreversible tranquilizers (azaperone or acepromazine) significantly reduced the amount of supplemental narcotic required over these extended periods. But Sawyer and Williams[11] also point out that these same tranquilizers (particularly acepromazine) prolonged recovery times. However, sea otters in the rehabilitation centers could be

reversed and held in a controlled setting, allowing them to be closely monitored during recovery.

Sea otters captured for sampling and instrumentation are generally handled immediately after capture and subjected to procedures lasting less than one hour. The initial reaction of a healthy, wild sea otter to capture includes a vigorous struggle. Animals in a highly excited state may require more drugs for initial induction.[12] Sea otters in our studies required higher doses of fentanyl than were recommended by Sawyer and Williams.[11] In addition, to reduce stress, immediate release was preferred to holding the animals after processing. Thus, use of long lasting tranquilizers is not advisable. We found fentanyl in combination with diazepam alone produced smooth inductions and provided anesthetic effects lasting at least 30 to 40 minutes. Diazepam is now reversible with flumazenil but we have not found this necessary, and in fact believe the residual diazepam actually may help reduce post-reversal stress.

Sea otters tolerated and sometimes required relatively high doses of fentanyl (one adult female required a dose of 0.75 mg/kg before she was adequately immobilized), but generally doses greater than 0.33 mg/kg provide little benefit in terms of improved anesthesia. Less narcotic can be used when biological sampling and tagging are the only purposes of capture. Electro-ejaculation procedures required dosages closer to surgical dosages because of the intense physical stimulation.

Fentanyl is known to have excitatory central nervous system effects.[6] Diazepam has been used to control tremor and seizures in sea otters.[1,11], and we found it more effective than azaperone for this purpose.

Naloxone is an effective narcotic antagonist with a history of use in sea otters.[14,15] However, it has a short half life compared with fentanyl, and others using naloxone have reported recycling, although with more potent narcotics.[5] For an animal such as the sea otter, which spends its life in the water, there is significant potential for narcotic recycling to cause fatalities. Sea otters experiencing the effects of recycling slowed to the point of resting quietly in the water. However, several animals began to roll face down in the water for at least several seconds. At this point supplemental naloxone was given, but without intervention the potential for drowning was clear. For sea otters immobilized with fentanyl and azaperone, and reversed with naloxone, the risk of renarcotization increased with increasing dose of azaperone.

To our knowledge, we did not experience any recycling-related mortalities during our studies. However, prior to 1990, 1 of 45 radio instrumented sea otters, reversed with naloxone and released immediately, disappeared and was not seen again. It is not known if the disappearance of this otter was due to recycling and drowning, death due to

complications from surgery, radio failure, or movement out of the study area. However, had the otter died and the body beached it likely would have been recovered if the radio was functional.

Naltrexone, like naloxone, is a pure antagonist but with a longer half life.[6] We observed no signs of renarcotization in 26 otters observed up to 3 hours post-reversal. Other researchers, however, have observed recycling when using naltrexone in low doses with more potent narcotics.[8] Our recommended naltrexone dose of 1.5-2X the fentanyl administered during processing (usually 0.44 mg/kg - 0.66 mg/kg) is much higher than 0.01 mg/kg recommended by Williams et al[15] for naloxone. However, because naltrexone has little agonistic effect and sea otters appear to tolerate relatively high doses, giving extra to prevent recycling seems prudent. Others have published similar recommendations.[5,8]

Generally, body temperatures of sea otters increased during processing, and monitoring temperature closely is critical to ensure well-being of the otters throughout the handling procedure. Loss of temperature control is common under anesthesia, particularly for an animal like the sea otter which has a dense pelage with extremely good insulating properties. Once an otter is removed from the water, its temperature can rise rapidly, depending primarily on environmental conditions. We have found that keeping otters wet prior to sedation, either by holding them in a net pen or by running water over them when they are held in capture boxes, is key to maintaining normal body temperatures throughout the handling period.

We had only one drug-related mortality, which involved a sea otter with lungs compromised by sea water aspiration. Impaired lung capacity is a known risk factor when using fentanyl,[6] but we did not realize the extent of injury at the time of anesthetization. Over-all, our capture and drug-related mortality rates appear to be well below what is often experienced when handling wild animals.

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Table 1. Mean (and standard deviation) dosages of fentanyl, diazepam and azaperone (mg/kg) administered to sea otters during captures for surgical instrumentation and biological sampling.

		Fentanyl						
		Not supplemented		Supplemented			Diazepam	Azaperone
Procedure	Mean handling time (min)	Dosage mg/kg (SD)	Percent of captures	Initial dosage mg/kg (SD)	Total dosage mg/kg (SD)	Percent of captures	Dosage mg/kg (SD)	Dosage mg/kg (SD)
Surgical *n* = 295	41	0.34 (0.08)	85	0.29 (0.06)	0.39 (0.09)	15	0.10 (0.008)	0.26 (0.02)
Sampling *n* = 303	30	0.23 (0.04)	90	0.21 (0.05)	0.30 (0.09)	10	0.07 (0.008)	0.25 (na)

Table 2. Results of logistic regression modeling the probability of that supplemental fentanyl will be required (insignificant interaction terms not included).

		Results of full model			Best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = supplemental	Intercept	0.03	0.86	367.3	1.00	0.32	365.3
	initial fentanyl	17.17	0.0001		17.17	0.0001	
	sedative type	3.89	0.05		4.03	0.05	
	capture type	18.75	0.0001		18.77	0.0001	
	initial temp.	0.003	0.96		–	–	

Table 3. Results of logistic regression modeling the probability of observing tremor or convulsion (insignificant interaction terms not included).

		Results of full model			Best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = tremor / convulsion	Intercept	0.32	0.57	281.9	0.45	0.50	276.5
	sedative type	3.47	0.06		39.33	0.0001	
	sedative dose	0.44	0.51		—	—	
	capture type	0.20	0.66		—	—	
	body temp.	0.16	0.69		—	—	

Table 4. Results of logistic regression modeling the probability of observing narcotic recycling while using naloxone for reversal. Significant interaction in full model dictated separating diazepam and azaperone anaesthetizations.

		Results of full model			2nd best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = narcotic recycling	Intercept	4.05	0.04	155.5	0.05	0.82	159.7
	initial naloxone dose	5.93	0.01		2.13	0.14	
	sedative type	2.12	0.14		14.23	0.0002	
	sed. type*nal. dose intactio	4.89	**0.03**		—	—	
	handling time	5.85	0.02		4.02	0.04	
	holding time	2.37	0.12		3.36	0.07	
			diazepam			azaperone	
Y = narcotic recycling	Intercept	0.77	0.38	115.5[1]	4.21	0.04	42.9[1]
	initial naloxone dose	0.45	0.50[2]		4.82	0.03[3]	naloxone
	handling time	1.86	0.17		4.38	0.04	
	holding time	0.53	0.47		2.26	0.13	

[1] Data from azaperone and diazepam anaesthetizations separated — AIC values not comparable between models or with full model.
[2] relationship similar using diazepam dose rate P = 0.38, AIC = 115.2.
[3] relationship similar using azaperone dose rate P = 0.03, AIC = 43.4.

Table 5. Results of logistic regression modeling the probability of an otter reaching a hyper thermic (>40 ˚C) state while sedated (insignificant interaction terms not included).

		Results of full model			Best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = hypothermia	Intercept	54.68	0.0001	164.3	57.123	0.0001	161.5
	fentanyl dose	0.01	0.92		—	—	
	sedative type	0.84	0.36		—	—	
	capture type	2.03	0.15		—	—	
	handling time	2.50	0.11		10.82	0.001	
	initial temp.	53.89	0.0001		56.26	0.0001	

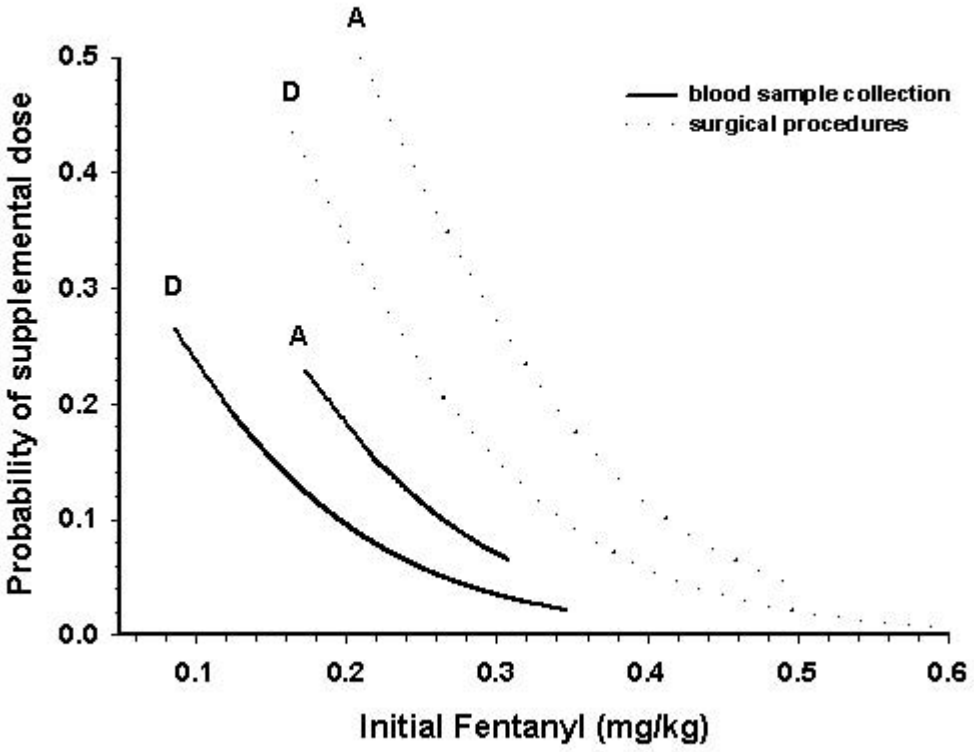

Figure 1. Probability that a sea otter will require supplemental fentanyl during processing in relation to the initial dose of fentanyl administer, the purpose of anesthesia (sample collection only vs. surgical instrumentation) and sedative used (D = diazepam vs. A = azaperone).

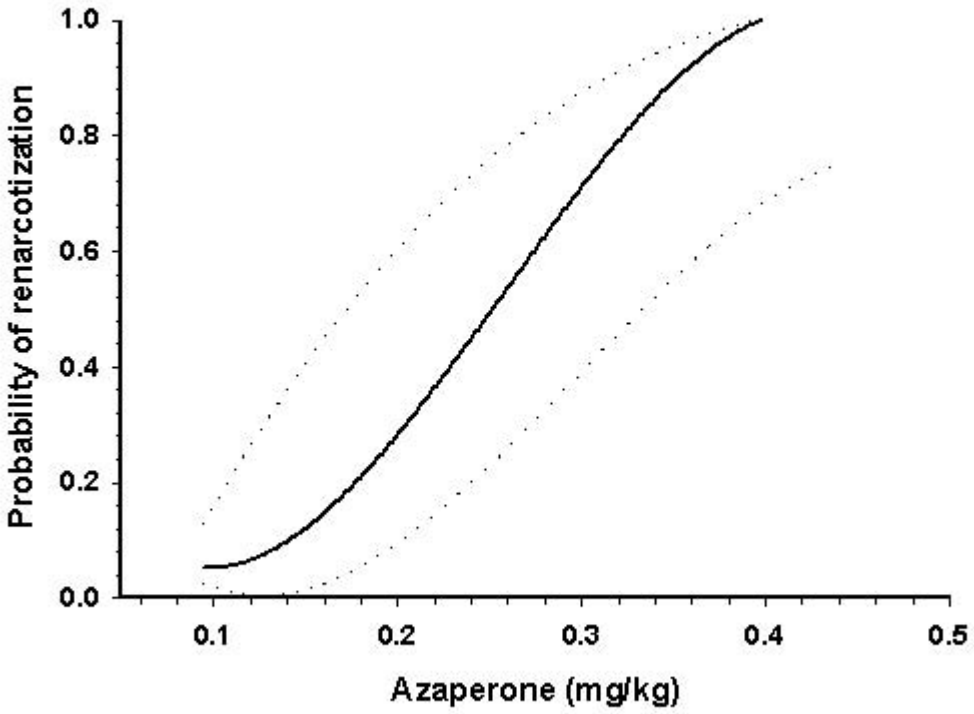

Figure 2. Probability of narcotic recycling for sea otters anesthetized with fentanyl and azaperone, and reversed with naloxone.

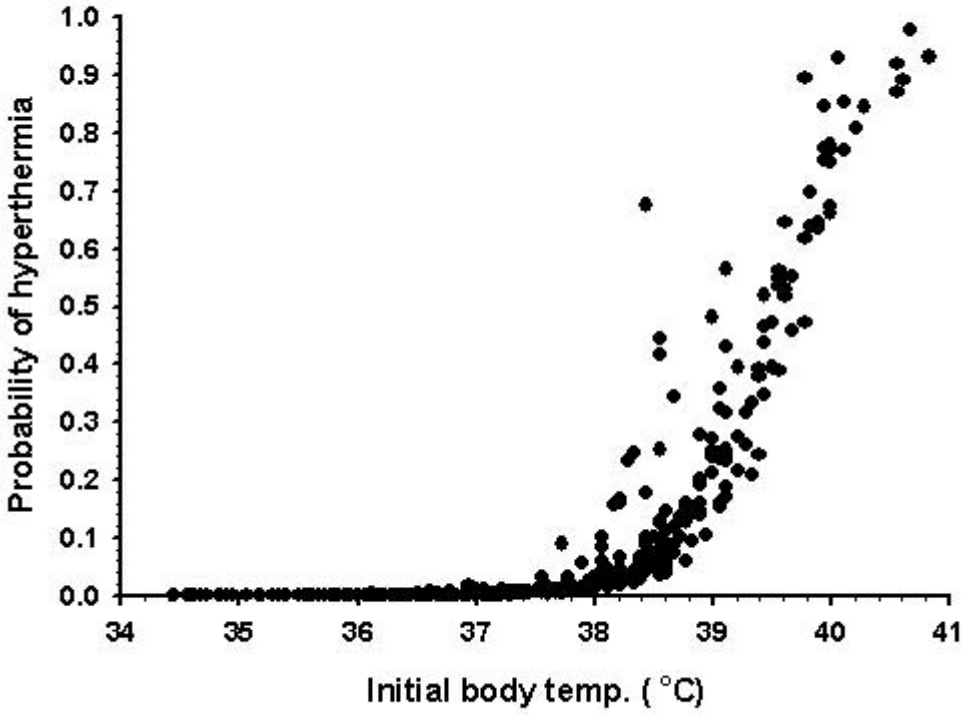

Figure 3. Probability of an anesthetized sea otter reaching a hyper thermic condition (>40 ˚C) in relation to its initial body temperature at the time of drugging.

APPENDIX SO-03

Long-term Changes in the Abundance and Growth of the Pacific Blue Mussel, *Mytilus trossulus*, in a Heavily Oiled Bay in Prince William Sound, Alaska[1]

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Long-term Changes in the Abundance and Growth of the Pacific Blue Mussel, *Mytilus trossulus* in a Heavily Oiled Bay in Prince William Sound, Alaska

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and Susan M. Saupe

Abstract

Since the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, several unrelated studies have reported reduced densities of *Mytilus trossulus* in oiled areas. To examine the long-term changes in mussel populations we combined the results of two projects that conducted research in the heavily oiled Herring Bay to create a data set spanning five years from 1993-1997. Both projects collected mussel samples from similar sites using randomly placed quadrats and carried out growth studies on tagged mussels. Significant differences in mussel density and growth were found between oiled and control sites and between years. Mussel density on oiled sites dropped to their lowest level in 1996-97. Mussel density at the control sites did not change significantly until 1997 when it increased compared to 1996. Mussel length-frequency distributions at oiled sites were more strongly skewed to the right than at control sites in 1994, '95 and '97 indicating that a larger proportion of the population was composed of young individuals at oiled sites than at control sites in those years. Where differences in mussel growth rates were observed over the course of the study, control mussels grew faster than those at oiled sites. Prolonged exposure to residual polynuclear aromatic hydrocarbons may have contributed to the decline of mussel populations at oiled sites in Herring Bay from 1993-1996. However, other factors such as heavy predation by whelks cannot be ruled out.

Keywords: *Mytilus trossulus*, *Exxon Valdez*, oil spill, Prince William Sound, population trends, long-term recovery, growth.

Introduction

After the *Exxon Valdez* oil spill in 1989 several projects studied the response of mussel, *Mytilus trossulus*, populations in Prince William Sound (PWS). These studies found mussels had reduced densities and growth rates in oiled areas. Houghton *et al*. (1993a, 1993b, 1993c, and 1993d) found differences in the abundance of *Mytilus* on oiled and control sites in western PWS resulting from shoreline cleaning during 1991-92. *Mytilus* had reduced density and biomass on sheltered rocky shores in PWS that were oiled by the spill (Highsmith *et al*. 1994). Highsmith *et al*. (1996) found reduced growth at oiled sites in Herring Bay beginning in 1993 that persisted at least until 1995. Babcock *et al*. (1998) observed a significant decline in mussel densities from 1994 to 1996 in both restored and oiled reference (unmanipulated) beds, but this decline was probably not caused by the spill.

In the decade since the *Exxon Valdez* oil spill, no one project has collected the data necessary to examine long-term changes in mussel population dynamics in oiled areas. To extend the time series of data available, we combined data from two projects that carried out research on mussels in the same location. The Herring Bay Restoration studies (HBR) initiated mussel studies during 1993-1995 which examined mussel density and growth in oiled versus non-oiled intertidal areas. The Nearshore Vertebrate Predator (NVP) project studied mussels in three major areas including Herring Bay during 1996-1998 and investigated their role as one of several species of prey that because of their relative scarcity may be limiting the recovery of predators such as sea birds and sea otters in oiled areas (Holland-Bartels 1998). Despite the different objectives of these two projects, comparable data were collected on mussel density and growth in Herring Bay covering the period 1993-1997.

Methods

Location

Herring Bay lies on the NE end of Knight Island near the middle of Prince William Sound, Alaska (Figure 1). The bay's shoreline is categorized as sheltered rocky habitat, and much of it was oiled by the *Exxon Valdez* oil spill. The HBR study sites where data was collected on mussel density, size, and growth were selected based on matched oil and control sections of shoreline. Similar site topography, slope, exposure and presence of comparable mussel beds were considered in site selection. The NVP project site selection was systematic, dividing Herring Bay's shoreline into 200 m segments. Data presented here will be limited to four sites (oil 1 /control 1 and oil 2 /control 2) which were common between the HBR project and the NVP project (Figure 2). An additional third paired site, which was part of the HBR study is not considered here because there was no matching NVP site pair.

Mussel Density and Size-Frequency Distribution

The HBR mussel study began in June 1993. The experimental design consisted of four vertical transects set perpendicular to the shoreline. The position of the first transect was randomly located systematically in the first quarter of the mussel zone. The other

three transects were located by consecutively adding one-fourth of the total mussel zone length to the first transect location. The width of the mussel band along each transect was measured and multiplied by a random number between 0 and 1 (representing a proportion of the width of the mussel zone) to establish the placement of the upper right corner of the sampling quadrat. Quadrat size varied from 0.01 to 0.1m^2 depending on the year. All mussels were removed from the quadrat, bagged and frozen for sorting in the laboratory. Sites were revisited in September 1993, May 1994, September 1994, and May 1995. Quadrats sampled after the first sample period were placed one meter to the left of the previous quadrats. In the laboratory, mussels were thawed and washed in a 0.5 mm sieve. Mussels retained by the sieve were counted and maximum shell length was measured to the nearest millimeter.

The NVP project began in 1996. Each site consisted of a 200 m long shore segment within which ten vertical transects were laid 20 m apart. The first transect to be sampled was placed a random distance between 0 and 20 m from the beginning of the segment. Each transect was laid from the upper limit to the lower limit of the mussel zone perpendicular to the shore. A 500 cm^2 quadrat was positioned a random distance along each transect. All mussels were removed from within the quadrat and handled in the same manner as the HBR study. The shore segments sampled in 1997 were offset 400 m from those sampled in 1996.

Mussel Growth

The HBR growth study removed mussels from the middle of the mussel zone near the sites where density estimates were made, bagged them separately in seawater and returned the mussels to a research vessel where each mussel was tagged or etched with a number on its shell and it's length measured to the nearest 0.05 mm with calipers. Mussels >15 mm were chosen for the growth study. Superglue gel was used to attach an individually numbered polyethylene shellfish tag (Hallprint, Pty. Ltd., Holden Hill, South Australia) to the shell or, alternatively, an engraving tool was used to etch a number directly into the shell. The tagged or etched mussels were stored in a flowing seawater tank until the next low tide, then returned to the sites from which they were removed. Thirty to sixty tagged mussels were placed inside a 1/4 inch, wire mesh cage (20 x 20 x 7 cm) which was attached to the substrate with anchor screws. Growth was measured as the difference in total length between visits. Initial measurements were taken in June 1993 and mussels were collected, measured, and returned to their cages in August 1993, May 1994, August 1994, May 1995, and August 1995. Mussels were caged because of high mortality by predators in an early test deployment. To test for differences in flow rates inside and outside cages, calcium sulfate dissolution cylinders were placed adjacent to each other, one cylinder was caged and the other uncaged. The cages were placed in the intertidal region in areas representing a wide range of flow rates.

In June 1997, the NVP researchers used a method of measuring growth which caused minimal disturbance to the mussel. Mussels were tagged *in situ* in the intertidal region between 1.2 - 2.0 m above mean lower low water. The tagging sites were a systematically selected subset of a series of mussel study sites, each a 200 m length of shore, that were distributed systematically along the entire shoreline of Herring Bay after the first site had been selected randomly. Mussels >15 mm in shell length were

haphazardly chosen to be tagged. Mussels <15 mm in length were too small to be tagged. Mussels were never densely packed at the tagging sites, therefore intraspecific inhibition of growth through overcrowding was not a factor in the mussel growth. Each mussel received the same type of shellfish tag used by the HBR studies and a 2 x 8 mm plastic, reference strip manufactured as ornamental fly-tying ribbon. Superglue gel was used to affix the numbered tag and reference strip to the mussel. Mussels were tagged in place. The reference strip was placed with its axis along the vector of maximum growth and flush with the posterior edge of the mussel's valve. Growth was measured to the nearest 0.1 mm from the posterior edge of the reference strip to the posterior edge of the shell. A 30 x 30 x 5 cm Vexar (high density polyethylene netting) cage with 3.2 mm mesh was bolted around 25 tagged mussels at each site. Twenty-five mussels were also placed directly next to the cage to test for a cage effect. The tagged mussels were revisited during July 1997 and June 1998 to measure growth using the reference strips and to replace lost mussels. At the end of the experiment, July 1998, the mussels were removed and transported to the laboratory for measuring. Growth was measured using calipers to the nearest 0.1 mm as the difference in length from the umbo to the end of the reference strip and to the posterior edge of the valve.

Statistical Analysis

Three-way analysis of variance was used to test for differences in mean mussel densities between years, oil and control treatments and individual sites. An F-test was used for planned comparisons of density between oiled and unoiled treatments in each year. The Tukey-Kramer *post hoc* test for differences in mean mussel density between years assuming equal variances and with unequal sample sizes was used to test for between-year differences in mussel density (treatments combined). Size-frequency data were grouped into 2 mm size classes and tested for differences between oil and control site pairs 1 and 2 from 1993-1997 using the two-sample Kolmogorov-Smirnov test on percent cumulative size-frequency data. The mussels used in the length-frequency analysis were removed from the quadrats for measurement. These mussels because of their close proximity to one another might not necessarily be expected to grow independently. However, our growth data showed highly variable growth between side-by-side individuals reaching differences as great as 4 mm mo^{-1} (unpublished data) and indicating that the length-frequency data was based on independent measurements. Moreover, because of the large number of individuals required for size-frequency analysis it would have been impractical to select each mussel randomly. Mussels <2 mm were enumerated differently by the two projects and were not included in the density data but were lumped as a size class for the size-frequency data.

Annual growth rates for the HBR studies (1993/94 and 1994/95) were analyzed using two-sample t-tests for comparisons of oiled sites with control sites. The NVP project annual growth data (1997/1998) were analyzed using a two-way analysis of variance between sites and between oil and control treatments. Welch's approximate t-test (for unequal variances and sample sizes) with Satterthwaite's adjusted degrees of freedom was used to test for differences in growth between oiled and control sites of each site pair (Day and Quinn 1989).

Results

Mussel Density

Mussel density showed significant differences between treatments (oil verses control) and years for mussels >2 mm (Table 1) (Figure 3). The highest mussel density on oiled sites occurred in 1994 and the lowest in 1996-97. Significantly greater densities of mussels on oiled sites compared to control sites occurred only in 1994 (two-tailed F-test, $p < 0.01$). The ANOVA revealed only one a significant interaction, year by treatment (Table 1). Therefore between-year differences in mussel density were compared separately at control sites and oiled sites. Densities of mussels on control sites showed no significant between-year differences from 1993 to 1996 (Tukey-Kramer test, $p > 0.05$). Density increased between 1996 and 1997 at control sites (Tukey-Kramer test, $p < 0.05$). Density at control sites was significantly greater than at oiled sites in 1997 (two-tailed F-test, $p < 0.05$). Mean mussel density tended to decrease at oiled sites after 1994, but between-year differences were not significant until 1996. Mussel density at oiled sites in both 1996 and 1997 was less than that at oiled sites in 1994 (Tukey-Kramer tests, $p < 0.01$).

Mussel Size-Frequency Distribution

Percent cumulative length-frequency curves between matched pair oil and control sites were significantly different during 1994, 1995, and 1997 (Table 2) (Figure 4). These differences were consistent in direction over all three years, and showed a greater concentration of individuals among smaller length classes at the oiled sites. The density of mussels >20 mm at all sites was lowest during 1995 and 1996 but all sites experienced strong recruitment of mussels (<5 mm in shell length) during 1996 (Figures 5, 6). The heaviest recruitment of mussels occurred on control site 2 during 1997, the last year of sampling.

Mussel Growth

The HBR studies found significantly higher mussel growth rates on control 2 than oil 2 during 1994/95 (two sample t-test, $p < 0.001$). At site pair 1 the oiled site did not differ from the control in the growth year 1993/94 or 1994/1995. The NVP mussel data for the 1997/98 growth year showed significantly greater growth at control sites 1 and 2 combined than at the oiled sites (Table 3, Figure 7). For that same growth year, further analysis showed that for both site pairs, oil/control 1 and oil/control 2, there was significantly greater growth at the control site than at the oiled site (Welch's approximate t-test with Satterthwaite's adjusted degrees of freedom; oil/control 1, $p< 0.01$, oil/control 2, $p< 0.001$). The mean growth rates obtained by the NVP project were two to three times greater than those found by the HBR studies (Figure 7). This suggests that some aspect of the HBR growth measurement methodology perhaps related to the removal of individuals twice a year may have introduced more stress in the tagged mussels and thereby reduced mussel growth rates compared to those obtained by the NVP project. Caution should be taken when interpreting the low mean growth rate of oiled site 2 during 1997/98 because

of the small sample size (n = 5), owing to heavy mortality of the tagged mussels at the site.

The calcium sulphate dissolution cylinders used by the HBR studies to test for caging effects revealed 4.94 ± 2.71 % (n = 6) less dissolution for caged cylinders. This indicates that there may have been a reduced volume of water, and thus particulates, that flowed past mussels in the cages verses mussels outside the cages. However, the NVP project showed no significant differences in growth between caged mussels and those outside cages (Two-way ANOVA, p = 0.188 with significant heteroscedasticity; Mann-Whitney U-test p = 0.521).

Discussion

Several studies found reduced populations of oiled mussels at various times in western Prince William Sound after the *Exxon Valdez* oil spill (Highsmith *et al.* 1994 and 1996; Houghton *et al*. 1993 and 1994; Babcock *et al*. 1998). Even though the combined data sets for the HBR studies and the NVP project were relatively small and represent only one bay in Prince William Sound, we were able to detect a downward trend in oiled mussel populations after 1994. The most pronounced result was in the reduction of individuals in all size classes between 1994 and 1996-97. Mussel densities reached their lowest point on oiled sites in 1996-97. Recruitment by, mussels <2 mm and the growth of individuals into larger size-classes was evident in 1996 and 1997.

Oiled sites experienced a decline in mussel density after 1994. Although density at control sites was less than that at oiled sites in 1994 a decline comparable to that at oiled sites did not occur at the control sites. Density increased between 1996 and 1997 at control sites. No such increase was observed at oiled sites between 1996 and 1997. Localized oiling and clean-up history on each site may have accounted for the difference in these results. One to two months after the *Exxon Valdez* oil spill the highest concentrations of total polynuclear aromatic hydrocarbons (PAH) were found in mussels suspended in the water column of Herring Bay compared to all other oiled areas (Short and Harris, 1996). Mussels exposed to increasing concentrations of crude oil from 0.12 mg 1^{-1} of 6 mg 1^{-1} showed reduced scope for growth (Gilfillan, 1975). Widdows *et al*. (1987) reported that an order of magnitude increase in mussel tissue concentration of two and three-ring aromatic hydrocarbons can account for an approximately 50% reduction in the growth potential of *Mytilus edulis*. Babcock *et al*. (1998) reported mean total PAH levels in Herring Bay in 1995 were 3,845 μg/g (wet wt.) in surface sediments of mussel beds and 4,191 ng/g (dry tissue wt.) in mussels. This indicates that PAHs were still bioavailable at sites in Herring Bay seven years after the spill, although concentrations were relatively low. Nevertheless, Thomas *et al.* (1999) found that oil-exposed mussels sampled in Herring Bay during 1996 had a significantly lower lethal tolerance (LT_{50}) for air survival than reference groups.

Physical characteristics could in part explain the decline of Herring Bay's mussel population. The HBR studies reported inherent differences between matched oil and control sites due to significantly lower sea surface temperature and salinity on control sites (van Tamelen and Stekoll 1996). It was also discovered using satellite photos during the winter months of 1989 and 1990 that the control sites can experience ice scour (van Tamelen and Stekoll 1996). The combination of temperature, salinity, and ice scour could

explain the lower densities of mussels found on control sites during 1994. During 1996 Prince William Sound experienced a strong El Niño, with sea surface temperatures frequenting 15°-17°C (unpublished data C-LAB Mid-sound buoy, Univ. of Alaska, Institute of Marine Science). This warming event was not catastrophic for *Mytilus trossulus* which has been reported to survive in temperatures ranging from –2°-16°C in the Port of Valdez (Blanchard and Feder 1997) but it may have contributed to higher than normal mortalities resulting in the lowest mussel density recorded on oiled sites in 1996-97. An indirect impact of a warming event may be a change in predation pressure. Sanford (1999) found that a slight decrease in water temperature dramatically reduced the effects of the sea star *Pisaster ochraceaus* on its principal prey *Mytilus californianus* and *Mytilus trossulus*.

Predation may have acted differentially on oil and control sites in Herring Bay. Two species of *Nucella*, *N. lima* and *N. lamellosa*, inhabit the intertidal region in Herring Bay. The HBR studies and the NVP project reported whelks and asteroids were the main predators of *Mytilus trossulus* in Herring Bay. Carroll and Highsmith (1996) conducting research in Kachemak Bay, Alaska, after a lethal winter freeze event, found the whelk, *Nucella lima*, was able to eliminate 60-90% of the mussels at a given site in one season. With *Nucella* present, the mussel population was significantly reduced for over three years. There is evidence of similar forces acting on the mussel population in Herring Bay. The HBR studies reported *Nucella* densities on separate population dynamic sites were approximately 5-10 individuals/m^2 between 1991-1995 (Highsmith *et al*. 1996). Ebert and Lees (1996) found reduced recapture rates in tagged *N. lamellosa* at oiled sites compared to unoiled sites in 1991-92 in Prince William Sound. Ebert and Lees (1996) did not measure *Nucella* density, therefore it is difficult to compare their recapture rate data with the *Nucella* density data of Highsmith et al. (1996) and the present study. Researchers observations also suggest local aggregations of *Nucella* at our mussel study sites in September 1994 (S. Saupe, personal observation). The NVP project reported a mean *Nucella* density of 1.2/m^2 throughout Herring Bay during 1996 and 2.4/m^2 in 1997 (Holland-Bartels *et al*. 1998). The higher densities of *Nucella* from 1992-1995 and their observed aggregations at our mussel study sites in 1994 suggest predation may have been a factor in the reduction of mussels on control sites compared to oiled sites in Herring Bay.

Conclusions

Mussels at oiled sites in Herring Bay during 1993-1997 had not recovered, possibly from a combination of disturbance events. Some residual effect of the oil spill in Herring Bay in combination with predation and possibly warming of sea surface temperatures may have been responsible for the continued decline in mussel abundance at oiled sites for more than eight years after the spill. Although similar decreases in mussel density between 1993 and 1997 have been reported by other investigators over the same period of time as our study, we do not know the complete geographical extent of this trend. *Mytilus trossulus* is the third most abundant taxon found in Prince William Sound's intertidal region (Lindstrom 1999) and a major food source for sea birds and sea otters. A long-term reduction of mussels could have a detrimental effect on their higher order consumers.

Acknowledgments

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Table 1. Three-way analysis of variance of *Mytilus trossulus* densities (no./m sq.) between years (1993-1997), sites and treatments (oiled [O], control [C]) for Herring Bay.

	DF	MS	F Value	P
Year	4	7.6×10^{7}	3.554	0.009
O/C	1	1.2×10^{8}	5.583	0.020
Site	1	1.4×10^{6}	0.076	0.797
Year * O & C	4	1.0×10^{8}	4.665	0.002
Year * Site	4	4.1×10^{6}	0.188	0.944
O & C * Site	1	1.8×10^{6}	0.087	0.769
Year * O & C * Site	4	3.0×10^{6}	0.141	0.967
error	106	2.1×10^{7}		

Table 2. Two-sample Kolmogorov-Smirnov statistics of *Mytilus trossulus* percent cumulative length-frequency data for oil and control (O/C) site pairs 1 and 2.

	O/C 1		O/C 2	
Year	D	P	D	P
1993	0.0581	ns	0.1401	ns
1994	0.4089	<0.001	0.0847	<0.001
1995	0.2142	<0.05	0.2781	<0.01
1996	0.0172	ns	0.2727	ns
1997	0.1936	<0.05	0.6493	<0.001

Table 3. Two-way analysis of variance of *Mytilus trossulus* 1997/98 annual growth rates (mm/mo) on oiled and control site pairs 1 and 2 in Herring Bay. O/C = oiled/control treatments.

	DF	MS	F Value	P
Site	1	9.997	19.039	<0.001
O/C	1	18.228	34.716	<0.001
Site * Oil	1	3.817	7.270	0.008
error	110	0.525		

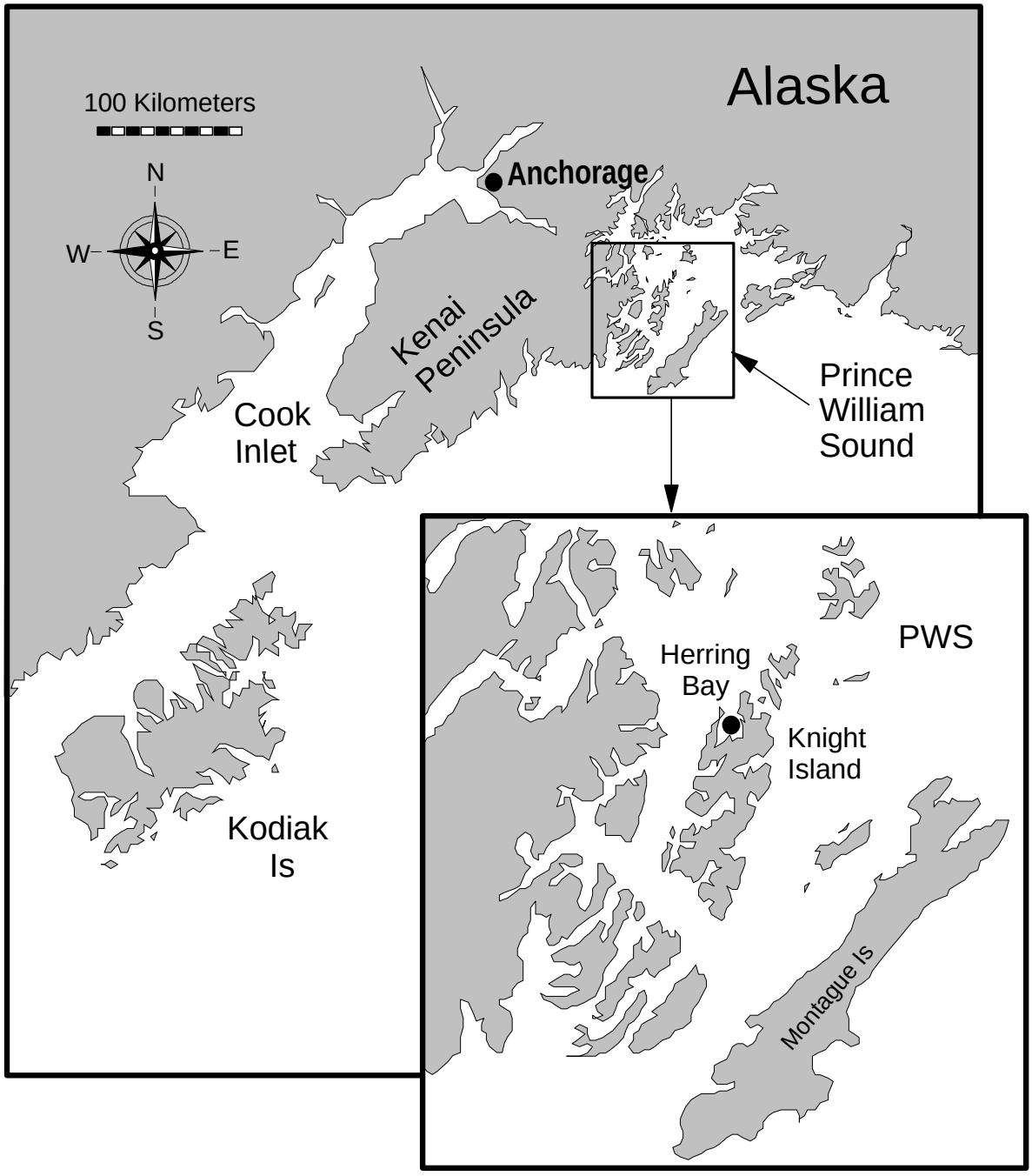

Figure 1. Map showing the location of Prince William Sound, Alaska, and Herring Bay.

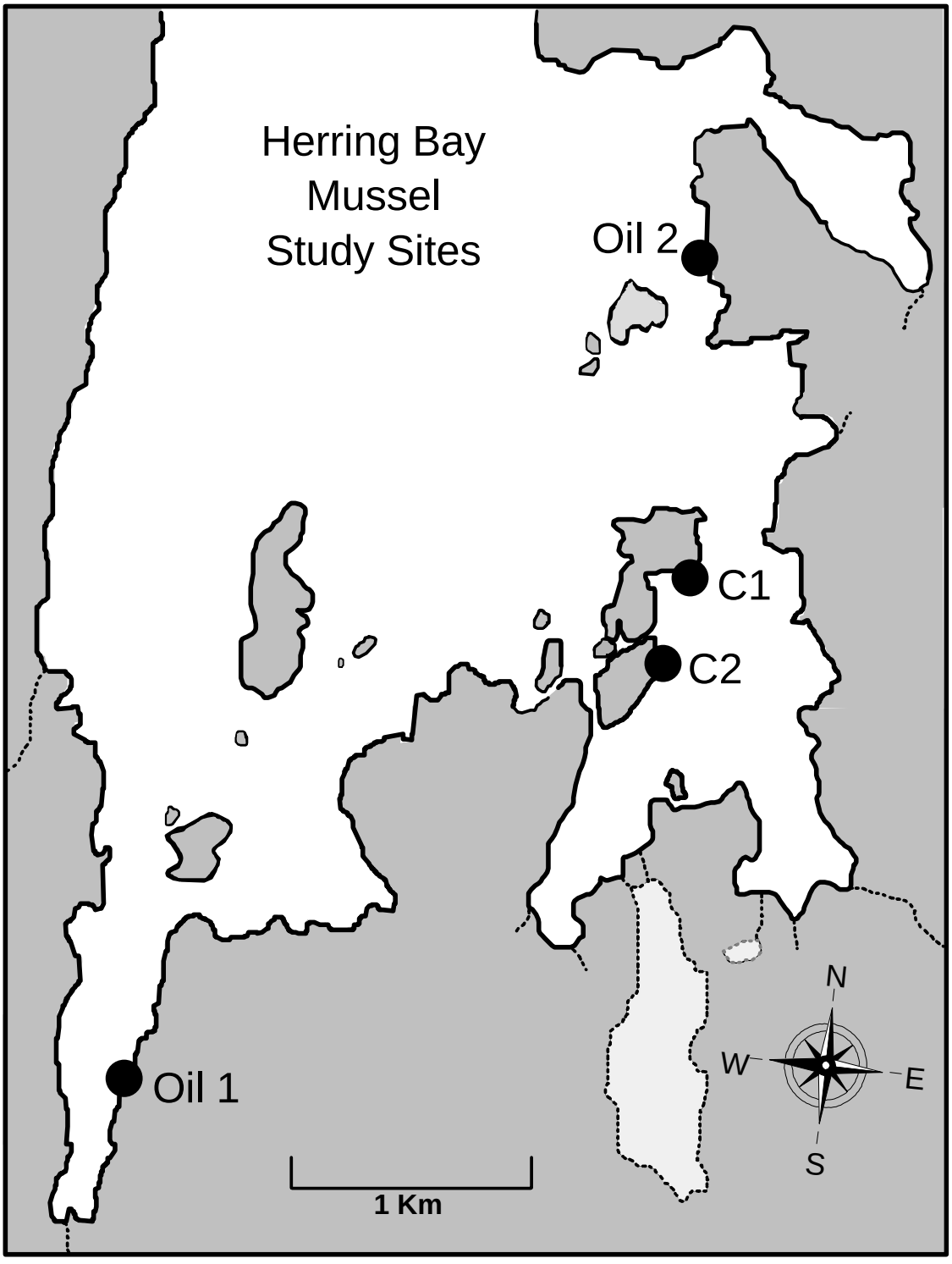

Figure 2. Map of oil and control site pairs 1 and 2 in Herring Bay, Prince William Sound, Alaska.

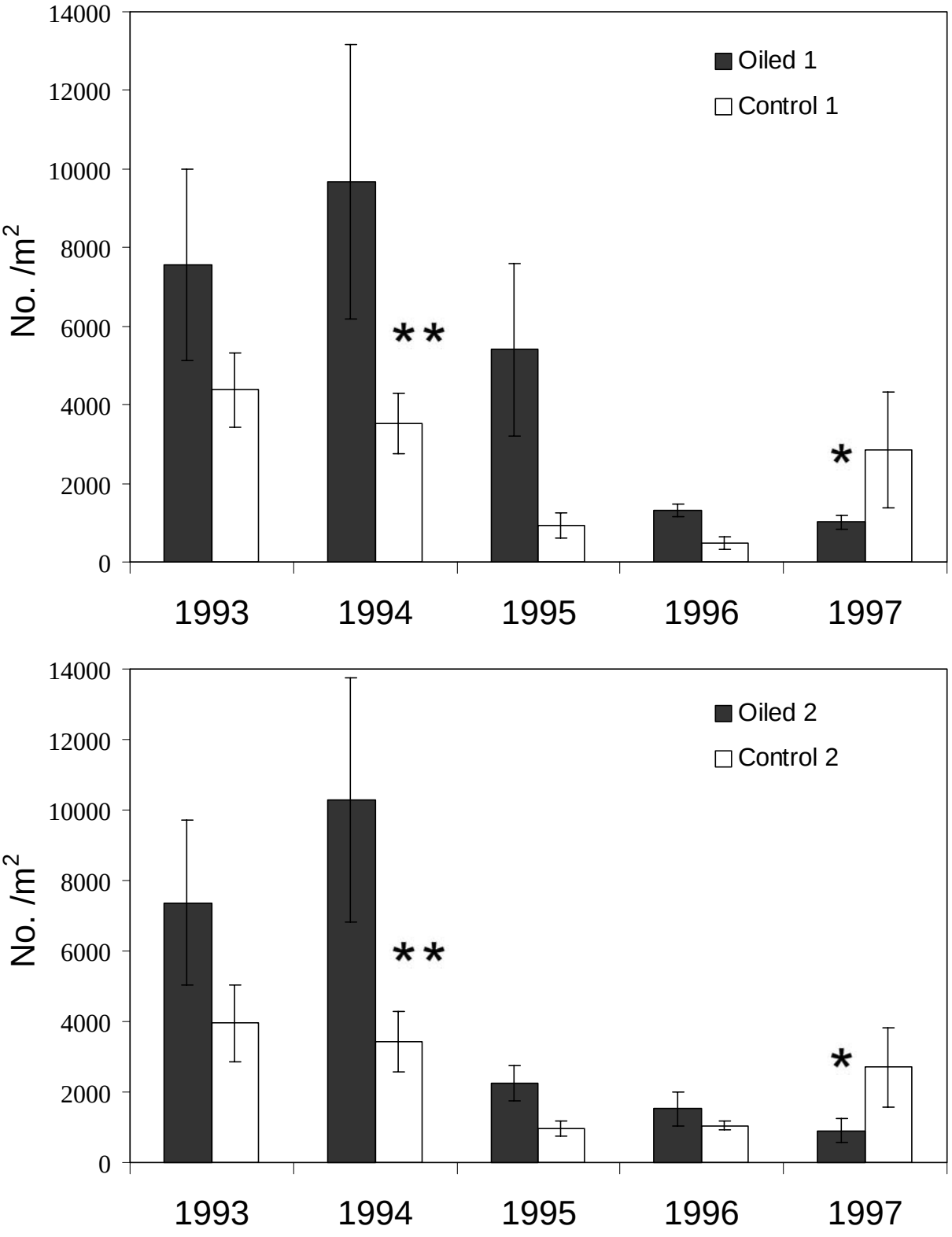

Figure 3. *Mytilus trossulus* densities (no./m^2) between 1993 and 1997 for oiled and control site pairs 1 and 2 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project. The asterisk indicates significance between oiled and control sites 1 and 2 combined (**, $p < 0.01$;*, $p < 0.05$).

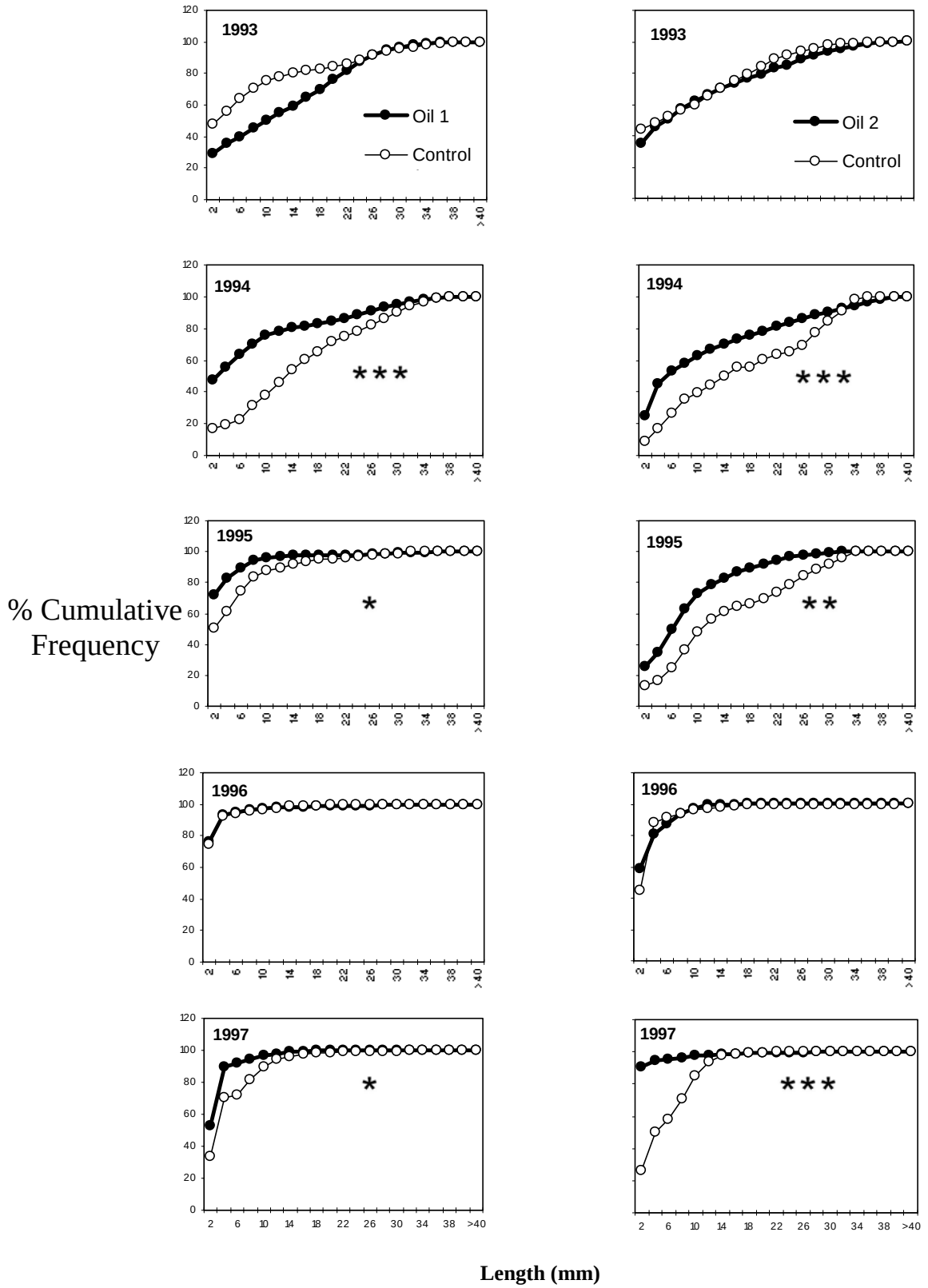

Figure 4. Percent cumulative size-frequency curves of *Mytilus trossulus* for oil and control site pairs 1 and 2 from 1993-1997 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project (***, $p < 0.001$;**, $p < 0.01$;*, $p < 0.05$).

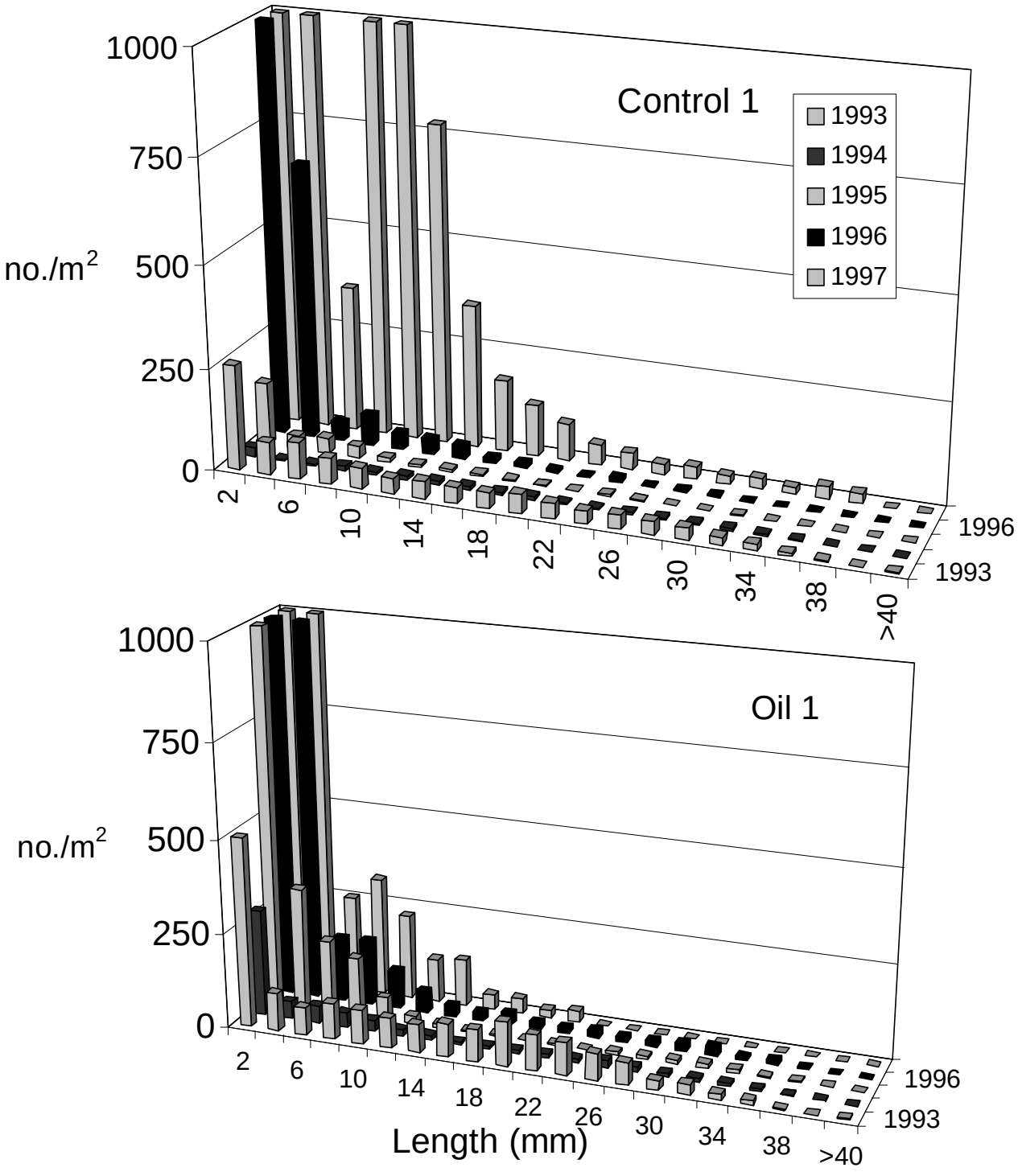

Figure 5. Size-frequency distribution of *Mytilus trossulus* for oil and control site pair 1 from 1993-1997 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project.

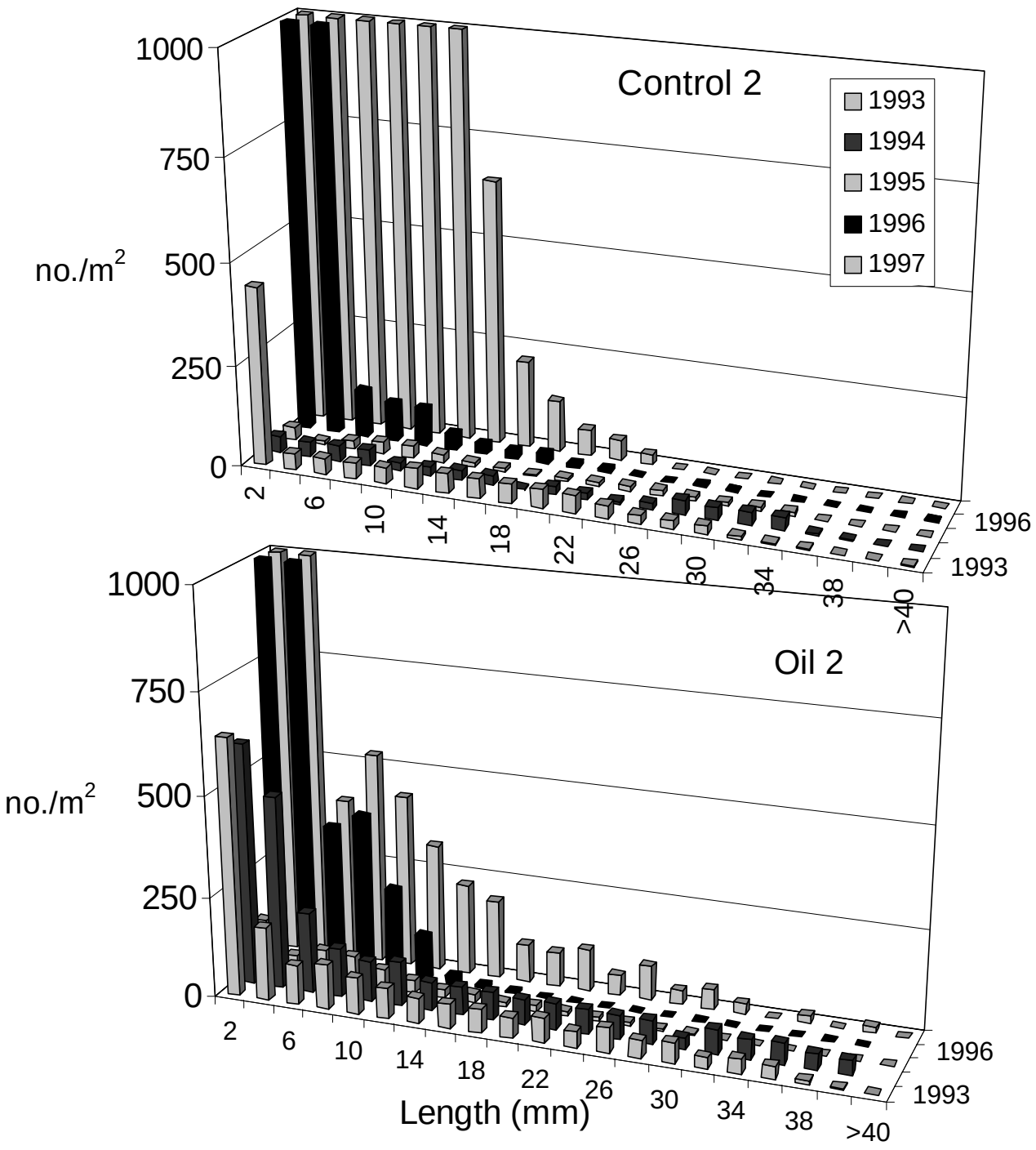

Figure 6. Size-frequency distribution of *Mytilus trossulus* for oil and control site pair 2 from 1993-1997 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project.

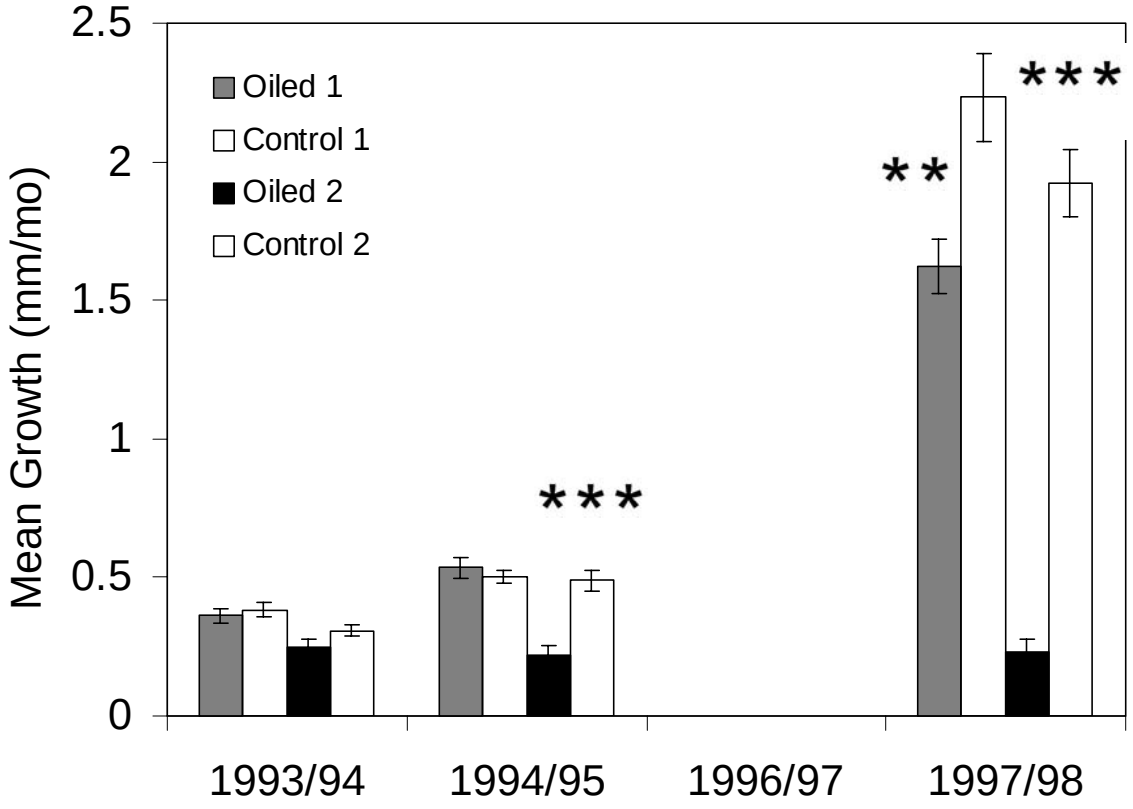

Figure 7. Mean annual growth (mm/mo) of *Mytilus trossulus* for oiled and control site pairs 1 and 2 in Herring Bay (***, $p < 0.001$;**, $p < 0.01$). Growth years 1993/94 and 1994/95 data were from the HBR studies. Growth year 1997/98 data were from the NVP project.

APPENDIX SO-04

Comparison of Age-length and Growth-increment General Growth Models of the Schnute Type in the Pacific Blue Mussel, Mytilus trossulus Gould[1]

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Comparison of Age-length and Growth-increment General Models of the Schnute Type in the Pacific Blue Mussel, Mytilus trossulus

Joshua Millstein and Charles E. O'Clair

Abstract

Models of Mytilus growth, based mostly on length-at-age data, have typically taken the form exemplified by the von Bertalanffy or Gompertz formulations. We examined growth in the Pacific Blue Mussel, Mytilus trossulus, in Prince William Sound, Alaska. Mussels were tagged with individually numbered, plastic tags and a plastic reference marker was glued at the posterior edge of the shell. The mussels were tagged at 13 sites in July 1997 and were collected in July 1998. Age was determined from surface growth rings on the shell, and shell length at maximum annulus was measured. Annual deposition of the growth rings was verified through radial sections of mussel valves, aided by acetate peels, in conjunction with in situ annual growth measurements. Growth-increment was measured from the reference marker to the posterior edge of the shell. Growth was modeled with the Schnute general growth model for age-length data which provides a convenient analytical method for selecting among all previously published growth models. An analog of the Schnute model designed for mark-recapture data (ie growth-increment data) was used to model growth measured in situ on the tagged mussels, as well as growth between the outermost annuli on mussel shells. Bootstrap confidence intervals were obtained for all parameters of the model and for model predicted lengths at each annulus. Confidence intervals of the between-annuli growth-increment model overlapped those of the age-length model at all annuli when growth over the entire range of ages in the population was estimated. Differences in growth model parameters between the age-length model and the mark-recapture analog could be accounted for solely by inherent differences in age-based versus length-based models. Growth estimates generated from between-annuli measurements were equivalent to growth estimates obtained from mark-recapture measurements of annual growth.

Keywords: Growth, Mytilus trossulus, Pacific Blue Mussel, Schnute Model, von Bertalanffy Model

1. Introduction

Accurate models of individual growth are fundamental to reliable estimates of secondary production. Growth is often estimated by relating the length of an individual to its age, which in bivalve mollusks is usually determined by examination of disturbances in the valves caused by changes in seasonal, tidal or circadian accretionary growth. Aging bivalves has usually involved counting rings on the shell surface produced during periods of greatly reduced or suspended growth, usually during winter months at higher latitudes (Haskin, 1954; Lubinsky, 1958; Seed, 1969; Andrews, 1972; Theisen 1973; Seed and Richardson, 1990). However, in mussels it is often necessary to examine growth lines in radial sections of the shell to obtain accurate and reliable estimates of age (Lutz, 1976; Thompson, 1984; Anwar et al., 1990). Improved resolution of growth lines can be achieved by inspection of acetate peels of shell sections (Rhoads and Pannella 1970).

Mytilus growth, usually measured as an increase in shell length with age, has typically been modeled with the von Bertalanffy or Gompertz formulations (Seed and Suchanek, 1992). These formulations assume that growth is determinate and, therefore, ceases at some fixed adult size. Asymptotic growth may not always be realized in the life span of Mytilus (Seed, 1980; Gardner and Thomas, 1987). Schnute (1981) proposed a general size-at-age growth model that incorporates the von Bertalanffy and Gompertz formulations as well as many others as submodels. The model has four (or fewer) parameters, the estimates of which are almost invariably statistically stable. Special cases of the model include not only asymptotic growth, but also linear, quadratic, or exponential growth. A model analogous to the Schnute model proposed by Baker et al. (1991) allows the use of growth increment data from mark-recapture studies to model growth if one of the parameters, usually the starting age, is specified beforehand. Although it would seem of value to employ growth-increment models to validate age-length estimates of growth, Francis (1988) cautions that age-length models are age-based whereas growth-increment models are length-based, and therefore the two techniques describe different population parameters. However, comparison of growth models that use between-annuli, growth-increment measurements with those that use growth-increment measurements from actual mark-recapture data could circumvent this problem. Once validated, between-annuli growth-increment measurements can serve as a surrogate for growth-increment measurements from mark-recapture methodology and can therefore reduce field time. Mark-recapture methods require the expenditure of time and resources to mark individuals, suffer from the risk of data loss from the mortality of marked individuals or tag loss, and require at least two visits to the field site over the course of at least one year to monitor growth. The annulus growth-increment method requires only one visit to the field when mussels are collected or measured in the field. The method also allows for the comparison of historical growth rates using growth-increment data from geologically preserved assemblages of shells.

We report here the results of a comparison of the length-at-age and growth-increment versions of the Schnute model applied to the same collection of mussels (Mytilus trossulus Gould 1850) from Prince William Sound, bordering the Northern Gulf of Alaska. We address the problem of using models based on mark-recapture data to validate age-length estimates of growth with the aid of a growth-increment model based on interannular distances on mussel shells. To our knowledge this is the first attempt to model growth in any

invertebrate by comparing size-at-age and size-increment versions of the same general growth model in the same population.

2. Methods

2.1 Tagging

Mussels, Mytilus trossulus, were tagged in situ in the intertidal region on the shores of Montague Island and Knight Island bordering Montague Strait, Prince William Sound, Alaska (Figure 1). Tagging sites were a systematically selected subset of a series of mussel study sites, each a 200-m length of shore, that were distributed systematically along the shoreline of the two islands after the first site had been selected randomly. Mussels were tagged between 1.2 and 2.0 m above mean lower low water at eight sites on Montague Island and five sites in Bay of Isles, Knight Island. To minimize the stress on the mussels associated with the common practice of removing them from the substrate, transporting them to a central location for tagging and then returning them to the site of collection and caging them until they reattach themselves to the substrate, and to avoid caging the mussels for a long period of time (which may affect growth), mussels were tagged, in place with minimal disturbance and were not caged. An individually numbered, flexible, polyethylene shellfish tag (Hallprint Pty. Ltd., Holden Hill, South Australia) was glued to a pre-dried spot on one valve of each mussel using superglue in gel form. The spot on the shell was usually dried by sequentially wiping with an alcohol wipe and a dry towel. If necessary, compressed air in a can was gently blown on the spot after it was swabbed with the alcohol wipe. A reference strip consisting of a 2 x 8 mm length of plastic, ornamental, fly-tying ribbon was also glued to the same valve. The reference strip was glued in place with its long axis along the vector of maximum growth of the valve and its beveled posterior edge sloped toward and flush with the posterior edge of the valve.

The mussels were collected approximately one year after they were tagged and were transported to the laboratory where they were shucked and the valves separated. Tag growth-increment, defined as the difference between length at collection (distance from umbo to posterior edge of valve along the vector of maximum growth) and length at tagging (distance from umbo to posterior edge of reference strip), was measured to the nearest 0.1 mm on the tagged valve of 110 surviving mussels. The mussels ranged in initial length from 16.5 to 43.4 mm. We tagged no mussels less than 16 mm in maximum shell length because of the mechanical difficulty of gluing tags and reference strips to small mussels. Mussels exceeding 40 mm in shell length were rare in our study area.

2.2 Aging and between-annuli length-increments

To relate the rings on the surface of the shell of Mytilus trossulus to the growth lines that are formed annually in the nacreous layer of the shell (Lutz, 1976) we made radial sections of the valves of 90 Mytilus collected in our study areas on Montague Island and Knight Island in 1996. As broad a size range of mussels as possible was selected haphazardly from samples of mussels collected at four to six sites chosen at systematic intervals along the shore within each study area. The mussel shells were prepared for aging using a method modified after Rhoads and Pannella (1970). All tissue was removed from the mussel shell, and the valve with the least erosion was selected for aging. The valve was washed with

detergent to remove any lipid residue, dipped briefly in a 1.0 % HCL-water solution, rinsed immediately with freshwater, and dried. Each valve was imbedded in a block of epoxy resin (Epofix; Struers, Westlake, Ohio). A radial section of the imbedded valve was made using a precision cut-off machine (Accutom-2) with a diamond blade. The section was made perpendicular to the surface of the valve along a plane that bisected the umbo and the posterior edge of the valve (the vector of maximum growth). The cut valve surface was wet-polished sequentially with 3600, 6000 and 12000 grit polishing paper on a grinding wheel and then buffed with 0.05 μm Alumina buffing powder on a felt buffing cloth on the wheel. The polished surface was etched with a 1 % HCL-water solution for 105 sec., then immediately blotted dry with paper wipers. The etched shell surface was flooded with acetone and a piece of sheet acetate 0.5 mm thick was applied to the etched surface. The acetate peel was removed from the shell surface after 6.0 min. and examined for growth lines under a compound microscope with phase contrast optics.

The increased resolution of the growth lines from the acetate peels allowed growth lines visible in the umbo to be followed through the nacreous and prismatic layers to the surface of the valve where they emerged as annuli (Figure 2). The strength of and the distance between the surface annuli varied with site, tidal height and age of mussel. In heavily eroded shells, erosion in the umbonal region rendered identification of the first annulus difficult. However, remnants of the surface annulus were usually present at the margins of the valve (Figure 2).

After we developed our aging technique by establishing the relationship between the annually-formed nacreous growth lines and surface annuli in the 90 sectioned shells, we aged the untagged valves of the 110 tagged mussels using surface annuli alone. Three workers independently aged all 110 untagged valves. Each mussel was aged and the length to the last annulus was measured to the nearest 0.1 mm with a digital caliper. To avoid confusion arising from the variation in ages of juvenile mussels at the start of their first winter, age was expressed as number of annuli instead of years. We also measured the difference between length to the last annulus and length to the next to the last annulus (termed here annulus growth-increment) on the untagged valve. Studies of bivalve growth often use data that include a series of length measurements to consecutive annuli for each bivalve. With this approach the successive measurements on each shell are not independent and cannot be treated statistically unless a technique for repeated measures is used. To ensure independence in each data set here, only one measurement was made on each mussel in each version of the growth model.

To compensate for the lack of tagged mussels less than 16 mm in shell length, we randomly selected 18 mussels from the 110 tagged survivors to measure growth in the first two years of life. In nine of these mussels we measured the distance from the umbo to the first annulus for the age-length analysis and the distance between the first and second annulus for the annulus growth-increment analysis. Similarly, in the remaining nine mussels we measured the distance from the umbo to the second annulus for the age-length analysis and the distance between the second and third annulus for the annulus growth-increment analysis. We present models fitted to data with and without these substituted measurements.

2.3 Growth models

2.3.1 Age-length model

Schnute's (1981) general growth model with four parameters was fit to the age-length data. The Schnute age-length model takes the form

$$Y(t) = \left[y_1^b + (y_2^b - y_1^b) \frac{1 - e^{-a(t-\tau_1)}}{1 - e^{-a(\tau_2-\tau_1)}} \right]^{1/b}$$

under case 1 where Y(t) is length Y at age t, y_1 and y_2 are lengths at ages τ_1 and τ_2. Parameters τ_1 and τ_2 are ages that are chosen and fixed by the biologist, usually among the youngest and oldest ages of observed animals. Parameters a and b define the shape of the curve and are unequal to zero under case 1. The parameters, a, b, y_1, and y_2 (or a subset of these in the submodels) are found by minimization of the sum of squares (S)

$$S = \sum_{i=1}^{n} [\hat{Y}_i - Y_i(y_1, y_2, a, b)]^2$$

based on an additive error assumption and where $\hat{Y}_i$ is the observed length at age t_i and Y_i is the length at age t_i predicted by the model. The multiplicative error structure is usually selected if variability about a fitted growth curve increases (Quinn and Deriso 1999) with increasing length. An additive error assumption was used here, because variability in the response did not consistently increase with the predictor. In fact, variability in the growth increment data often tended to decrease with mussel length. Baker et al. (1991) found that parameter and standard error estimates for their data were not greatly affected by the choice between additive and multiplicative error structures. They also questioned the stability of the multiplicative model after experiencing difficulties obtaining consistent parameter and standard error estimates.

Cases 2, 3, and 4 are submodels of case 1 with 3, 3, and 2 parameters, respectively, that define situations where b = 0, a = 0, and a = b = 0 (see Appendix A for equations). The case 1 model with a > 0 and b fixed at 1 reduces to the von Bertalanffy model with a equal to the von Bertalanffy parameter k. The case 2 model with a > 0 is equivalent to the Gompertz model (see Schnute, 1981 for a discussion of these and other submodels). Initially, the four parameter case 1 model is fit to the data. If the parameters appear to approach limiting values that correspond to another case, then the appropriate submodel is selected with the aid of an F-test of the variance ratio

$$F = \frac{\left(\frac{RSS_j - RSS_i}{df_j - df_i} \right)}{R} MS_i$$

which is approximately F-distributed when the sample size is large (Schnute 1981). Two submodels, case I and case j, have residual sums of squares RSS_i and RSS_j with df_i and df_j

degrees of freedom. RMS_i is the residual mean square of case I, where case I is the submodel with the greater number of parameters. We rejected the null hypothesis that the submodels were the same at the $p = 0.05$ level if the F-statistic was greater than $F_{0.05}(df_i - df_I, df_i)$. If two submodels had the same number of free parameters, and if both submodels were determined by the F-test to be more parsimonious (ie. had fewer parameters) than the four parameter case 1 model, then the submodel with the smallest residual sum of squares was chosen.

2.3.2 Growth-increment model

Baker et al. (1991) developed a growth model for mark-recapture data analogous to the Schnute (1981) model that incorporates size at marking, length of time at large, and size at recapture. Because absolute age is not included in the data set, the model can only predict size at relative age. The biologist must supply one parameter, usually size at initial age (y_1), decreasing the number of estimated parameters in the Schnute (1981) four-parameter model to three. Here we obtained this parameter by taking the mean y_1 from the age-length results. The case 1 analog to the Schnute model takes the form

$$Y_r = \left[Y_m^b e^{-a(t_r - t_m)} + (y_2^b - y_1^b e^{-a(\tau_2 - \tau_1)}) \frac{1 - e^{-a(t_r - t_m)}}{1 - e^{-a(\tau_2 - \tau_1)}} \right]^{1/b}$$

where Y_m and Y_r are sizes at t_m, age at marking, and t_r, age at recapture, respectively (cases 2, 3, and 4 are given in Appendix B). The parameters, a, b, y_1, y_2, τ_1, and τ_2 are the same as those in the Schnute (1981) age-length growth model. Standard errors and confidence limits were calculated using bootstrap methods (Efron and Tibshirani, 1986).

3. Results

3.1 Precision of age estimation

Aging of the tagged mussels revealed individuals with up to 12 annuli (11+ yr olds) ranging in shell length at last annulus from 16.8 mm to 43.2 mm (Figure 3). The discrepancy in annulus count between agers was one or fewer in 77.2% of the mussels aged, and complete agreement between at least 2 two agers in 85.4% (Table 1). Mean length at annulus differed between agers by less than one standard deviation in almost all cases (Table 2).

Nearly all of the 110 mussels that were tagged and left in situ for one year clearly showed exactly one annulus in the region of new shell growth posterior to the reference marker. Only one mussel showed no distinguishable annulus in the region of new shell growth. Nine mussels showed a single ‘check’, or non-annular shell disturbance in addition to the annulus. These checks could be distinguished from annuli by a discontinuity or a fading-out of the check toward the edge of the valve.

3.2 Age-length model

The case 2 Schnute age-length submodel for the age at length to last annulus (F-test; mussels with three or more annuli) was selected for all three agers in the absence of very small mussels with only one or two annuli in the original group of tagged mussels (Tables 3 and 4). However, the von Bertalanffy submodel (with parameter b fixed at 1 and parameter a free) was also determined by the F-test to be more parsimonious than the case 1 model for all three agers. The RSS's of the von Bertalanffy submodels were greater than the case 2 submodels by only about 0.1% or less, and the RSS 95% confidence intervals were almost coincident. For these data sets the two submodels produce such similar fits that the choice between them based on the RSS was somewhat arbitrary. The 95% confidence intervals for age-length curves overlapped each other at all annuli (Figure 4). When length measurements to the first and second annuli substituted from a randomly selected subset of the tagged mussels were added to the model, the von Bertalanffy submodel was selected for all three agers (Tables 4 and 5). For consistency, all curves presented in Figures 4, 7 and 8 are constructed from the von Bertalanffy submodel.

3.3 Growth-increments

Shell growth in the tagged mussels ranged from 0.4 to 12.8 mm over one year. Growth tended to decrease with increasing initial length (Figure 5). No growth-increment data were available for mussels <16 mm in initial length because none were tagged (Figure 6). The origin of the growth-increment curve was defined by the parameter $\bar{y}_1$, which was the mean y_1 from the appropriate age-length curves. Hence, the growth-increment curve was the predicted length at age relative to the initial length $\bar{y}_1$ at age τ_1. The growth-increment model was applied to both tag growth-increment and annulus growth-increment data. Case 3 of the Schnute model was selected for the tag growth-increment data. The von Bertalanffy submodel was also determined by the F-test to be more parsimonious than the case 1 model, and the RSS was only 0.5% greater than the RSS of the case 3 submodel. Without the augmentation of first and second year growth, the von Bertalanffy submodel was selected for the annulus growth-increment data from one ager, the case 3 submodel was selected for the second ager, and the case 2 submodel was selected for the third ager. The RSS's for the von Bertalanffy fit to the data of the second and third ager were less than 1% more than both other submodels (Tables 4, 6 and 7).

When the growth-increment analog of the Schnute model was fit to the first and second year augmented annulus growth-increment data the von Bertalanffy submodel with $b = 1$ was the best fit for two agers, and the case 1 Schnute model with four parameters was the best fit for the remaining ager (Table 8). Although the fit of the von Bertalanffy submodel to the data of one ager was not more parsimonious than the case 1 model (F-test), the von Bertalanffy RSS was less than one SE greater than the case 1 RSS.

3.4 Model comparisons

Schnute growth curves, fit to age-length data from annuli three to eight for the three agers, fell significantly below the curve generated from tag growth-increment data (Figure 4). The 95% confidence intervals for age-length curves overlapped each other at all annuli but

overlapped those of the tag growth-increment curve only at annulus four. When we fit the Schnute model analog for mark-recapture data to the annulus growth-increment data, the growth curve was much closer to that of the tag growth-increment model (Figure 7). In fact, confidence intervals for the tag growth-increment and annulus growth-increment models overlapped at all five annuli. Comparison of the age-length and annulus growth-increment curves using the augmented data sets revealed that the initial slope of the age-length curve was steeper than that of the annulus growth-increment curve, but that growth in older mussels slowed more rapidly under the age-length model (Figure 8).

4. Discussion

By comparing age-length and growth-increment data from the same group of mussels, we were able to distinguish differences in growth estimates owed to growth estimation technique and aging bias as well as eliminate differences owed to sampling bias. Agreement between annulus growth-increment and tag growth-increment data (Figure 7) indicated that the deposition of surface rings on the shells of Mytilus trossulus at our study sites in Prince William Sound occurred with approximately annual periodicity. The appearance of one new annulus in tagged mussels left in situ for one year, also supports this interpretation. This relationship was validated only for the size range of mussels tagged (16.5 - 43.4 mm), although there is no obvious reason why there would be any difference for mussels outside this size range. The correspondence between growth layers in the shell, which were found to be annually deposited in Mytilus edulis by Lutz (1976), and surface annuli is also consistent with the interpretation of annual deposition of surface rings (Figure 2). This implies that most of the differences between the age-length and tag growth-increment curves can be attributed to differences in the growth estimation techniques. If growth rate varies with age (and it seems reasonable to assume that older mussels grow more slowly than equally-sized, younger mussels), then size-specific growth rate estimates will be influenced by year-class strength. Effectively, a growth curve based on length-increment data will be weighted by year-class strength for year-classes with overlapping size ranges. Such a model should generate a less biased prediction of growth based on the length of an individual from the same population than a model constructed from age-length data, because growth rate at a given length will be weighted properly to reflect the age distribution of the population. Indirectly, this type of model could also provide a less biased estimate of age from length (if y_1 is chosen correctly), because factors such as year-class strength that could influence the probability of an individual of a given size being a certain age would be incorporated into the model. Alternatively, to avoid bias when estimating age from length with age-length data, the growth model used must be structured to have length as the predictor and age as the response. Conventional growth curves constructed from age-length data will not be affected by year-class strength, but instead will be influenced by size-selective mortality as described by Lee's phenomenon (Lee, 1912; Ricker, 1958). Hence, these models should generate less biased estimates of length from age than curves constructed from growth-increment data. Age (really τ_1 + time-at-large) is still an appropriate independent variable in a mark-recapture growth model because time is under the control of the investigator.

The annulus growth-increment curves allow us to compare the tag growth-increment data to growth data obtained from surface annuli, and to avoid the difficulties discussed above. The only difference between the annulus growth-increment curve and the tag growth-

increment curve should have been caused by the difference in the environmental regime in the time periods during which growth occurred. It is clear from Figure 7 that these curves were in fact very close. Annulus growth-increment data are also more powerful for studying temporal and year-class effects on growth than age-length data, because the growth estimates are based on a more restricted period of time.

If age is a better predictor of growth rate than length, we should expect growth rate to be more sensitive to changes in age than changes in length. In Figure 8 changes in slope of the annulus-increment curves represent changes in growth rate with mussel length, but changes in slope of the age-length curves represent changes in growth rate with mussel age. The greater degree of curvature in the age-length curves may reflect the greater sensitivity in growth rate to age than to length. At annuli one through three the growth rate from the age-length model was greater than that from the annulus growth-increment model, but the reverse was true for greater ages. This difference should occur if growth rate estimates for older individuals from the annulus growth-increment model have an upward bias owing to the presence of young, large, fast-growing mussels in the population, while growth rate estimates for younger individuals have a downward bias caused by the presence of old, small, slow-growing mussels.

The Schnute model provides an added level of information about the general shape of the growth curve because it does not constrain the curve by assumptions like asymptotic size, thereby allowing the curve to conform more closely to the actual data. Conventional parameters such as l_∞ or t_0 can still be calculated if they exist (see Schnute 1981 for formulae). Comparisons between submodels can be made more directly. Our initial choice of the Schnute growth model over the von Bertalanffy submodel, for example, seems justified because it allowed us to compare the fit of many other submodels and to reach a final decision based on the character of the data itself. Selection of the von Bertalanffy submodel for the age-length and annulus growth-increment data in most cases where $\tau_1 = 1$ as well as the relatively good fit for the remaining data sets suggests that the use of this model to construct growth curves may be generally appropriate for mussels (Tables 4, 5 and 8).

5. Conclusion

This study revealed that the growth rings on the outer surface of the shell of Mytilus trossulus in Prince William Sound, Alaska were deposited annually. The Schnute general growth model for age-length data provided a convenient analytical method for selecting among all previously published growth models. An analog of the Schnute model designed for mark-recapture data (ie growth-increment data) was useful for modeling growth of tagged mussels measured in situ in Prince William Sound, as well as growth between the outermost annuli on mussel shells. The von Bertalanffy submodel was the most appropriate general model for our data. This result supports the common tendency by previous workers to use the von Bertalanffy model for mussel growth. Comparison between age-length and tag growth-increment models, with the entire range of ages in the population considered, revealed that the accumulation of positive and negative differences in growth rate tended to balance out over the lifetime of the mussel resulting in similar length predictions of both models for the older individuals. Differences in growth model parameters between the age-length model and the mark-recapture analog could be attributed solely to inherent differences in the estimation techniques. These discrepancies can be avoided by replacing age-length data with annulus

growth-increment data, which generates growth estimates equivalent to estimates from mark-recapture data, to construct growth curves.

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Table 1. Level of agreement and discrepancy in number of annuli counted by three agers on 110 tagged mussels, Mytilus trossulus, from Prince William Sound.

Category	No. of Mussels	% of Total
Level of Agreement[1]		
3	27	24.5
2	67	60.9
0	16	14.5
Discrepancy between agers[2]		
0	27	24.5
1	58	52.7
2	21	19.1
3	3	2.7
4	1	0.9

[1]Number of agers (of three total) agreeing on the number of annuli on each mussel shell after independent counts.
[2]Number of annuli by which agers differ on each mussel shell.

Table 2. Mean length (mm) at annulus of tagged mussels, Mytilus trossulus, from Prince William Sound. Mean length to last annulus is shown for mussels with three or more annuli. Mean length to first or second annulus of a randomly selected subset of older mussels is shown for annuli 1 and 2. SD, standard deviation; n, number of mussels.

Annulus	Ager 1			Ager 2			Ager 3		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
1	3.6	1.3	9	4.5	1.6	9	5.1	2.3	9
2	10.6	3.0	9	12.2	2.2	9	11.4	3.7	9
3	20.5	2.4	6	22.2	2.4	6	21.2	3.7	4
4	23.7	4.0	12	22.6	4.4	15	23.9	3.9	12
5	26.6	3.6	22	27.6	3.8	30	27.1	4.8	33
6	29.0	4.4	36	29.3	4.1	37	29.1	4.4	41
7	30.2	5.6	19	34.1	6.5	14	32.9	4.5	13
8	34.5	3.0	9	34.3	4.4	3	31.2	2.5	3

Table 3. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for Schnute case 2 (b = 0) and the von Bertalanffy submodel (LVB1; b = 1) parameters of age-length growth model of tagged mussels, Mytilus trossulus, from Prince William Sound. All tagged mussels had three or more annuli. $\tau_1 = 3$, $\tau_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Ager 1				Ager 2				Ager 3			
	Value	SE	LL	UL	Value	SE	LL	UL	Value	SE	LL	UL
Case 2												
SS	1969	279	1512	2406	1829	233	1404	2178	2127	263	1655	2526
a	0.27	0.10	0.12	0.45	0.25	0.09	0.11	0.42	0.21	0.12	0.03	0.44
y_1	20.4	1.3	18.3	22.5	20.4	1.2	18.2	22.3	21.3	1.4	18.7	23.5
y_2	30.8	0.52	29.9	31.6	32.0	0.58	31.0	32.9	31.7	0.63	30.6	32.7
LVB1												
SS	1972	273	1462	2375	1836	234	1418	2190	2128	261	1646	2513
a	0.20	0.09	0.07	0.37	0.16	0.09	0.04	0.33	0.13	0.12	-0.04	0.34
y_1	20.3	1.34	17.9	22.4	20.3	1.30	18.0	22.2	21.2	1.53	18.6	23.6
y_2	30.8	0.50	29.9	31.5	31.8	0.55	31.0	32.8	31.6	0.63	30.5	32.6

Table 4. Statistical tests (F- test) of the best submodel (case) by ager of the Schnute age-length and analogous annulus growth-increment models, and the best case of the tag growth-increment model for tagged mussels, Mytilus trossulus, from Prince William Sound. LVB1, von Bertalanffy submodel (b = 1); τ_1, age at beginning.

Ager	Model	Case j / Case i	df_j	df_i	F	P
1	Age-length, $\tau_1 = 3$	2/1	107	106	0.07	>0.05
	Age-length, $\tau_1 = 1$	LVB1/1	107	106	0.03	>0.05
	Annulus Growth-incr., $\tau_1 = 3$	LVB1/1	108	107	0.77	>0.05
	Annulus Growth-incr., $\tau_1 = 1$	2/1	108	107	4.38	<0.05
2	Age-length, $\tau_1 = 3$	2/1	106	105	1.06	>0.05
	Age-length, $\tau_1 = 1$	LVB1/1	106	105	0.15	>0.05
	Annulus Growth-incr., $\tau_1 = 3$	3/1	107	106	0.18	>0.05
	Annulus Growth-incr., $\tau_1 = 1$	LVB1/1	107	106	3.88	>0.05
3	Age-length, $\tau_1 = 3$	2/1	107	106	0.09	>0.05
	Age-length, $\tau_1 = 1$	LVB1/1	107	106	0.04	>0.05
	Annulus Growth-incr., $\tau_1 = 3$	2/1	108	107	0.20	>0.05
	Annulus Growth-incr., $\tau_1 = 1$	LVB1/1	108	107	2.52	>0.05
-	Tag Growth-increment, $\tau_1 = 3$	3/1	108	107	0.19	>0.05

Table 5. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for Schnute case 1 parameters with b = 1 (von Bertalanffy submodel) of the age-length growth model of tagged mussels, Mytilus trossulus, from Prince William Sound. Length to first or second annulus estimated from a randomly selected subset of 18 mussels with three or more annuli. $\tau_1 = 1$, $\tau_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Value	SE	LL	UL
Ager 1				
SS	1764	285	1267	2193
a	0.28	0.04	0.22	0.35
y_1	3.5	1.2	1.5	5.3
y_2	30.9	0.47	30.1	31.7
Ager 2				
SS	1482	211	1122	1804
a	0.23	0.04	0.17	0.30
y_1	4.7	1.1	2.8	6.4
y_2	32.0	0.52	31.1	32.8
Ager 3				
SS	1934	252	1478	2314
a	0.24	0.05	0.17	0.33
y_1	4.8	1.2	2.8	6.8
y_2	31.9	0.60	30.9	32.9

Table 6. Parameter value and bootstrap estimates of standard error and 95% confidence intervals of case 3 ($\underline{a} = 0$) and the von Bertalanffy submodel (LVB1; b = 1) of the tag growth-increment model of tagged mussels, Mytilus trossulus, from Prince William Sound. $\bar{y}1$ = mean $\underline{y}_1$ of age-length models (see Table 3), $\underline{\tau}_1 = 3$, $\underline{\tau}_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Value	SE	LL	UL
Case 3				
SS	465	68.0	350	572
$\underline{b}$	2.6	0.37	2.0	3.2
$\bar{y}_1$	20.7	-	-	-
$\underline{y}_2$	35.4	0.67	34.3	36.6
LVB1				
SS	468	75.6	340	588
$\underline{a}$	0.21	0.04	0.14	0.29
$\bar{y}_1$	20.7	-	-	-
$\underline{y}_2$	35.6	0.62	34.6	36.6

Table 7. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for annulus growth-increment (growth between next to the last and last annulus) growth model of tagged mussels, Mytilus trossulus, in Prince William Sound. The model that best fit the data of each ager was: Ager 1, von Bertalanffy submodel (b = 1); Ager 2, case 3 ($\underline{a} = 0$); Ager 3, case 2 ($\underline{b} = 0$). $\bar{\underline{y}}_1$ = mean $\underline{y}_1$ of age-length models (see Table 3), $\underline{\tau}_1 = 3$, $\underline{\tau}_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Value	SE	LL	UL
Ager 1				
SS	366	45.4	288	438
$\underline{a}$	0.21	0.03	0.16	0.27
$\underline{b}$	1.0	-	-	-
$\bar{\underline{y}}_1$	20.7	-	-	-
$\underline{y}_2$	34.8	0.59	33.8	35.8
Ager 2				
SS	343	44.3	267	413
$\underline{a}$	0	-	-	-
$\underline{b}$	2.0	0.2	1.7	2.4
$\bar{\underline{y}}_1$	20.7	-	-	-
$\underline{y}_2$	35.2	0.66	34.1	36.2
Ager 3				
SS	339	43.2	264	408
$\underline{a}$	0.27	0.03	0.22	0.33
$\underline{b}$	0	-	-	-
$\bar{\underline{y}}_1$	20.7	-	-	-
$\underline{y}_2$	34.7	0.58	33.8	35.6

Table 8. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for the Schnute case 1 submodel [von Bertalanffy submodel ($b = 1$) for agers 2 and 3] of the annulus growth-increment model of tagged mussels, Mytilus trossulus, from Prince William Sound. Length to first and second annuli estimated from a randomly selected subset of mussels with three or more annuli. $\tau_1 = 1$, $\tau_2 = 7$. SE, standard error; LL, lower limit; UL, upper limit.

Parameter	Value	SE	LL	UL
Ager 1				
SS	363	45.6	283	430
a	0.30	0.06	0.20	0.41
b	0.41	0.20	0.07	0.72
y_1	4.3	-	-	-
y_2	32.9	0.69	31.7	34.0
Ager 2				
SS	399	50.5	312	478
a	0.14	0.03	0.10	0.19
b	1.0	-	-	-
y_1	4.3	-	-	-
y_2	33.0	0.81	31.7	34.3
Ager 3				
SS	424	59.6	322	521
a	0.15	0.03	0.11	0.19
b	1.0	0	-	-
y_1	4.3	-	-	-
y_2	32.2	0.83	30.7	33.5

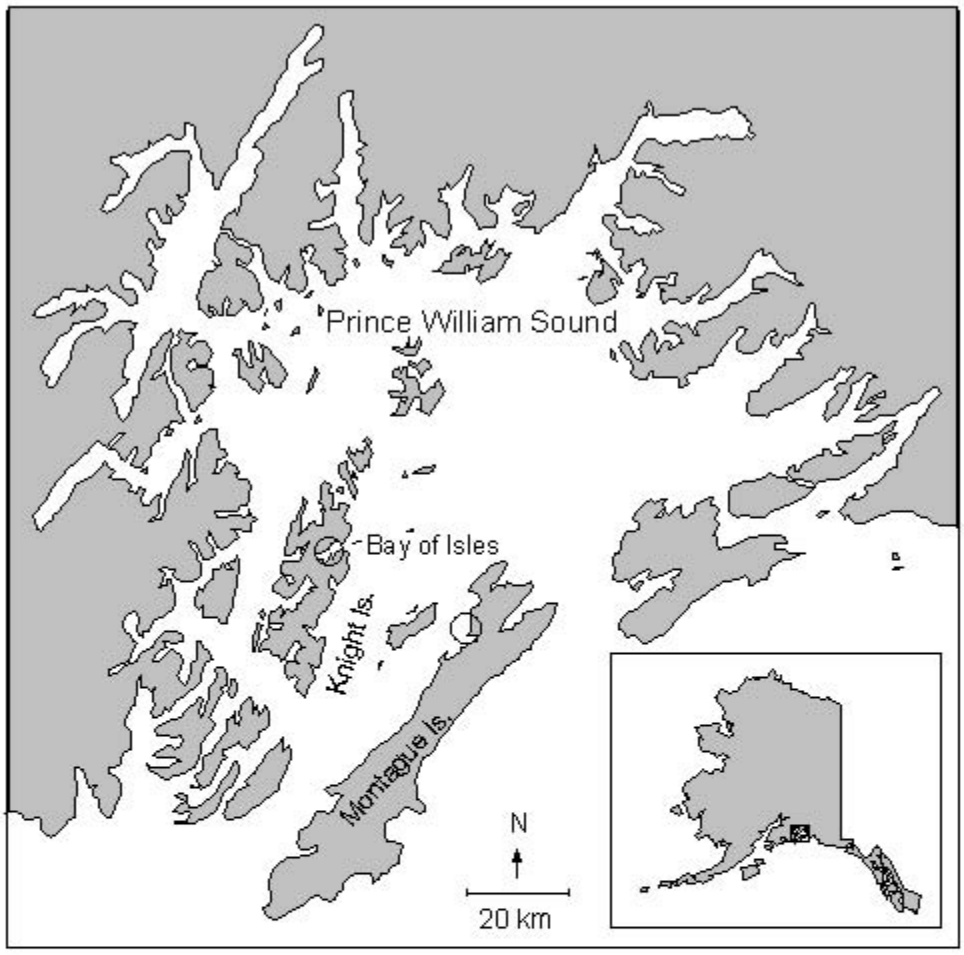

Figure 1. Map of Prince William Sound, Alaska showing study areas (circled).

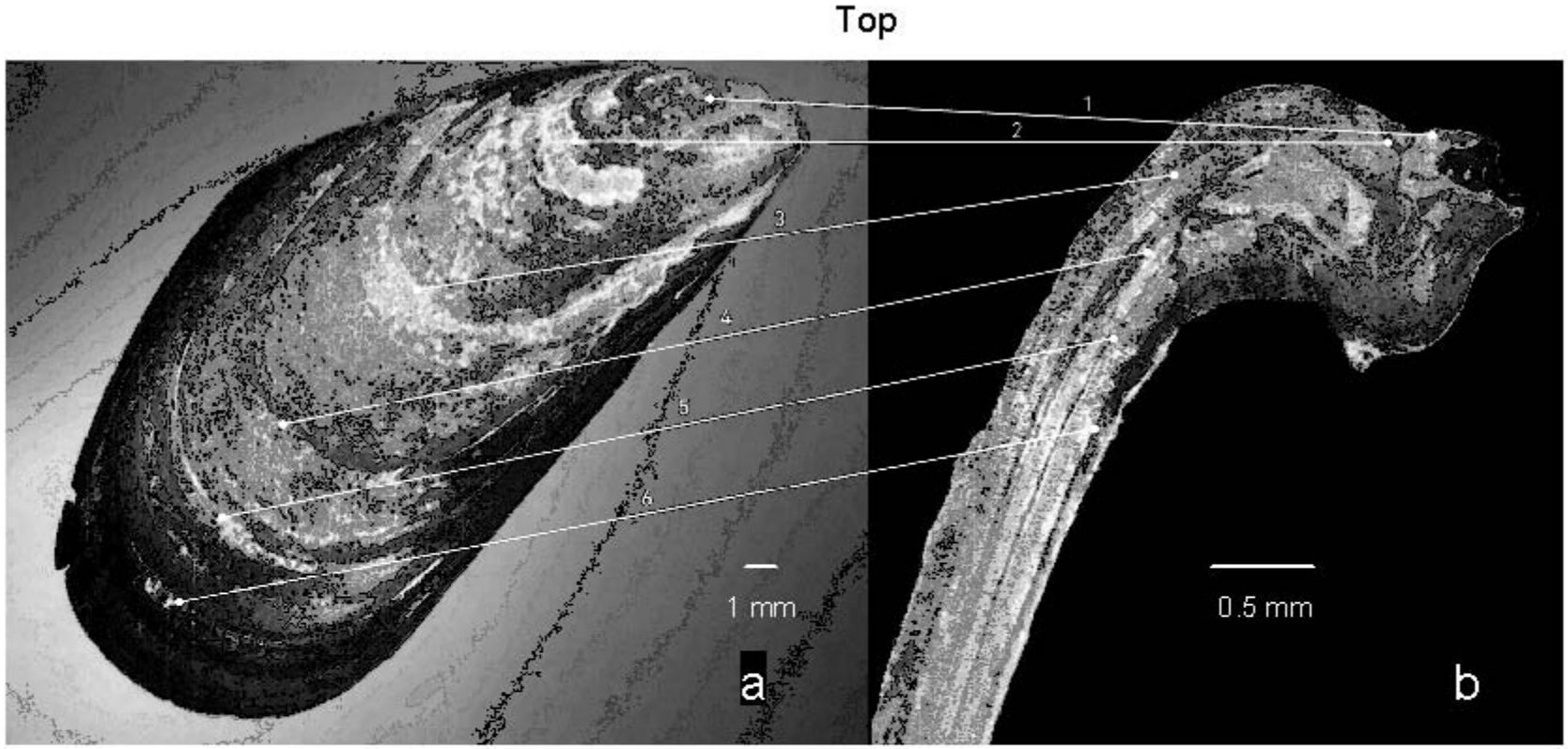

Figure 2. Right valve (a) and acetate peel (b) of radial section through umbonal region of corresponding left valve of a 29.4 mm mussel, Mytilus trossulus, from Prince William Sound. Numbered lines connect annual rings on the valve surface with corresponding growth lines in the inner nacreous layer of the sectioned valve.

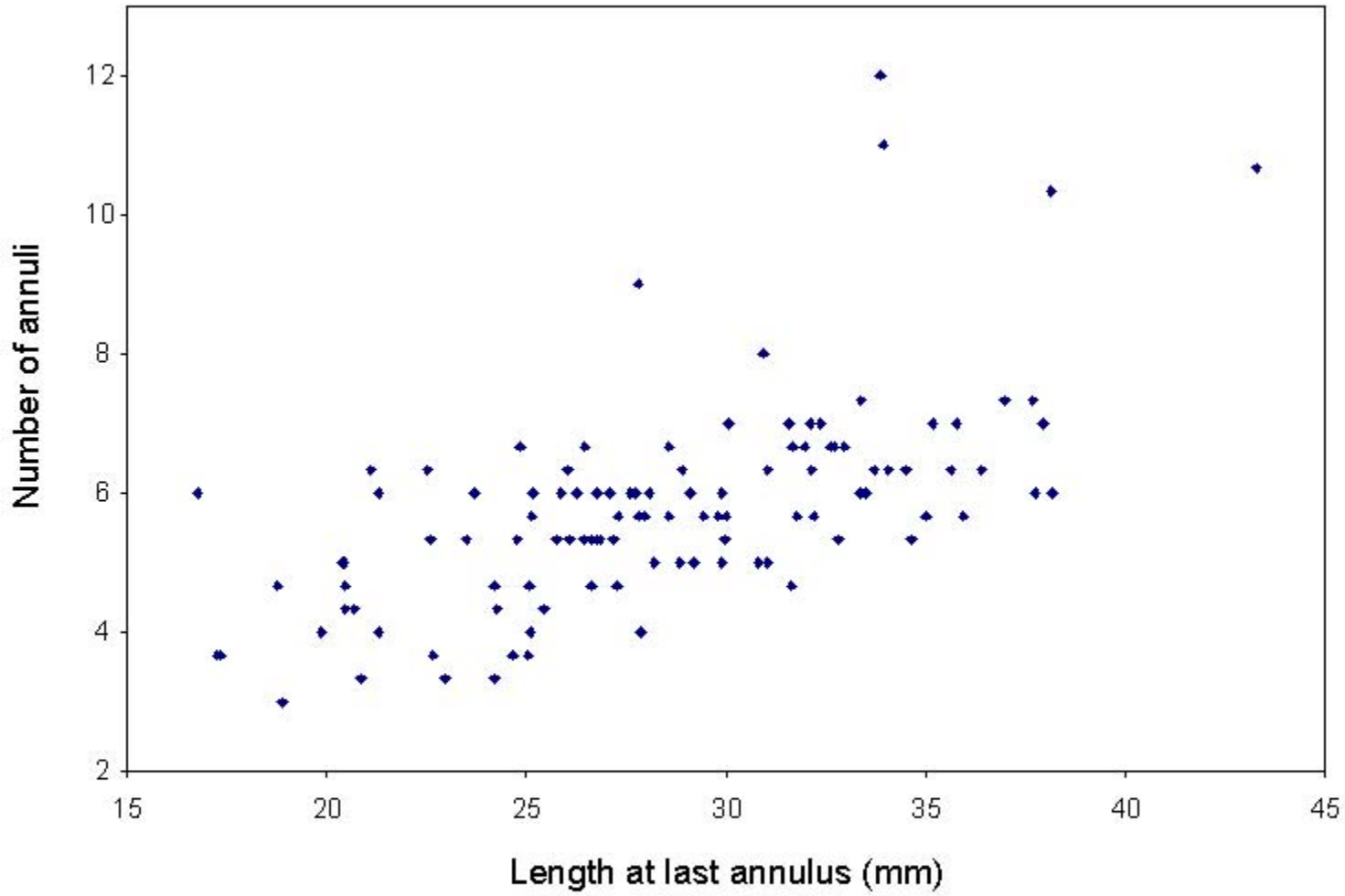

Figure 3. Number of annuli versus shell length at last annulus for 110 Mytilus trossulus tagged at Montague Island and Knight Island in Prince William Sound, Alaska. Each point is the mean of independent observations by three agers of number of annuli and length at last annulus for each mussel.

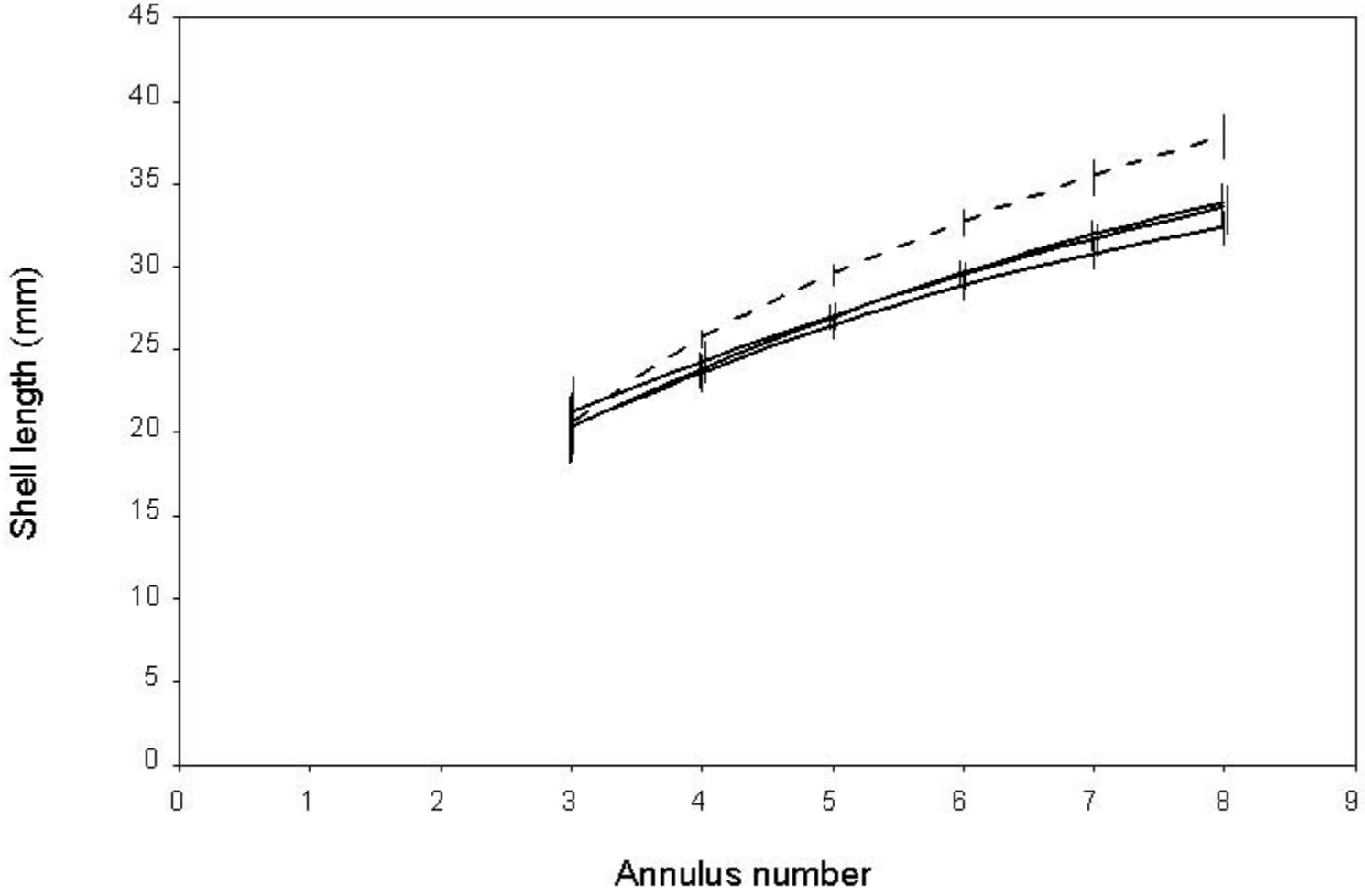

Figure 4. Curves of growth of 110 tagged Mytilus trossulus at Montague Island and Knight Island in Prince William Sound, Alaska depicting growth from age-length data estimated by three observers and modeled by the Schnute equations (solid lines) and from tag growth-increment data modeled by the Schnute analog growth equation for mark-recapture data (dashed line; see Tables 2 and 5 for parameter values). Vertical bars are bootstrap estimates of 95% confidence intervals.

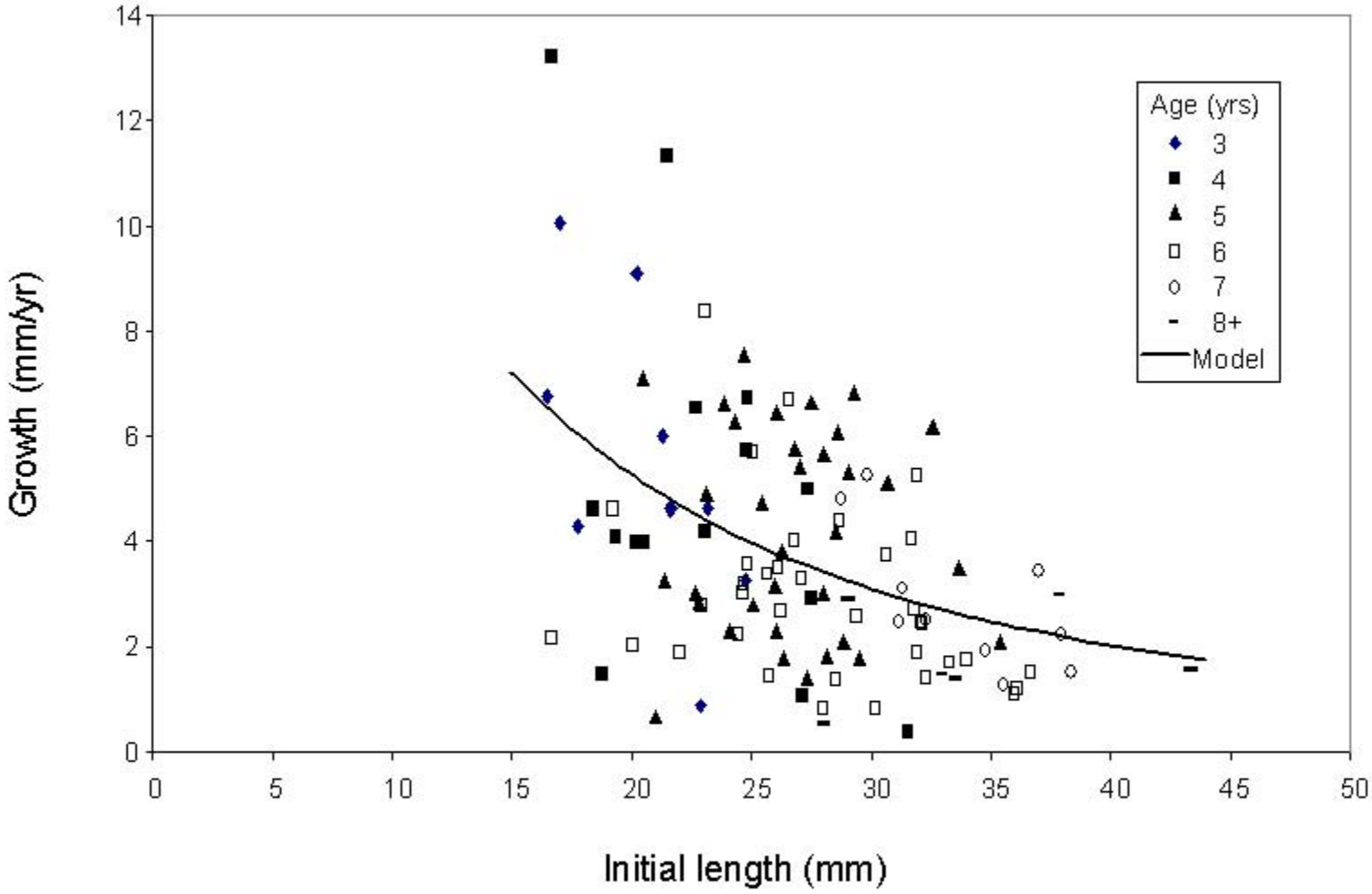

Figure 5. Increase in shell length measured one year after tagging versus initial shell length of 110 Mytilus trossulus aged 3 to 8+ yrs at Montague Island and Knight Island in Prince William Sound, Alaska.

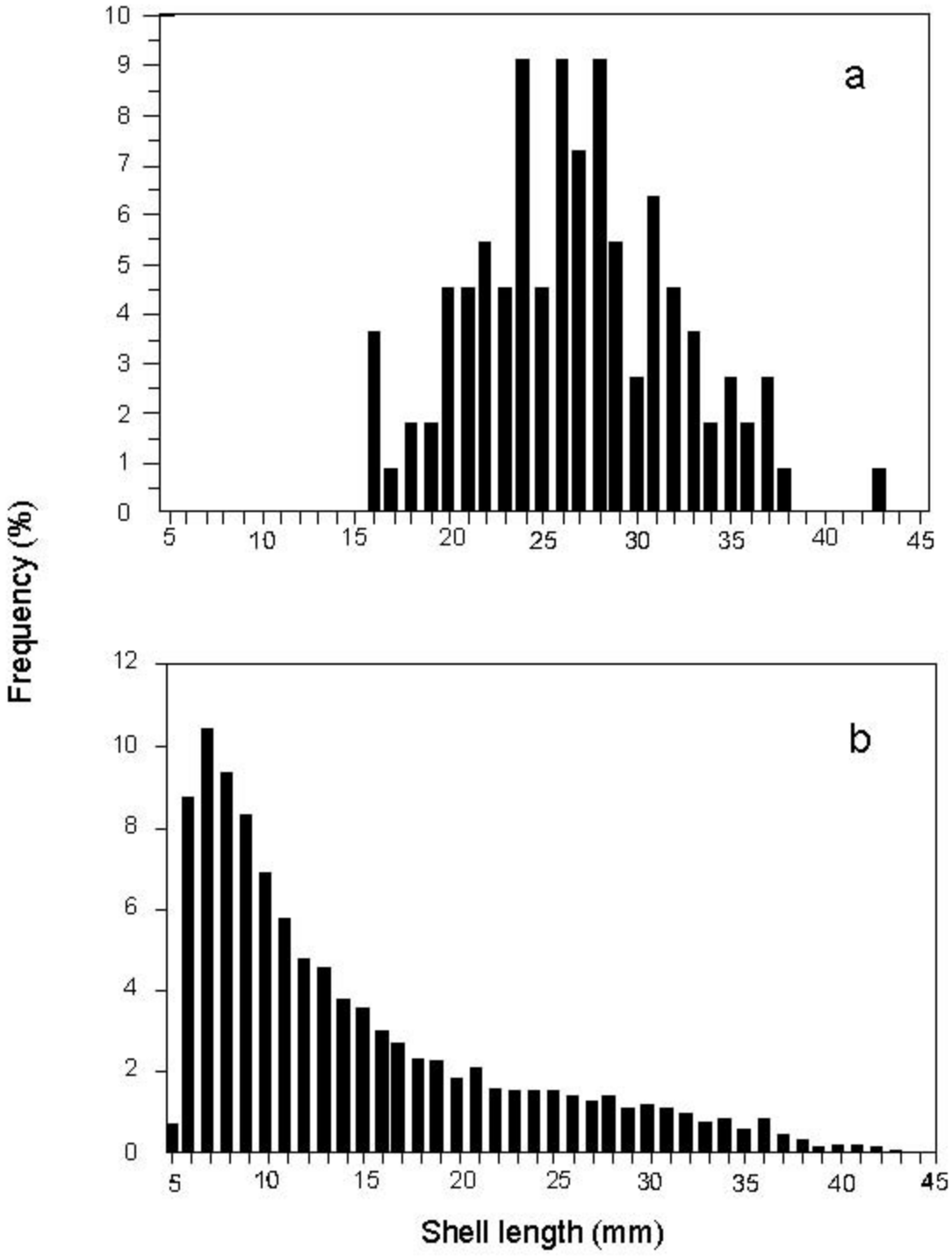

Figure 6. Length-frequency distribution of (a) tagged mussels, Mytilus trossulus, (n = 110) and (b) mussels (n = 18,196) randomly sampled between 0.6 and 1.2 m above mean lower low water at tagging sites on Montague Island and Knight Island in Prince William Sound, Alaska.

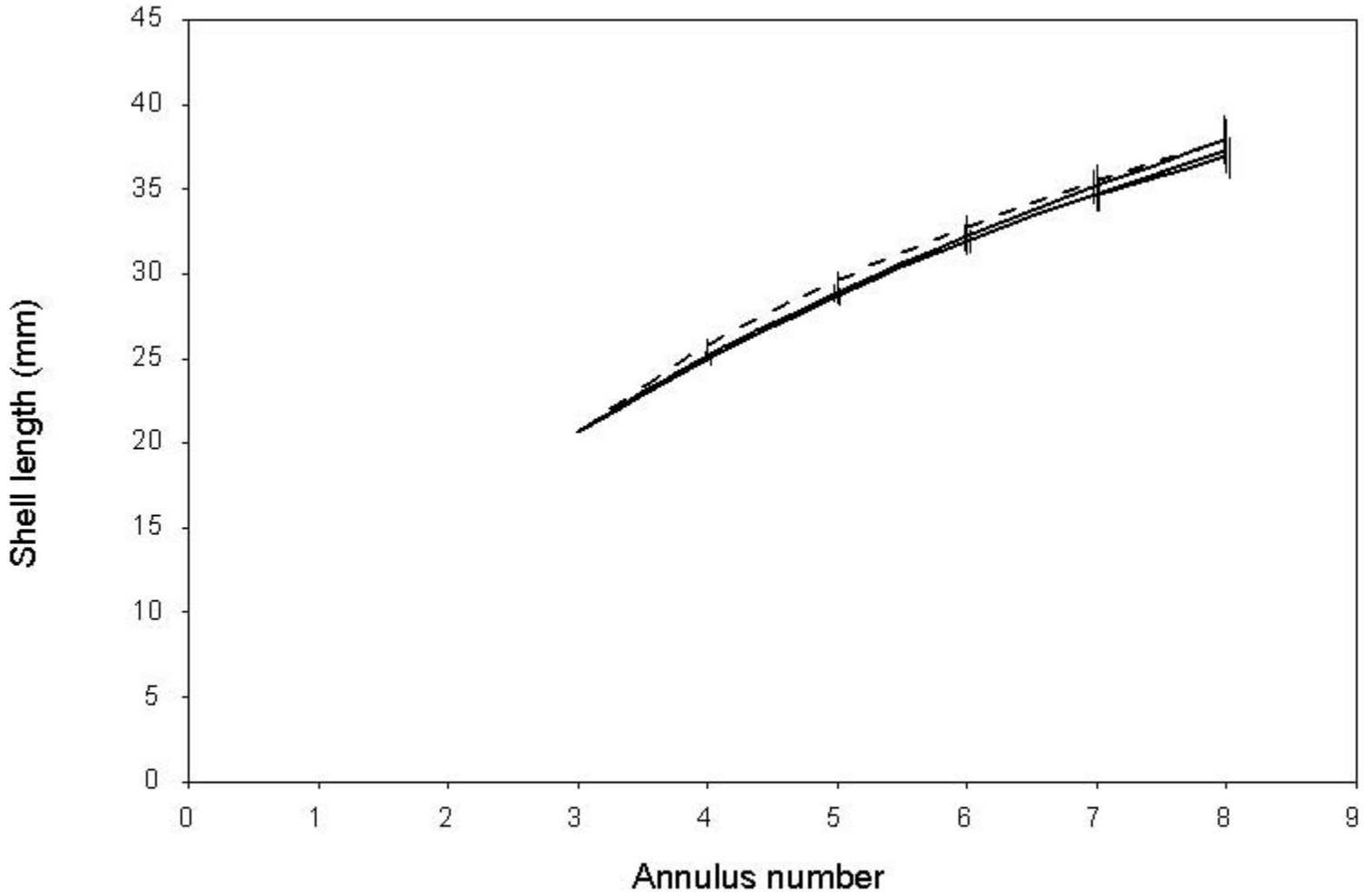

Figure 7. Curves of growth of 110 tagged Mytilus trossulus at Montague Island and Knight Island in Prince William Sound, Alaska depicting growth from annulus growth-increment data estimated by three observers (solid lines) and from tag growth-increment data (dashed line). Both sets of data were modeled by the Schnute analog growth equation for mark-recapture data. Vertical bars are bootstrap estimates of 95% confidence intervals.

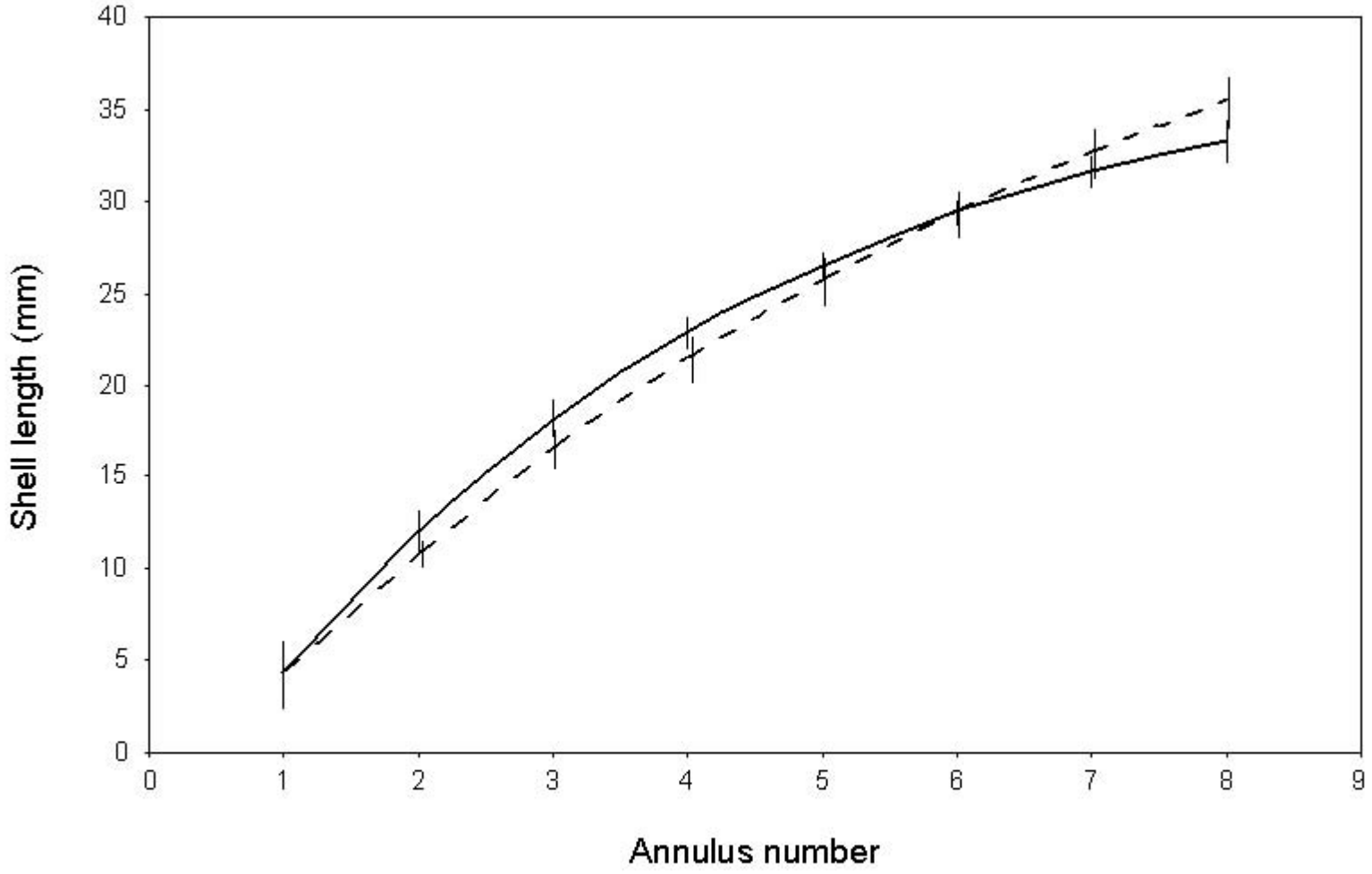

Figure 8. Curves of growth of 110 tagged Mytilus trossulus at Montague Island and Knight Island in Prince William Sound, Alaska depicting growth from age-length data modeled by the Schnute equation (solid line) and from annulus growth-increment data modeled by the Schnute analog growth equation for mark-recapture data (dashed line). Each curve depicts means of three observers. Vertical bars are bootstrap estimates of 95% confidence intervals (see Tables 4 and 7 for parameter values and confidence intervals). The curves include growth at the 1st and 2nd annuli estimated by substituting measurements to these annuli in a subset of the tagged mussels.

Appendix A

Submodels of the Schnute Growth Equation
(See Methods for definitions of terms)

Case 2: a ≠ 0, b = 0

$$Y(t) = y_1 \exp\left[\log\left(\frac{y_2}{y_1}\right)\frac{1 - e^{-a(t-\tau_1)}}{1 - e^{-a(\tau_2-\tau_1)}}\right]$$

Case 3: a = 0, b ≠ 0

$$Y(t) = \left[y_1^b + (y_2^b - y_1^b)\frac{t-\tau_1}{\tau_2-\tau_1}\right]^{1/b}$$

Case 3: a = 0, b = 0

$$Y(t) = y_1 \exp\left[\log\left(\frac{y_2}{y_1}\right)\frac{t-\tau_1}{\tau_2-\tau_1}\right]$$

Appendix B

Submodels of the Baker et al. (1991) Analog to the Schnute Growth Model for Mark-recapture Data
(See Methods for definitions of terms.)

Case 2: a ≠ 0, b = 0

$$Y_r = \exp\left[\ln Y_m e^{-a(t_r - t_m)} + (\ln y_2 - \ln y_1 e^{-a(\tau_2 - \tau_1)}) \frac{1 - e^{-a(t_r - t_m)}}{1 - e^{-a(\tau_2 - \tau_1)}}\right]$$

Case 3: a = 0, b ≠ 0

$$Y_r = \left[Y_m^b + (y_2^b - y_1^b) \frac{t_r - t_m}{\tau_2 - \tau_1}\right]^{1/b}$$

Case 4: a = 0, b = 0

$$Y_r = \exp\left[\ln Y_m + (\ln y_2 - \ln y_{1)} \frac{t_r - t_m}{\tau_2 - \tau_1}\right]$$

APPENDIX SO-05

Mesoscale Differences in Mussel, *Mytilus trossulus*, Population Structure in Prince William Sound, Alaska, in Relation to Oiling History and Predation Intensity[1]

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Running title: Predation and size structure of prey after disturbance

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Mesoscale Differences in Mussel, *Mytilus trossulus*, Population Structure in Prince William Sound, Alaska, in Relation to Oiling History and Predation Intensity

Charles E. O'Clair, Joshua Millstein and Mandy Lindeberg

ABSTRACT: To assess the relative importance of direct and indirect (sea otter, *Enhydra lutris*, and *Nucella* spp. predation) effects of the *Exxon Valdez* oil spill (EVOS) on mussel, *Mytilus trossulus*, population structure 7-8 yrs after the spill, we sampled mussel populations on 1,076 transects in May/June and July 1996 and 1997 on Knight Island (KI; oiled) where sea otter numbers were greatly reduced by the spill and on the northwestern shore of Montague Island (MI; unoiled) where sea otters were unaffected by the spill. The density of all mussels (≥ 5 mm in shell length) at KI exceeded that at MI in both years of our study, but the majority of mussels at KI were small, having recruited to the population in the previous 1-2 yrs. The length-frequency and biomass (ash-free dry mass [AFDW]) distributions of the mussels at KI were strongly skewed to the right in both years. The density and biomass of mussels ≥ 20 mm in shell length did not differ between study areas in 1996, but was higher at MI than at KI in 1997. The density of ≥ 40 mm mussels (the size range preferentially consumed by sea otters) was very low at both study areas. The density did not differ between study areas in 1996, but in 1997 was greater at MI than at KI. The mean biomass density of mussels ≥ 40 mm at KI slightly exceeded that at MI in 1996, but it was less than 1/6 that at MI in 1997. The results indicate that release from sea otter predation on Knight Island after the EVOS did not result in an increase in the abundance of large mussels there 7-8 yrs after the spill as would be predicted if sea otters controlled the size-structure of mussels in Prince William Sound. There is no evidence that the direct effects of oil on the mussel population of KI extended beyond 1995. The mean density of *Nucella lima* on KI exceeded that on MI by 2.4 x in 1996 and by 6.6 x in 1997, and the mean density of *N. lamellosa* on KI exceeded that on MI by 80 x in 1996 and by over 350 x in 1997. Estimates from laboratory feeding rate studies on *N. lima* and *N. lamellosa* of the total consumption of *Mytilus* by *Nucella* in our study areas were ≤0.3% and about 4% of the total mussel population in the study areas at MI and KI, respectively. The level of predation of *Nucella* on *Mytilus* at KI may have contributed to the creation of a bottleneck in the supply of large mussels in the population despite the release of the large mussels from sea otter predation at KI in the 7-8 yrs after the EVOS.

KEY WORDS: *Mytilus trossulus*, Sea otter, *Nucella*, Predation, *Exxon Valdez* oil spill, Population structure, Size structure

INTRODUCTION

The rate of recovery of populations of marine organisms after catastrophic disturbance, whether natural or anthropogenic in origin, depends on factors intrinsic to the organism such as reproductive rate and recruitment potential, but may also be strongly influenced by interspecific interactions or the level of chemical or physical stress in the environment unrelated to the disturbance (Loya 1976, Harris et al. 1984, Suchanek 1993, Davenport et al. 1995, Carroll & Highsmith 1996). Interspecific interactions may facilitate or inhibit population recovery after disturbance (Harris et al. 1984, Carroll & Highsmith 1996). Identifying important links in the interaction web of an intertidal community through small-scale experiments though useful to understanding community organization (Paine 1980, Menge 1995), may have limited utility for the prediction of recovery from broad-scale catastrophic disturbance (McCook & Chapman 1997).

The *Exxon Valdez* oil spill (EVOS) of 24 March 1989 and subsequent cleanup efforts represented a widespread, catastrophic disturbance to rocky intertidal communities in western Prince William Sound (PWS), Alaska (Highsmith et al. 1996, Hooten & Highsmith 1996, Houghton et al. 1996, Lees et al. 1996, Stekoll et al. 1996, van Tamelen & Stekoll 1996). The mussel, *Mytilus trossulus* Gould 1850, suffered reduced abundance and biomass to various degrees or not at all on oiled shores depending on habitat type and tidal level (Highsmith et al. 1996). On shores treated to remove oil, mussels generally suffered greater reductions in abundance than on oiled but untreated shores. The impact on the mussel population depended on the type of treatment; mussels exposed to high-pressure, hot-water treatment suffered up to a 96% reduction in percent cover after treatment (Houghton et al. 1996, Lees et al. 1996). By 1995, six years after the spill, mussel populations at oiled sites (including those on treated shores) showed fluctuations in abundance indistinguishable from those at reference sites (Houghton et al. 1997, Coats et al. 1999).

In addition to the direct effects of oil on mussels, *Mytilus* population size and structure might have been influenced indirectly by the effects of oil on mussel predators. Mussels of the genus *Mytilus* are preyed upon by a wide variety of mammalian, avian and marine invertebrate species. In addition to limiting mussel abundance and influencing local patterns of mussel distribution, predators such as the sea otter, *Enhydra lutris*, some shorebirds, crabs and gastropods prey on particular size ranges of *Mytilus*, thereby potentially influencing the size structure of mussel populations (see Seed & Suchanek 1992 for review). On the west coast of North America *Mytilus trossulus* ranges from Alaska to California (Seed 1992). It co-occurs and hybridizes with *M. galloprovincialis* in parts of Washington and California, but is the only bay mussel in Alaska (McDonald & Koehn 1988, Rawson & Hilbish 1995, Suchanek et al. 1997). *M. californianus* overlaps in distribution with *M. trossulus* in Alaska, but inhabits exposed outer coasts, appears unable to tolerate freezing conditions, and is not abundant north of Sitka, Alaska (Seed & Suchanek 1992). Populations of *M. trossulus* in PWS are subject to predation by sea otters, gulls (*Larus glaucescens* and *L. canus*), seaducks (*Bucephala islandica* and *Histrionicus histrionicus*), shorebirds (*Haematopus bachmani* and *Aphriza virgata*) dogwinkles (*Nucella lamellosa* and *N. lima*) and seastars (*Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata*, and *Leptasterias* sp.; Paul & Feder 1975, Garshelis 1983, O'Clair & Zimmerman 1986, Van Blaricom 1988, Bishop et al. 1998, Chapter 3 Part B,

Chapter 4). Of these predators, only sea otters have been shown to control mussel size-structure under some circumstances in PWS. Sea otter predation shifts the size-distribution toward smaller mussels as the larger ones are preferentially consumed (VanBlaricom 1987, 1988). Prior to the EVOS mussels represented up to 40% of the diet of sea otters at Green Island, PWS (Estes et al 1981, VanBlaricom 1987, 1988). Mussels were a major part of the diet of females with large dependent pups and independent pups there (Garshelis 1983, Van Blaricom 1988). At locations where female sea otters with dependent pups and independent pups were abundant, sea otter predation influenced the size distribution of *Mytilus*, shifting the distribution toward smaller individuals as the larger mussels (chiefly those ≥ 40 mm in shell length) were preferentially consumed (VanBlaricom 1987, 1988). Foraging observations of sea otters at northern Knight Island and northwestern Montague Island in 1996-97 revealed that clams were the most commonly observed group of prey organisms in sea otter diets, and mussels represented a relatively small percentage (10-13%) of the diet of sea otters (Chapter 3 Part B). However, Dean et al. (Chapter 3 Part B) did not break their foraging observations down by the sex, age or reproductive status of the sea otter, and no observations were made in winter when sea otters might forage nearer protected shores to avoid storms. Therefore, it is not clear what influence sea otters may have had on mussel population structure in western PWS a decade after the EVOS.

That segment of the sea otter population in the path of the oil spill in western PWS suffered heavy mortality. The northern Knight Island region was in the direct path of the EVOS and extensive stretches of the shoreline of the islands in the region were heavily oiled (Galt et al. 1991). Sea otter mortality in the region approximated 90% immediately after the spill (Bodkin & Udevitz 1994). Montague Island was not in the path of the EVOS and no oil came ashore within our study area there (Galt et al. 1991). The sea otter population at Montague Island was not reduced after the EVOS (Chapter 3 Part A). The number of sea otters in the northern Knight Island region remained low in the decade following the EVOS. Through 1998 sea otter numbers in the region did not exceed about half the pre-spill numbers whereas at Montague Island sea otter numbers remained high (Chapter 3 Part A, Chapter 3 Part B). Presumably, the mussel population in the northern Knight Island region was subject to a much reduced level of sea otter predation in the 7-8 yrs. after the EVOS compared to the population on Montague Island, and might be expected to respond with an increase in the abundance of large mussels relative to Montague Island.

Here we compare abundance and size-structure in mussel populations in an oiled and an unoiled area of PWS. We assess several factors that may have influenced mussel populations after the EVOS, and report the relative contribution of direct and indirect (principally sea otter predation) effects of the EVOS on the mussel populations.

METHODS

Study sites. The study was conducted on two islands in Prince William Sound (PWS), Alaska. The environment of one study area on the northwestern coast of Montague Island (MI) was not contaminated by oil from the EVOS and populations of vertebrate predators remained at pre-spill levels after the oil spill. The other study area included two locations on Knight Island (KI), Herring Bay and Bay of Isles, in the path of the oil spill, and where numbers of vertebrate

predators declined after the spill. Mussels were sampled within 200-m long segments of shore (sites) in the two study areas (Figure 1). The site was the sampling unit. The MI study area contained 250 contiguous sites; the KI area contained 155 contiguous sites in Bay of Isles and 187 contiguous sites in Herring Bay. The length of the shoreline including nearshore islands within each study area was measured on aerial photographs using a digital planimeter. The shoreline lengths were 51.5 km and 68.4 km at the MI and KI study areas, respectively. The dates of sampling were 29 May to 8 June and 2-12 and 19-30 July in 1996 and 18-28 May, 17-27 June, and 16-27 July in 1997.

Sampling design and mussel collection. Following cost and power analysis performed on preliminary mussel density data collected in 1995 a sample size of 60 sites per study area was chosen. Power analysis revealed that this sample size would allow the detection of a difference of 42 mussels 500 cm^{-2} (55% of the mean) at the $\alpha = 0.05$ significance level with power $1 - \beta = 0.8$. The actual number of sites sampled was 51 on MI and 57 on KI in 1996. In 1997 the number of sites sampled was 55 in each study area.

The sites were numbered sequentially throughout the study areas, beginning with the northern end of the MI. Each study area included the shorelines of islands adjacent to shore. The first site sampled in each study area was selected with a random number generator. The remaining sites were sampled systematically such that every 4th to 6th segment was sampled, depending on the study area.

Mussels were sampled in quadrats on ten vertical transects laid 20 m apart within each site. The first transect was placed a random distance between 0 and 20 m from the boundary of the site. The remaining nine transects were laid systematically at 20-m intervals starting from the first. Each transect was laid perpendicular to shore from the upper limit to the lower limit of the realized distribution of *Mytilus trossulus*, ie from the uppermost to the lowest mussel on the vertical transect. A 500 cm^2 quadrat was positioned a random distance along each transect. All mussels were removed from within the quadrat, placed in a plastic bag and frozen within 3-4 h. A total of 1,054 and 1,108 quadrats were sampled in 1996 and 1997, respectively.

Our density estimate for *M. trossulus* was restricted to the realized distribution of the mussel in the two study areas because it would have been unrealistic to attempt to estimate density over the potential distribution of the species based on habitat characteristics. *Mytilus trossulus*, like its congener *M. edulis*, is capable of inhabiting a broad range of intertidal habitats from protected habitat to that exposed to heavy wave action and including substrates such as bedrock or sediments ranging from boulder to mud (CEO, personal observations; Seed and Suchanek 1992). Moreover, the potential vertical distribution of mussels is modified by many physical and biological factors chief among which may be temperature extremes, desiccation and predators (Seed and Suchanek 1992). We were simply not able to adequately quantify the effect of site-specific differences in the magnitude of physical factors and biotic influences on mussel distribution to adjust the realized distribution of *M. trossulus* and thereby obtain an estimate of the potential distribution of the mussel at each site.

Several environmental variables were measured in conjunction with the mussel sampling. Invertebrate predators of mussels (asteroids and *Nucella* spp.) were counted 1 m either side of each transect. The width of the mussel zone was measured with a surveyor's tape. The slope of

the shore on each 200-m segment was measured with a clinometer. The substrate in each quadrat was classified according to the Wentworth grain-size scale (Holme & McIntyre 1984) . The segments were post-stratified into two strata based on substrate: 1) rocky (including bedrock and boulder areas) and 2) unconsolidated or mixed substrate (including various mixtures of silt, sand, granules, pebbles and cobble).

Mussel density, size and biomass. Mussel samples were sieved in the laboratory using 4 mm, 2 mm, 1 mm and 0.5 mm mesh sieves. Small mussels were counted in two size classes (0-2 mm and 2-5 mm) based on shell length. The shell length of all mussels ≥ 5 mm was measured to the nearest 0.1 mm with a digital caliper connected to a datalogger. A total of 58,432 mussels were measured for size-frequency analysis in 1996; 78,554 were measured in 1997.

A total of 280 mussels were selected from the main study locations (MI, Bay of Isles and Herring Bay) for the measurement of mussel mass. Mussels were selected from both mixed and rocky strata in as wide a range of shell lengths as possible. Each mussel was drip dried on a paper towel and then weighed. All measurements of mass were taken to the nearest mg on a precision balance. After the wet mass was obtained the mussels were placed in a drying oven at 60˚C, weighed at 24 h intervals until the weights stabilized, and then ashed in a muffle furnace for 5 hours at 550˚C. The ash-free dry mass (AFDM) was calculated by subtracting the mass of the ash from the dry mass of the mussel (Palmerini and Bianchi 1994).

***Nucella* laboratory feeding experiments**. Separate 60-day feeding experiments, run sequentially, were conducted in the laboratory in which *Nucella lima* or *N. lamellosa* were held together with *Mytilus trossulus* in perforated containers in a tank under flow-through conditions. The *N. lima* experiment was conducted from November to January; that with *N. lamellosa* from March to May. The mussels and dogwinkles were collected by hand at low tide or using SCUBA in or near Auke Bay, Alaska (lat 58˚22' N, long 134˚39'W) and transported to the Auke Bay Laboratory for the experiments. Three size classes of *Mytilus* were used in three treatments: 1) shell length (SL), 5-20 mm; 2) SL, 20.1 - 40 mm; SL, >40 mm. Ten mussels of the appropriate size class were haphazardly assigned to each container. Each treatment was run in triplicate. In the first experiment 10 *N. lima* were randomly assigned to each container (30 snails treatment^{-1}) using a computer random number generator. The snails averaged 21.3 mm in shell height (range, 14-29 mm). Because of the larger size of *N. lamellosa* (mean shell height, 37.4 mm; range, 24-45 mm), five snails were randomly assigned to each container in the second experiment. The experiments were monitored daily for drilled mussels, and a running mean predation rate expressed as no. of mussels eaten snail^{-1} d^{-1} was calculated. Mussels were replaced as they were consumed. Mean water temperature in the *N. lima* and *N. lamellosa* experiments was 7.8˚C (range, 4.8˚C - 8.9˚C) and 5.3˚C (range, 4.7˚C - 6.0˚C), respectively.

Quantitative analysis. Separate regressions of AFDM on shell length were calculated for each study location. The mass of each mussel ≥ 5 mm in shell length was calculated using the appropriate regression for the location where the mussel was collected. The density of mussels or mass M_l of mussel tissue per unit area in each study area was

$$M_l = \frac{\left(\sum_{j=1}^{s} \frac{\left(\sum_{i=1}^{q} \sum_{h=1}^{n} m_h \right)}{q} \right)}{s}$$

where m_h = the hth mussel or the mass of the hth mussel in the ith quadrat depending on whether density or biomass are being estimated, q = the number of quadrats of unit area in the jth shore segment, and s = the number of segments in the lth study area. The area of the mussel zone in each study area was the product of the length of the shoreline and the mean width of the mussel zone in the study area.

The number N_{kl} of mussels consumed by each species k of *Nucella* in each study area l was

$$N_{kl} = n_{kl} \times U_{kl}$$

where U_{kl} was the number of *Nucella* of species k in study area l, and the number n_{kl} of mussels eaten in one year by one *Nucella* of species k in study area l was

$$n_{kl} = \frac{1}{\sum_{a=1}^{3} \frac{p_a}{r_a}}$$

estimated from the proportion p_a of each of three size classes of mussels (shell lengths [SLs], 5-20 mm; SLs, 20.1-40 mm; SLs, >40 mm) and the rate r_a of consumption of mussels in each size class by each species of *Nucella* in one year, determined in the laboratory (see Results). The estimate of Carroll and Highsmith (1996) for the mid-intertidal feeding season in south central Alaska of 30 weeks was used to calculate r_a. Our estimate of n_{kl} rested on three simplifying assumptions: 1) that each species of *Nucella* preyed on *M. trossulus* in the study areas at the same rate as they did in the laboratory where alternative prey species were not available to them, 2) that the size classes of mussels were distributed randomly with respect to one another in the study areas, and 3) that *Nucella* exhibited no prey size selection of *Mytilus* at the study areas.

Analysis of variance was used to test for differences between study areas, years and strata for variables of mussel zone characteristics and mussel density and biomass. Homogeneity of variance was tested with Levene's test (Levene 1960). If necessary, transformations (log [y+1], $y^{-0.5}$, $y^{0.054}$) were used to stabilize variances. Transformations, except log (y+1), were derived according to the method of Taylor (1961). Planned comparisons were made with F-tests or Welch's approximate t-test with Satterthwaite's adjusted degrees of freedom for unequal variances (Day and Quinn 1989). Unplanned comparisons were made with the Kramer modification of Tukey's test for equal variances and unequal sample sizes (Day and Quinn 1989). Because the density and biomass data for mussels ≥ 40 mm in length and the density data for *Nucella lamellosa* on Montague Island contained many zeros we used the Mann-Whitney U-test to test for differences in density and biomass between study areas for these data. Analysis

of covariance was used to test for a difference in the slope of the relationship between log-transformed values of mussel shell length and AFDM between study locations. A two-tailed t-test was used to test the significance of the Pearson formulation of the product-moment correlation coefficient (r) describing the relation of *Nucella* spp. density with the modal length of the mussel length-biomass distribution at each site (Sokal & Rohlf 1995). The data were transformed (log [y+1]) for the test. Error values in the text and figures are one standard error of the mean.

RESULTS

Characteristics of mussel zone

The characteristics of the mussel zone and the substrate type available for mussel attachment differed between study areas. The mussel zone width averaged 4.3 x greater at Montague Island (MI; 37.2 ± 5.3 m) than at Knight Island (KI; 8.7 ± 0.6 m; data from 1996 and 1997 combined; Tables 1 and 2). The difference between study areas was not as pronounced in areas with rocky substrates as in areas with mixed substrates. As a result of the difference in mussel zone width between study areas the total area of the mussel zone at MI (1.53 km^2) exceeded that at KI (0.57 km^2) by 2.7 x. The mean slope of the shore within the mussel zone at MI (5.8° ± 0.4°) was one fourth as steep as that at KI (25.7° ± 1.7°; Tables 1 and 2). A greater proportion (63%) of the shore segments at MI were composed predominately of mixed substrate, whereas most (74%) of the shore segments at KI were predominantly rocky.

Mytilus density and biomass

Mytilus density differed between study areas depending on size-class, stratum and year. The mean density of all mussels ≥ 5 mm in shell length was higher at KI than at MI in 1996 and 1997 (Figure 2). Density of mussels ≥ 5 mm at KI (1,430 ± 245 m^{-2}) was 1.7 x that on MI (862 ± 191 m^{-2}) in 1996 (Tables 3 and 4; $F = 8.20$; $p < 0.01$). In 1997 the density at KI (2,020 ± 288 m^{-2}) exceeded that at MI (873 ± 125 m^{-2}) by 2.3 x ($F = 18.2$; $p < 0.01$). Significant study area by stratum and study area by stratum by year interactions for the ≥ 5 mm mussels indicated that pairwise comparisons of stratum means should be examined. When broken down to the stratum level only the rocky stratum at KI in 1997 significantly exceeded strata at MI (Tables 4 and 5).

The mean density of mussels ≥ 20 mm in shell length was higher at MI than at KI in 1997 but not 1996. Although no interaction terms were significant in the ANOVA, a two-tailed, planned comparison revealed that mean density of mussels ≥ 20 mm at MI (215 ± 36.1 m^{-2}) did not differ significantly from that at KI (134 ± 19.2 m^{-2}) in 1996 (Figure 2, Tables 3 and 4; $F = 2.53$; $p > 0.05$). In 1997 mean density at MI (280 ± 55.4 m^{-2}) exceeded that at KI (113 ± 24.2 m^{-2}) by 2.5 x ($F = 10.8$; $p < 0.01$). The ANOVA indicated a stratum effect. Within-year paired comparisons revealed that density was greater in mixed substrates (286 ± 60.1 m^{-2}) than on rocky substrates (127 ± 27.1 m^{-2}; Tukey-Kramer test; $p < 0.05$) in 1997 but not 1996 (mixed, 237 ± 31.6 m^{-2}; rocky, 122 ± 24.4 m^{-2}; Tukey-Kramer test; $p > 0.05$).

The density of large mussels (shell length ≥ 40 mm) was very low at both study areas (Figure 2). A large percentage of the quadrats at both areas contained no mussels in this size range (61% at MI, 63% at KI), therefore the data were not normally distributed. The Mann-Whitney U-test revealed no difference in mean density of large mussels between KI and MI in 1996 (test, $p = 0.07$; Figure 2). In 1997, the density at MI exceeded that at KI (Mann-Whitney U- test, $p = 0.004$; Figure 2). However, care must be taken in the interpretation of this result because of unequal variances (Levene's test, $p = 0.02$) in the large mussel data in 1997. When data from 1996 and 1997 were combined density did not differ between study areas (Mann-Whitney U-test, $p = 0.416$; Levene's test, $p = 0.027$).

The difference in mussel biomass between study areas depended on the size class of mussels and the year of sampling. The relationship of mussel mass (AFDM) to shell length did not differ between study areas (ANCOVA, study area x shell length interaction, $F = 0.132$, $p = 0.716$; Figure 3). The ANOVAS of biomass density of mussels in the size ranges ≥ 5 mm and ≥ 20 mm revealed in no significant effect of study area (Table 6). We then conducted paired *a priori* F- tests of biomass density of ≥ 5 mm and ≥ 20 mm mussels between study areas in 1996 and 1997. We observed no differences in biomass density of ≥ 5 mm between study areas in 1996 ($F = 0.27$, $p > 0.05$) or 1997 ($F = 2.91$, $p > 0.05$; Figure 4). Mean biomass density of ≥ 20 mm mussels at MI exceeded that at KI ($F = 10.7$, $p < 0.01$) in 1997, but not 1996 ($F = 1.06$, $p > 0.05$; Figure 4). Mean biomass density of ≥ 20 mm mussels also differed between strata (Table 6). *Post hoc* tests revealed greater biomass density in mixed sediment (29.0 ± 7.2 gm^{-2}) than on rocky substrate (10.3 ± 2.3 g m^{-2}; Tukey-Kramer test; $p < 0.01$) in 1997, but not in 1996 (mixed, 23.1 ± 3.2 g m^{-2}; rocky, 10.7 ± 1.9 g m^{-2}; Tukey-Kramer test; $p > 0.05$).

The effect of study area on the biomass density of large mussels (≥ 40 mm) varied between years. In 1996 mean biomass density of mussels ≥ 40 mm at KI exceeded that at MI by 43% (Mann-Whitney U-test, $p = 0.042$; Figure 4). In 1997 the relationship reversed and mean biomass density of large mussels at MI exceeded that at KI by 6.3 x (Mann-Whitney U-test, $p = 0.006$; Figure 4).

Mytilus length and biomass distribution

The length-frequency distribution of mussels ≥ 5 mm at both areas was skewed to the right in 1996 and 1997 (Figure 5; Table 7). Length-frequency modes occurred at shell lengths ranging from 6 to 8 mm at the two study areas over both years. The distribution at KI was more strongly skewed than that for MI indicating that mussel abundance was more strongly concentrated in the small size classes at KI (Table 7; Figure 5).

Differences between study areas in the distribution of biomass with mussel size were more pronounced than were differences between study areas in length frequency. At MI the distribution of mussel mass showed modes at shell lengths of 27 and 31 mm in 1996 and 1997, respectively (Figure 6). At KI modes occurred at shell lengths of 11 and 9 mm in 1996 and 1997, respectively (Figure 6). The MI distribution was skewed to the left in both years (Table 7). That at KI was skewed to the right in both years, and in 1996 was strongly platykurtic tending toward bimodal (Figure 6; Table 7).

Invertebrate predator density

A suite of invertebrate predators including two species of *Nucella* (*N. lima* and *N. lamellosa*) and five species of generalist seastar (*Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata*, and *Leptasterias* sp.) occur in the intertidal region in PWS and are known to prey on mussels or are potential mussel predators. The mean density of *Nucella lima* on KI exceeded that on MI in 1996 and 1997. In 1996 *N. lima* density on KI exceeded that on MI by 2.4 x (Figure 7, Table 8; Welch's t-test, $p<0.05$). In 1997, *N. lima* density on KI exceeded that on MI by 6.6 x (Figure 7, Table 8; Welch's t-test, $p<0.01$). Density of *N. lima* did not differ between years nor with substrate (Table 8).

Nucella lamellosa was rare at MI. It was observed on only nine (20%) shore segments there in 1996 and only 5 (9%) segments in 1997. The large number of zeros in the data set of *N. lamellosa* density precluded the use of parametric statistics. The mean density of *N. lamellosa* on KI exceeded that on MI by 80 x in 1996 (Figure 7; Mann-Whitney U-test, $p<0.001$). In 1997, *N. lamellosa* density on KI exceeded that on MI by over 350 x (Figure 7; Mann-Whitney U-test, $p<0.001$). The density of *N. lamellosa* on rocky substrate exceeded that on mixed substrate in 1996 by 476 x (rocky, 1.54 ± 0.48 m^{-2}; mixed, 0.003 ± 0.002 m^{-2}; Mann-Whitney U-test, $p<0.001$; study areas combined) and in 1997 by 701 x (rocky, 1.38 ± 0.36 m^{-2}; mixed, 0.002 ± 0.002 m^{-2}; Mann-Whitney U-test, $p<0.001$).

The density of *Nucella* spp. (both species combined) at the study sites was inversely correlated with the modal length of the mussel length-biomass distribution at the sites at KI in 1996 ($r = -0.633$, $p<0.01$) and in 1997 ($r = -0.463$, $p<0.01$; Figure 8). However, at MI these two variables were uncorrelated in both years (1996, $r = -0.068$, $p>0.05$; 1997, $r = -0.023$, $p>0.05$).

The density of large asteroids (*Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata*) on our transects did not differ significantly between study areas in 1996 or 1997 (Figure 7). Densities of the four species of seastars were lumped for the analysis. *Leptasterias* sp. was eliminated from the analysis because it is small and cryptic, and was difficult to count accurately. Abundance estimates of *Leptasterias* were made on the ordinal scale. Variances could not be stabilized with transformation of the seastar density data for the ANOVA (Table 8). Welch's approximate t-test was therefore used for within-year *post hoc* tests. The mean density of large asteroids was similar on KI (0.059 ± 0.019 m^{-2}) to that on MI (0.040 ± 0.013 m^{-2}) in 1996 (Welch's t-test, $p>0.05$) and 1997 (KI, 0.031 ± 0.006 m^{-2}; MI, 0.016 ± 0.007 m^{-2}; Welch's t-test, $p>0.05$; Figure 7). The density of large seastars on rocky substrate exceeded that on mixed substrate in 1996 (rocky, 0.076 ± 0.019 m^{-2}, mixed, 0.013 ± 0.005 m^{-2}; Welch's t-test, $p<0.01$) and 1997 (rocky, 0.037 ± 0.008 m^{-2}; mixed, 0.006 ± 0.002 m^{-2}; Welch's t-test, $p<0.001$; Table 8).

Nucella predation in the laboratory

Feeding rate was inversely related to mussel size in *Nucella lima*. On average, about one third of the *Nucella lima* fed at any particular time over the course of the experiment regardless of the size class of the mussels eaten (Table 9). However, feeding rate was highest for *N. lima* feeding on small (shell length, 5 - 20 mm) mussels. The mean feeding rate (averaged over 60 d) on small mussels was more than 4 x greater than that on medium (21.1 - 40 mm) mussels

(ANOVA, $F = 507$, $p < 0.001$; *a priori*, paired comparison, $F = 629$, $p < 0.001$; Table 9). The feeding rate on medium mussels was, in turn, more than twice that on large (> 40 mm) mussels (*a priori*, paired comparison, $F = 20$, $p < 0.01$; Table 9).

The relationship between feeding rate in *N. lamellosa* and mussel size class was similar to that for *N. lima*. The mean feeding rate on small mussels was 3x greater than that on medium mussels (ANOVA, $F = 43.9$, $p < 0.001$; *a priori*, paired comparison, $F = 40.1$, $p < 0.001$; Table 9). On average, a smaller percentage of the *Nucella lamellosa* fed at any given time than did *N. lima*. This was especially true of those feeding on large mussels (Table 9). The feeding rate of *N. lamellosa* on medium mussels was more than an order of magnitude greater than that on large (> 40 mm) mussels (*a priori*, paired comparison, $F = 7.9$, $p < 0.05$; Table 9).

Total annual consumption of *Mytilus* by *Nucella*

Estimates of total annual consumption of *M. trossulus* by *Nucella* spp. were markedly higher at KI than at MI in 1996 and 1997. The total number of mussels eaten by both species of *Nucella* combined at KI was about an order of magnitude greater than that at MI in both years (Table 10). The number of mussels consumed by *Nucella* spp. in 1996 represented 0.3% and 3.8% of the total number of mussels at the study areas on MI and KI, respectively. In 1997 *Nucella* spp. consumed 0.2% and 3.7% of the total number of mussels at MI and KI, respectively.

DISCUSSION

The direct effects of oil from the EVOS on mussel populations depended on habitat type and tidal level. Highsmith et al. (1996) estimated the abundance of *Mytilus* in western PWS in spring/summer 1990 and spring 1991. They found reduced mussel density throughout the vertical range studied at oiled sites compared to reference sites on coarse-textured substrate in spring 1990. In sheltered rocky and estuarine habitats only at lower tidal levels was mussel density reduced at oiled sites. In summer 1990 and spring 1991 mussel density was reduced at oiled sites in coarse-textured habitat at upper (summer 1990) and lower (spring 1991) tidal levels and in estuarine habitat at mid-tidal levels (summer 1990). The reduction in mean density at oiled sites relative to reference sites ranged from 20% to 90% depending on habitat, tidal level and year (Highsmith et al. 1996). Lees et al. (1996) estimated the abundance of *Mytilus* at 21 locations in western PWS in 1991-92 and found changes resulting from shoreline cleaning. They examined the effects of dispersants and beach cleaners, low-pressure warm water and high-pressure hot water (HP-HW) treatments on *Mytilus* abundance as well as that of other organisms on heavily oiled cobble beaches. Reductions in mussel percent cover after treatment ranged from 50% to 69% depending on the type of treatment used (Lees et al. 1996). The HP-HW treatment had the greatest impact on mussel cover. On a bedrock shore in a heavily oiled bay mussel percent cover was reduced 96% by HP-HW treatment (Houghton et al. 1996).

The direct effects of *Exxon Valdez* oil on mussel populations in PWS probably extended no more than a few years after the spill. The length-frequency distributions of *Mytilus trossulus* that Houghton et al. (1993) present for May, July and September 1991 in one heavily oiled bay (Herring Bay) show little evidence of recruitment there in 1991 (although the bimodal July distribution showed one mode at 5 mm). However, by 1993 mussels began recruiting to some

sites in Herring Bay. The length-frequency distributions of Highsmith et al. (1996) show evidence of good recruitment in June 1993, September 1994 and May 1995 at several sites in Herring Bay. In 1992 and 1993 Thomas et al. (1999) removed mussels from beds overlying sediments oiled by the EVOS in PWS. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in the tissues of the mussels from the oiled beds ranged from about 0.2 to 6 $\mu g\ g^{-1}$, exceeding the tissue concentrations of TPAH in reference mussels by 20-95 x. Nevertheless, byssal thread production, condition index, clearance rate and glycogen content did not differ between mussels from the oiled beds and those from reference beds (Thomas et al. 1999). Highsmith et al. (1996) found reduced growth in mussels at oiled sites in parts of one heavily oiled bay (Herring Bay) that persisted until 1995. However by 1995, fluctuations in mussel abundance at oiled sites (including those treated to remove oil) were indistinguishable from those at reference sites (Houghton et al. 1997, Coats et al. 1999).

Among the indirect effects of the EVOS on mussel population structure, release from sea otter predation seemed a likely possibility to us. Before the EVOS VanBlaricom (1987, 1988) had shown that sea otter predation (especially that by female sea otters with dependent pups and independent pups) controlled the size distribution of *Mytilus* at Green Island near northwest Montague Island. Sea otter predation shifted the size distribution of *M. trossulus* toward smaller individuals as the large mussels (chiefly those ≥ 40 mm in shell length) were preferentially consumed. Immediately after the spill, sea otter mortality in the oiled northern Knight Island region approximated 90% (Bodkin & Udevitz 1994). Through 1998 sea otter numbers in the region did not exceed about half the pre-spill numbers (Chapter 3 Part A). At unoiled Montague Island the sea otter population was not reduced after the EVOS and numbers remained high in the decade after the spill (Chapter 3 Part A, Chapter 3 Part B). Presumably, the mussel population in the northern Knight Island region was subject to a much reduced level of sea otter predation in the 7-8 yrs. after the EVOS compared to the population on Montague Island, and might be expected to respond with an increase in the abundance of large mussels relative to Montague Island.

Our results did not support the hypothesis that the mussel population on northern Knight Island in 1996-97 responded in the predicted way to a 7-8 yr release from sea otter predation. Although the density of all mussels ≥ 5 mm in shell length at KI exceeded that at MI in both years of our study, the majority of the mussels at KI were small, having recruited to the population in the previous 1-2 yrs. As a result, the length-frequency distribution of mussels ≥ 5 mm was more strongly skewed to the right at KI than at MI in 1996 and 1997.

The density and biomass of large mussels either showed no differences between study areas or were greater at MI than at KI. The density and biomass of mussels ≥ 20 mm in shell length did not differ between study areas in 1996, but was higher at MI than at KI in 1997. Perhaps the most revealing comparison of mussel density and biomass between study areas is that of mussels ≥ 40 mm in shell length. In his pre-spill study in PWS VanBlaricom (1987, 1988) compared the length-frequency distribution of mussels in an area harboring female sea otters with dependent pups and independent pups with that in an area containing adult male otters only and found that where sea otter predation structured mussel populations, mussels ≥ 40 mm suffered the greatest mortality. The density of ≥ 40 mm mussels was very low at both of our study areas. The density of this size class did not differ between study areas in 1996. In 1997 the density of ≥ 40 mm mussels at MI actually exceeded that at KI. The mean biomass density of

mussels ≥ 40 mm at KI exceeded that at MI somewhat in 1996, but in 1997 the relationship reversed and mean biomass density of ≥ 40 mm mussels at MI exceeded that at KI by 6.3 x. Our results therefore indicate that release from sea otter predation on Knight Island after the EVOS did not promote an increase in the abundance of large mussels there.

Failure of the mussel population at KI to respond to release from sea otter predation in the predicted way was probably not a result of inadequate time for newly recruited mussels to grow into larger size classes at KI. If good recruitment of *Mytilus* to heavily oiled shores on KI after the EVOS were delayed until 1993 (see discussion of direct effects above), then these recruits would have had time to grow into the ≥ 20 mm size class at KI by 1996-97, according to the models of mussel growth for the region (Millstein & O'Clair 2001). Of course, juvenile and adult mussels that survived the oil spill would presumably continue to grow at some level and should further augment the large size class at KI by 1996-97.

Alternatively, *Nucella* predation may have structured the mussel population on KI. Carroll & Highsmith (1996) found that predation by *Nucella lima* prevented recovery of *Mytilus trossulus* at some sites in Kachemak Bay, Alaska where mussel abundance had been reduced by a severe winter freeze. They estimated that *N. lima* could eliminate 60-90% of mussels at a given site in one season. Suchanek (1978) reported similarly high levels of *Nucella* (= *Thais*) predation on *M. trossulus* (= *M. edulis* in Alaska) in southeastern Alaska where the average percentage of drilled mussel shells on the low shore ranged from 61% on protected shores to 95% on wave-exposed shores at sites with high numbers of *Nucella* spp. (*N. canaliculata*, *N. emarginata*, *N. lamellosa* and *N. lima*). In the present study, our estimate of the percentage of mussels at the study areas consumed by *Nucella* spp. in 30 weeks (the estimated mid-intertidal feeding season in south central Alaska determined by Carroll & Highsmith [1996]) was much lower than that of Carroll & Highsmith (1996), ranging from 0.3% to 3.8% (rather than 60-90%) depending on the study area. However, our estimate was based, in part, on an estimate of the mean density of *Nucella* spp. over lengths of shore of 51.5 km and 68.4 km at MI an KI, respectively, whereas the estimate of Carroll & Highsmith (1996) was at a site with a high density of *N. lima*. Nevertheless, we may have underestimated the number of mussels consumed in our study areas. Our estimate of the feeding rate of *Nucella* spp. on *M. trossulus* in the laboratory ranged from 0.0056 to 0.189 mussels d^{-1} depending on mussel size-class and the species of *Nucella*. This estimate was lower than those of Seed (1976; 0.31mussels d^{-1}) and Stickle et al. (1985; 0.1-0.6 mussels d^{-1} [see Seed and Suchanek 1992] depending on shell thickness and temperature) for *N. lapillus* preying on *M. edulis*. Our estimate was closer to that of Wickens and Griffiths (1985; 0.02-0.12 mussels d^{-1}) for *N. cingulata* feeding on *Aulacomya ater* and that of Hunt and Scheibling (1998; 0.1 mussels d^{-1}) for post-recruit *N. lapillus* feeding on *M. edulis/M. trossulus*. Hunt and Scheibling's (1998) estimate of the feeding rate of *N. lapillus* on *Mytilus* (0.1 mussels d^{-1} and 0.156 mussels d^{-1} for tidepools and emergent rock, respectively) in the field was similar to the estimate they obtained in the laboratory, indicating that the feeding rate of *Nucella* spp. at our study sites may not have been markedly different from the feeding rate estimate we obtained in the laboratory.

The negative correlation between the modal length of the mussel length-biomass distribution and the density of *Nucella* spp. at the study sites on KI is indicative of an effect of predation by *Nucella lima* and *N. lamellosa* on the population structure of *Mytilus trossulus* there. The lack of a correlation between these two variables at MI indicates that *Nucella* densities

on MI may have been too low to produce a detectable effect on mussel population structure. Although *Nucella lima* densities did not differ between substrate types, densities of *N. lamellosa* were found to be far greater in rocky areas (particularly on large immobile rock surfaces) than on mixed substrate, thus the increased *Nucella* spp. densities found at KI may be partially explained by the increased percentage of rocky shoreline there.

The strongly right-skewed length-frequency distribution of mussels resulting from a higher density of 5 - 20 mm mussels, in combination with the greater *Nucella* densities at KI (2.4 x - 6.6 x that at MI for *Nucella lima* and 80 x to over 350 x that at MI for *N. lamellosa*, depending on the year) was also consistent with, but not necessarily indicative of an effect of *Nucella* predation on mussel population structure at KI. Increased mussel recruitment alone at KI may have produced the strongly right-skewed length-frequency distribution of mussels there. However if mussel recruitment alone were responsible for the higher density of 5-20 mm mussels on KI one would not necessarily expect a relationship between the modal length of the mussel length-biomass distribution and the density of *Nucella* spp. at the study sites on KI. Alternatively, the differences in the mussel length-frequency distributions between study areas may have been the result of the interplay of two mechanisms: 1) an increase in the number of refuges for mussels of small size at KI and 2) prey size selection by *Nucella* spp.

The substrate at KI may have favored *M. trossulus* recruits. The shores of KI were predominantly rocky and included a large amount of bedrock with barnacle cover. This type of surface contained many small crevices and pits, which can provide refuge from *Nucella* for small mussels. As a mussel grows too large to obtain refuge in the pits and crevices in rock and between barnacles it can become prey to *Nucella*. By contrast, the predominantly mixed intertidal substrate at MI may not have provided a similar size refuge for mussels.

Nucella may prefer larger mussels thereby enhancing the size refuge provided by the habitat at KI. We did not examine size-selection of *M. trossulus* by *N. lima* or *N. lamellosa*. Experienced *Nucella lapillus*, a species comparable in adult size to *N. lima*, were found to prefer *Mytilus edulis* of 20-25 mm shell length (SL) in the laboratory (Hughes & Dunkin 1984). In Yorkshire, UK, Hughes & Burrows (1991) found that *N. lapillus* primarily preyed on mussels 10-20 mm SL. However, in Nova Scotia, predation by *N. lapillus* post-recruits ($\geq$5 mm SL) on mussels < 5 mm SL was not uncommon (Hunt & Scheibling 1998).

Nucella predation on KI may have contributed in large part to the failure of mussels of large size to increase in abundance at KI despite being released from sea otter predation during the 7-8 yr period after the EVOS. Mussel recruitment may have been enhanced at KI by habitat complexity. However, although *Nucella* spp. consumed < 4% of the estimated total population of *M. trossulus* in our study area at KI (compared to $\leq$ 0.3% at MI), that level of predation may have contributed to the creation of a bottleneck in the supply of large mussels by increasing mortality in mussels of intermediate size, thereby reducing the number of mussels growing to large size enough to compensate for the reduced mortality experienced by large mussels as a result of release from sea otter predation.

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Table 1. Mussel zone characteristics at study areas (Montague Island vs. Knight Island) in strata (rocky vs. mixed sediment substrates) in 1996 and 1997 in Prince William Sound.

			Montague Island			Knight Island		
Characteristic	Year	Stratum	Mean	SD	N	Mean	SD	N
Mussel zone width (m)	1996	Mixed	34.7	40.2	34	11.7	2.9	14
		Rocky	14.0	10.2	17	6.5	3.3	43
	1997	Mixed	64.6	82.2	35	19.2	10.6	14
		Rocky	16.7	14.6	21	6.3	3.3	41
Slope of Shore (˚)	1996	Mixed	5.3	2.8	34	12.7	6.3	14
		Rocky	7.4	5.4	17	21.4	12.6	43
	1997	Mixed	5.0	3.5	35	16.2	14.4	14
		Rocky	6.6	4.1	21	37.8	21.0	41
Lower Edge of Mussel	1996	Mixed	0.606	0.622	34	0.405	0.608	14
Zone (m)†		Rocky	1.369	0.544	17	0.938	0.491	43
	1997	Mixed	0.399	0.529	35	-0.021	0.491	15
		Rocky	1.215	0.531	21	0.739	0.634	41
Upper Edge of Mussel	1996	Mixed	2.560	0.563	34	2.497	0.257	14
Zone (m)†		Rocky	2.542	0.366	17	2.781	0.342	43
	1997	Mixed	2.643	0.651	35	2.516	0.731	15
		Rocky	2.635	0.260	21	2.917	0.601	41

Notes: SD, standard deviation; N, sample size.
† Height above mean lower low water.

Table 2. Analysis of variance of mussel zone characteristics in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in two years (1996 and 1997) in Prince William Sound.

Mussel Zone Parameter Source of Variation	df	MS	F	P
Mussel Zone Width				
Data transformed ($y^{-0.5}$); Levene's Test[a], P = 0.347				
Study area	1	0.406	61.4	<0.001
Year	1	0.041	6.23	0.013
Stratum	1	0.584	88.2	<0.001
Study area x Year	1	0.001	0.139	0.710
Study area x Stratum	1	0.034	5.12	0.025
Year x Stratum	1	0.008	1.26	0.263
Study area x Year x Stratum	1	0.002	0.348	0.556
Error	210	0.006		
Slope of Shore				
Data transformed (Log[y+1]); Levene's Test[a], P =0.002				
Study area	1	9.32	145.5	<0.001
Year	1	0.080	1.26	0.262
Stratum	1	2.07	32.3	<0.001
Study area x Year	1	0.324	5.05	0.026
Study area x Stratum	1	0.332	5.18	0.024
Year x Stratum	1	0.188	2.94	0.088
Study area x Year x Stratum	1	0.119	1.85	0.175
Error	211	0.064		
Lower Edge of Mussel Zone				
Untransformed data; Levene's Test[a], P = 0.486				
Study area	1	71.8	21.1	<0.001
Year	1	29.9	8.77	0.003
Stratum	1	253.9	74.5	<0.001
Study area x Year	1	2.14	0.630	0.428
Study area x Stratum	1	2.51	0.737	0.392
Year x Stratum	1	2.41	0.707	0.401
Study area x Year x Stratum	1	0.921	0.270	0.604
Error	212	3.41		

Table 2 (cont.)

Mussel Zone Parameter Source of Variation	df	MS	F	P
Upper Edge of Mussel Zone				
Untransformed data; Levene's Test[a], P = 0.002				
Study area	1	3.38	1.19	0.277
Year	1	3.37	1.18	0.278
Stratum	1	13.4	4.70	0.031
Study area x Year	1	0.012	0.004	0.947
Study area x Stratum	1	15.6	5.46	0.020
Year x Stratum	1	0.482	0.169	0.681
Study area x Year x Stratum	1	0.354	0.124	0.725
Error	212	2.85		

a. Test of homogeneity of variances.

Table 3. Mussel density (No./m^2) in three size classes in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in 1996 and 1997 in Prince William Sound.

			Montague Island			Knight Island		
Characteristic	Year	Stratum	Mean	SE	N	Mean	SE	N
Mussels ≥ 5 mm in shell	1996	Mixed	637	78.1	32	1098	148	15
length		Rocky	1243	491	19	1545	327	42
	1997	Mixed	974	184	35	693	120	14
		Rocky	705	129	21	2473	359	41
Mussels ≥ 20 mm in shell	1996	Mixed	231	43.0	32	250	38.6	15
length		Rocky	187	65.2	19	92.1	18.6	42
	1997	Mixed	340	81.7	35	149	32.0	14
		Rocky	178	52.8	21	101	30.6	41
Mussels ≥ 40 mm in shell	1996	Mixed	3.1	1.4	32	5.8	1.3	15
length		Rocky	0.43	0.34	19	1.8	0.65	42
	1997	Mixed	11.3	5.5	35	2.2	0.59	14
		Rocky	0.49	0.28	21	0.65	0.35	41

Notes: SE, standard error of the mean; N, sample size.
† Height above mean lower low water.

Table 4. Analysis of variance of mussel density (No./500 cm^2) in two size classes in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in two years (1996 and 1997) in Prince William Sound.

Mussel Size Class Source of Variation	df	MS	F	P
Mussels ≥ 5 mm in Shell Length				
Data transformed (Log[y+1]); Levene's Test[a], P =0.052				
Study area	1	3.07	15.9	<0.001
Year	1	0.017	0.089	0.766
Stratum	1	0.212	1.10	0.296
Study area x Year	1	0.005	0.028	0.868
Study area x Stratum	1	0.811	4.20	0.042
Year x Stratum	1	0.219	1.13	0.289
Study area x Year x Stratum	1	1.34	6.96	0.009
Error	211	0.193		
Mussels ≥ 20 mm in Shell Length				
Untransformed data; Levene's Test[a], P =0.117				
Study area	1	811.8	4.83	0.029
Year	1	0.444	0.003	0.959
Stratum	1	1152	6.86	0.009
Study area x Year	1	249.8	1.49	0.224
Study area x Stratum	1	0.0001	0.000	0.999
Year x Stratum	1	0.434	0.003	0.960
Study area x Year x Stratum	1	350.3	2.086	0.150
Error	211	167.9		

a. Test of homogeneity of variances.

Table 5. Difference between study areas (Knight Island - Montague Island) in density (No. x [500 cm^2]$^{-1}$) of mussels ≥ 5 mm in shell length within/between strata (rocky or mixed sediment substrates) in 1996 and 1997 in Prince William Sound and significance of Tukey-Kramer post hoc test. Data transformed (log [y+1]) for ANOVA. * = $p < 0.05$

			Montague Island			
			1996		1997	
Study Area	Year	Stratum	Mixed	Rocky	Mixed	Rocky
Knight Island	1996	Mixed	22.4	-7.0	6.0	19.1
		Rocky	44.1	14.7	27.8	40.8
	1997	Mixed	2.7	-26.7	-13.6	-0.6
		Rocky	89.1*	59.7*	72.8*	85.8*

Table 6. Analysis of variance of mussel biomass density (AFDW g/500 cm^2) in two size classes in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in two years (1996 and 1997) in Prince William Sound.

Mussel Size Class Source of Variation	df	MS	F	P
Mussels ≥ 5 mm in Shell Length				
Untransformed data; Levene's Test[a], P =0.312				
Study area	1	2,417	0.788	0.376
Year	1	30.8	0.010	0.920
Stratum	1	8,242	2.68	0.103
Study area x Year	1	2,414	0.787	0.376
Study area x Stratum	1	345	0.113	0.738
Year x Stratum	1	206	0.067	0.795
Study area x Year x Stratum	1	11,993	3.91	0.049
Error	211	3,067		
Mussels ≥ 20 mm in Shell Length				
Untransformed data; Levene's Test[a], P =0.078				
Study area	1	5,640	2.93	0.088
Year	1	1.1	0.001	0.981
Stratum	1	17,393	9.04	0.003
Study area x Year	1	4,611	2.40	0.123
Study area x Stratum	1	117	0.061	0.805
Year x Stratum	1	26.2	0.014	0.907
Study area x Year x Stratum	1	3,914	2.04	0.155
Error	211	1,924		

a. Test of homogeneity of variances.

Table 7. Sample statistics for skewness (g_1) and kurtosis (g_2) of length-frequency and biomass distributions of mussels at Montague Island (MI) and Knight Island (KI) in 1996 and 1997, and results of the t-test of significance of the deviation of the statistics from their parametric values under a normal distribution. s_{g1} and s_{g2} are standard errors of g_1 and g_2, respectively; t_s is the estimated t statistic.

Area	Year	g_1	s_{g1}	t_s	P	g_2	s_{g2}	t_s	P
Length-frequency Distribution									
MI	1996	0.866	0.115	7.53	<0.001	0.074	0.229	0.32	n.s.
	1997	0.700	0.119	5.88	<0.001	-0.330	0.237	-1.39	n.s.
KI	1996	1.892	0.093	20.34	<0.001	3.933	0.186	21.14	<0.001
	1997	2.007	0.078	25.73	<0.001	4.802	0.156	30.78	<0.001
Biomass Distribution									
MI	1996	-0.130	0.022	-5.91	<0.001	-0.430	0.044	-9.77	<0.001
	1997	-0.178	0.019	-9.37	<0.001	-0.469	0.038	-12.34	<0.001
KI	1996	0.154	0.023	6.70	<0.001	-1.010	0.046	-21.96	<0.001
	1997	0.544	0.023	23.65	<0.001	-0.482	0.047	-10.26	<0.001

Table 8. Analysis of variance of *Nucella lima* and large seastar[a] densities (No. m^{-2}) in rocky and mixed sediment substrates at Montague Island and Knight Island in 1996 and 1997 in Prince William Sound.

Predator Group Source of Variation	df	MS	F	P
Nucella lima				
Data transformed (log[y+1]); Levene's Test[b], P = 0.05				
Study area	1	0.168	8.98	0.003
Year	1	0.034	1.81	0.18
Stratum	1	2.9×10^{-5}	0.002	0.97
Study area x Year	1	0.020	1.06	0.30
Study area x Stratum	1	4.1×10^{-4}	0.02	0.88
Year x Stratum	1	0.012	0.66	0.42
Study area x Year x Stratum	1	1.6×10^{-4}	0.008	0.93
Error	200	0.019		
Large seastars				
Data transformed ($y^{0.054}$); Levene's Test[b], P < 0.001				
Study area	1	0.164	1.18	0.28
Year	1	0.633	4.53	0.04
Stratum	1	3.63	26.0	<0.001
Study area x Year	1	0.057	0.41	0.52
Study area x Stratum	1	0.365	2.61	0.11
Year x Stratum	1	0.038	0.28	0.60
Study area x Year x Stratum	1	0.470	3.36	0.07
Error	200	0.140		

a. Includes *Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata.*

b. Test of homogeneity of variances.

Table 9. Mean percent feeding and day 60 running mean feeding rate of *Nucella lima* and *N. lamellosa* on *Mytilus trossulus* in a 60-d laboratory experiment. N_D, number of days feeding observations were made. Means are presented with standard errors of the mean as $\bar{x} \pm SE$. Feeding rates are means of three replicates.

	N. lima			*N. lamellosa*		
Mussel length class (mm)	N_D	Feeding (%) $\bar{x} \pm SE$	Feeding rate (mussels snail^{-1} day^{-1}) $\bar{x} \pm SE$	N_D	Feeding (%) $\bar{x} \pm SE$	Feeding rate (mussels snail^{-1} day^{-1}) $\bar{x} \pm SE$
5 - 20	58	35.6 ± 1.7	0.189 ± 0.005	56	22.9 ± 1.8	0.171 ± 0.019
20.1 - 40	58	32.5 ± 1.7	0.045 ± 0.004	56	22.7 ± 1.2	0.056 ± 0.011
> 40	58	36.9 ± 1.5	0.019 ± 0.003	56	9.8 ± 1.1	0.004 ± 0.004

Table 10. Total annual consumption of *Mytilus trossulus* in three size classes by *Nucella lima* and *N. lamellosa* at Montague Island (MI) and Knight Island (KI) in 1996 and 1997.

			Individual annual consumption (No. snail^{-1} yr^{-1})				Total mussels consumed (No. yr^{-1})			
Year	Study area	Predator	MLC1[a]	MLC2	MLC3	Total	MLC1	MLC2	MLC3	Total
1996	MI	*N. lima*	16.5	5.4	0.05	22.0	3.5×10^6	1.1×10^6	1.1×10^4	4.7×10^6
		N. lamellosa	17.1	5.6	0.06	22.8	7.1×10^5	2.3×10^5	2.3×10^3	9.5×10^5
		Nucella spp.	-	-	-	-	4.2×10^6	1.4×10^6	1.4×10^4	5.6×10^6
	KI	*N. lima*	27.4	2.8	0.06	30.3	4.3×10^6	4.4×10^5	9.6×10^3	4.8×10^6
		N. lamellosa	26.1	2.6	0.06	28.8	2.5×10^7	2.5×10^6	5.5×10^4	2.8×10^7
		Nucella spp.	-	-	-	-	2.9×10^7	3.0×10^6	6.5×10^4	3.2×10^7
1997	MI	*N. lima*	13.3	6.0	0.16	19.2	2.7×10^6	1.2×10^6	3.3×10^4	4.0×10^6
		N. lamellosa	13.0	5.9	0.16	19.0	1.1×10^5	4.9×10^4	1.3×10^3	1.6×10^5
		Nucella spp.	-	-	-	-	2.8×10^6	1.3×10^6	3.5×10^4	4.2×10^6
	KI	*N. lima*	31.7	1.9	0.01	33.6	1.4×10^7	8.1×10^5	5.9×10^3	1.4×10^7
		N. lamellosa	30.1	1.8	0.01	31.9	2.8×10^7	1.6×10^6	1.2×10^4	2.9×10^7
		Nucella spp.	-	-	-	-	4.1×10^7	2.4×10^6	1.8×10^4	4.4×10^7

a. Mussel length classes (MLCs) were: MLC1, 5-20 mm; MLC2, 20.1-40 mm; MLC3, >40 mm.

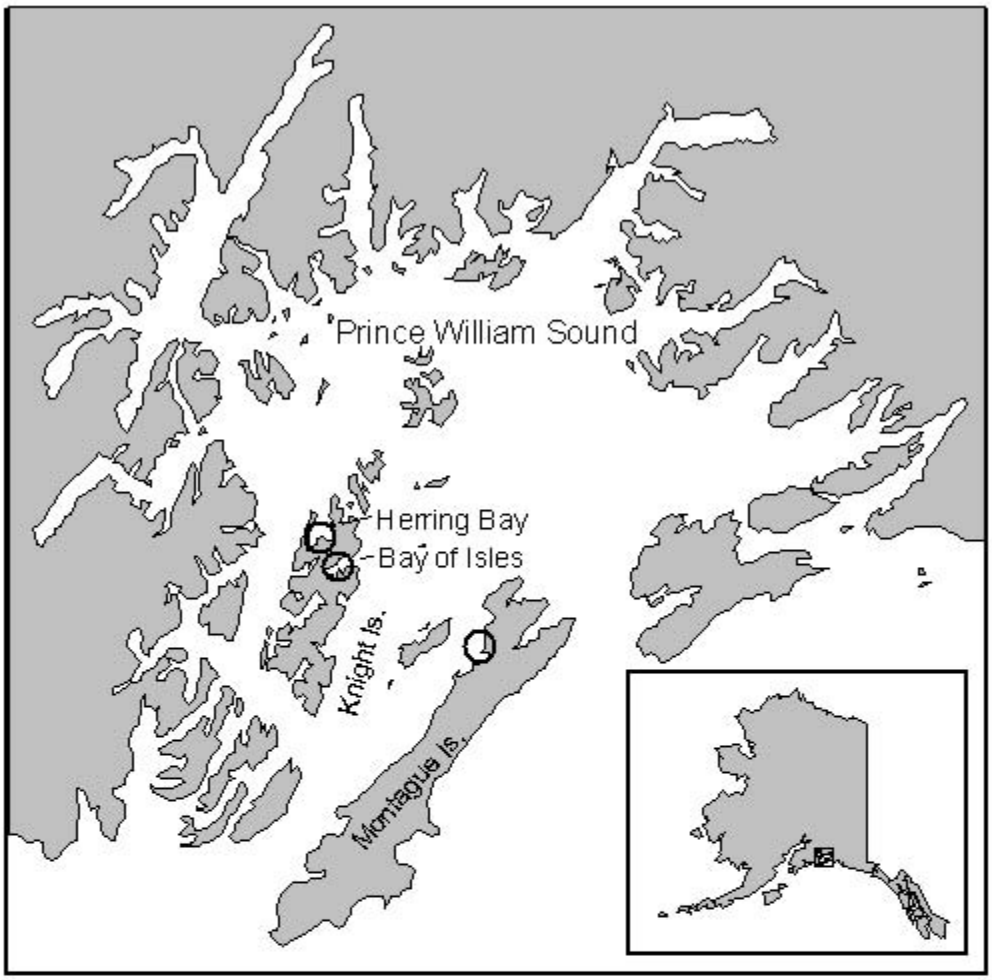

Figure 1. Location of study areas (circled) in Prince William Sound, Alaska.

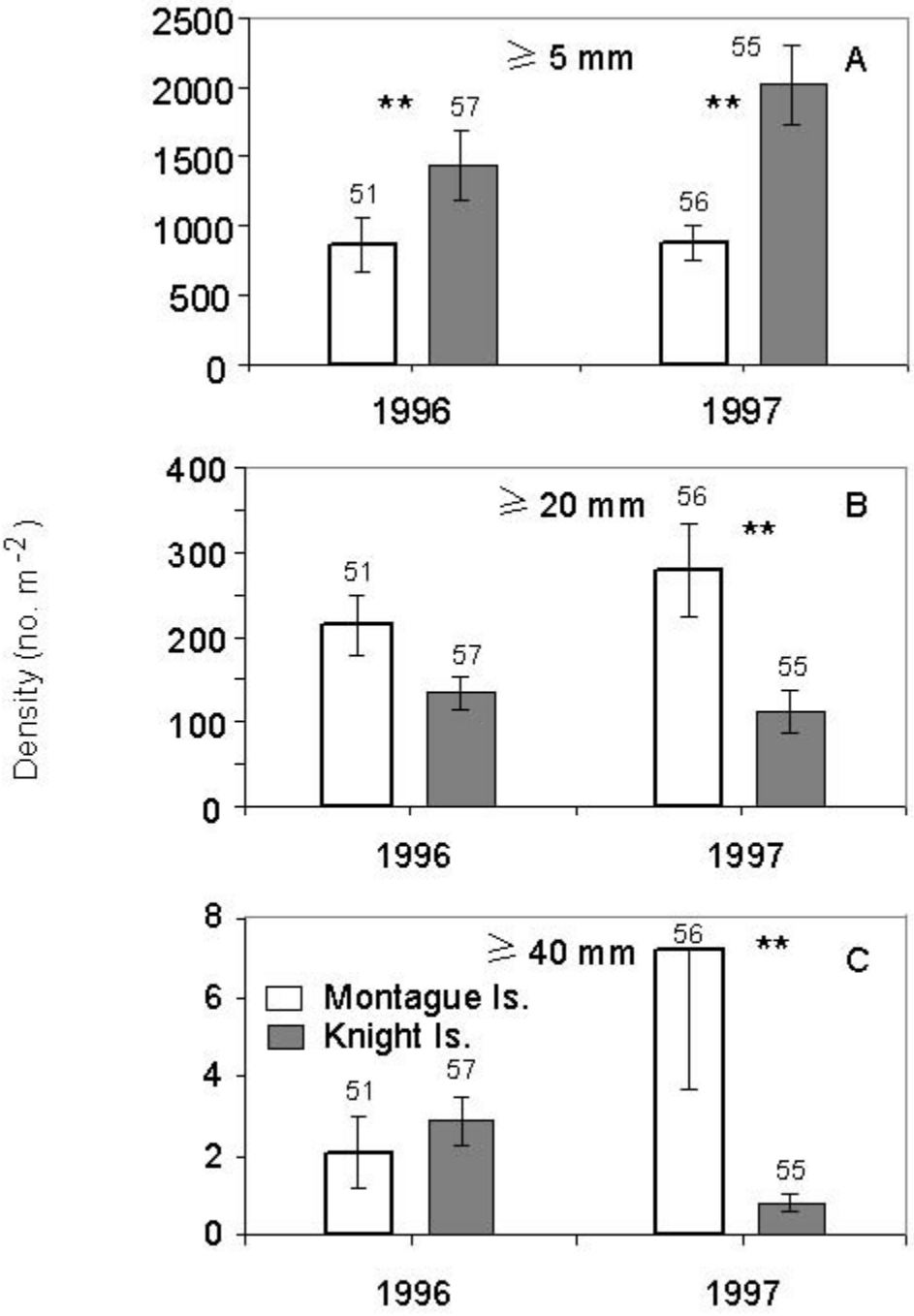

Figure 2. Mean density of mussels ≥ 5 mm (A), ≥ 20 mm (B) and ≥ 40 mm (C) in shell length at Montague Island and Knight Island in May-July 1996 and 1997. Error bars are one standard error of the mean. Numbers above the bars are sample sizes (no. of shore segments). **, $p<0.01$.

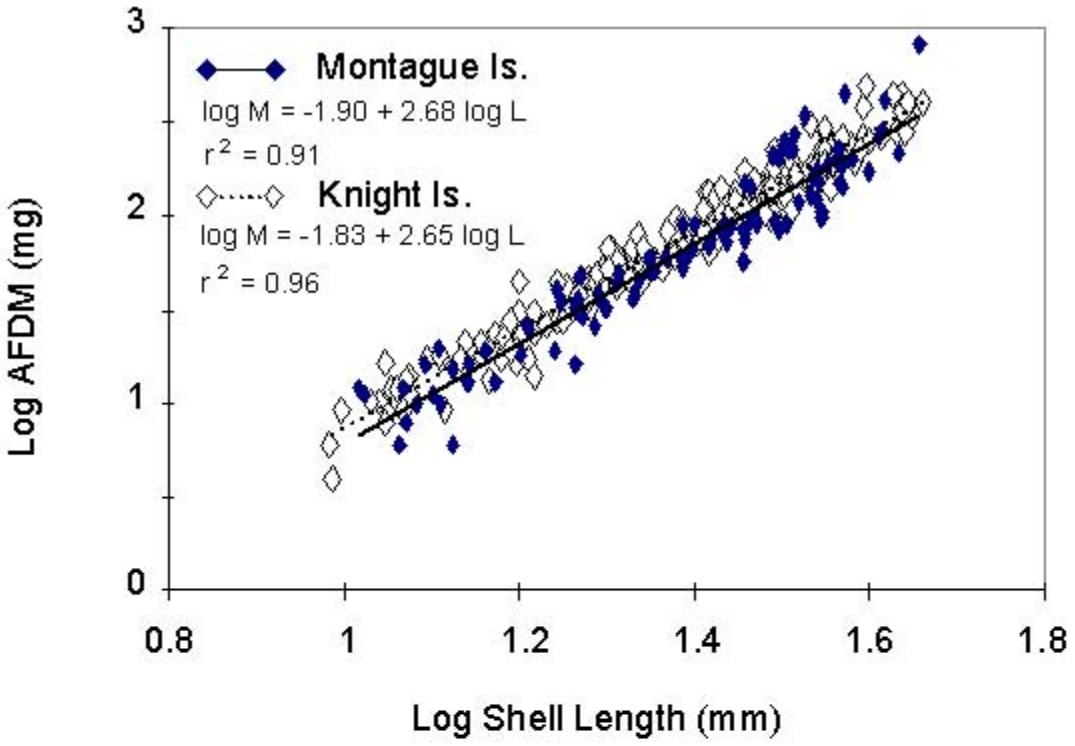

Figure 3. Regression of mussel ash-free dry mass (AFDM) with shell length (log scale) for Montague Island and Knight Island. Regression equation and the coefficient of determination (r^2) are shown for each location.

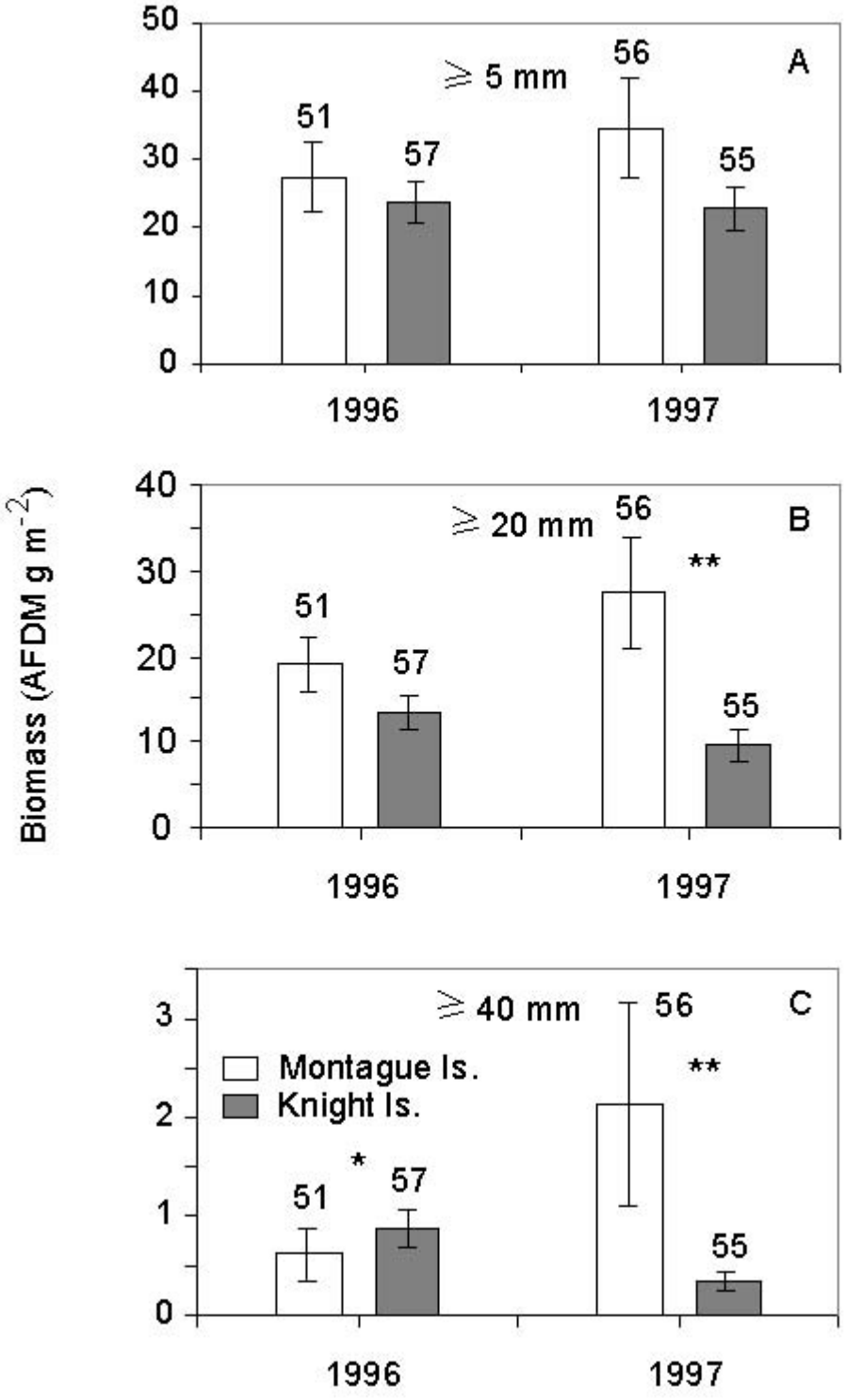

Figure 4. Mean biomass (ash-free dry mass) of mussels ≥ 5 mm (A), ≥ 20 mm (B) and ≥ 40 mm (C) in shell length at Montague Island and Knight Island in May-July 1996 and 1997. Error bars are one standard error of the mean. Numbers above the bars are sample sizes (no. of shore segments). *, $p<0.05$; **, $p<0.01$.

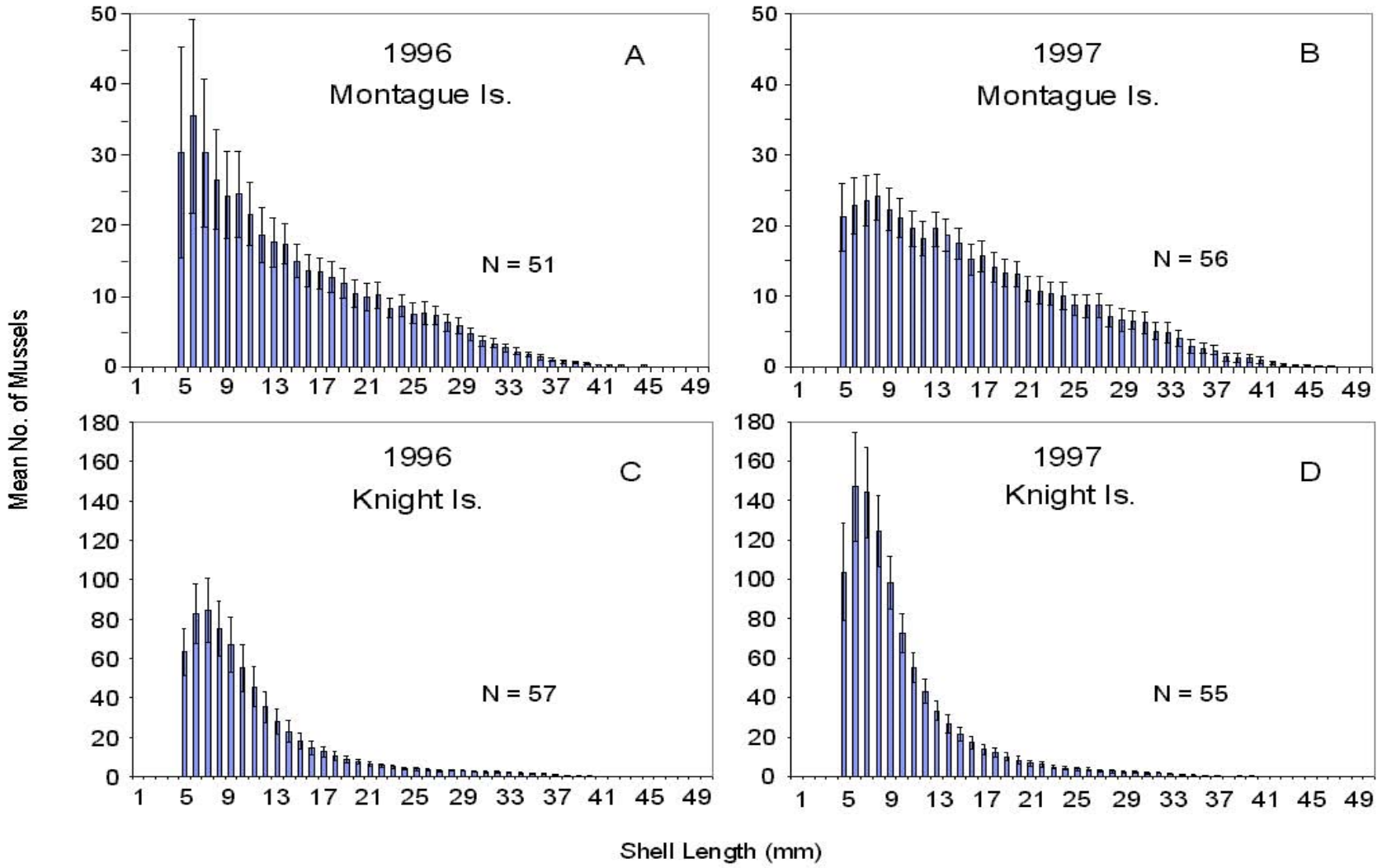

Figure 5. Length-frequency distribution of mussels ≥ 5 mm in shell length at Montague Island and Knight Island in 1996 and 1997. Error bars are one standard error of the mean. N = no. of shore segments sampled. Number of mussels measured was 20,722 (A), 24,693 (B), 37,710 (C) and 53,861 (D).

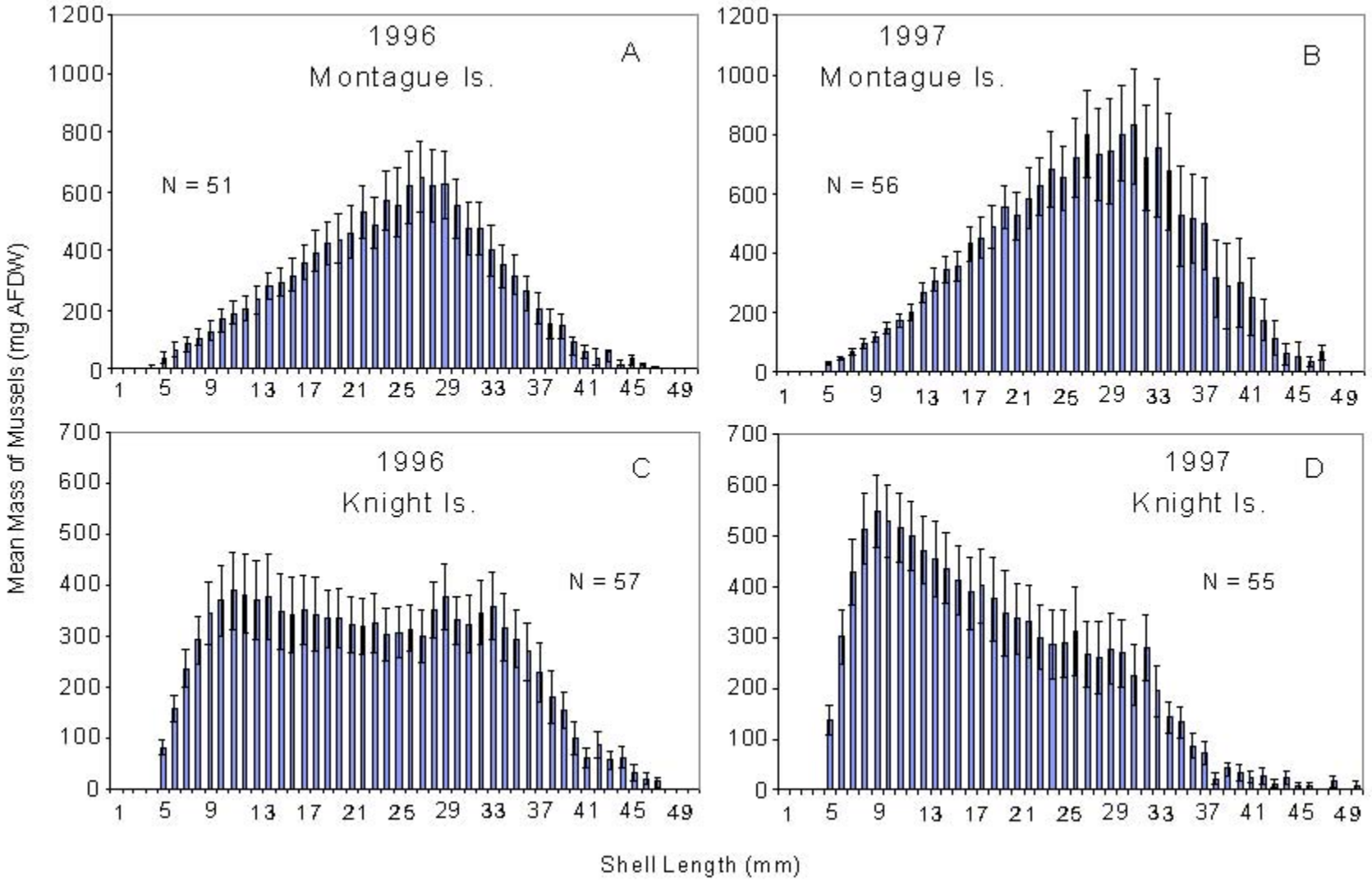

Figure 6. Distribution of mussel mass with shell length for mussels ≥ 5 mm in shell length at Montague Island and Knight Island in 1996 and 1997. Error bars are one standard error of the mean. N = no. of shore segments sampled. Number of mussels included was 20,722 (A), 24,693 (B), 37,710 (C) and 53,861 (D).

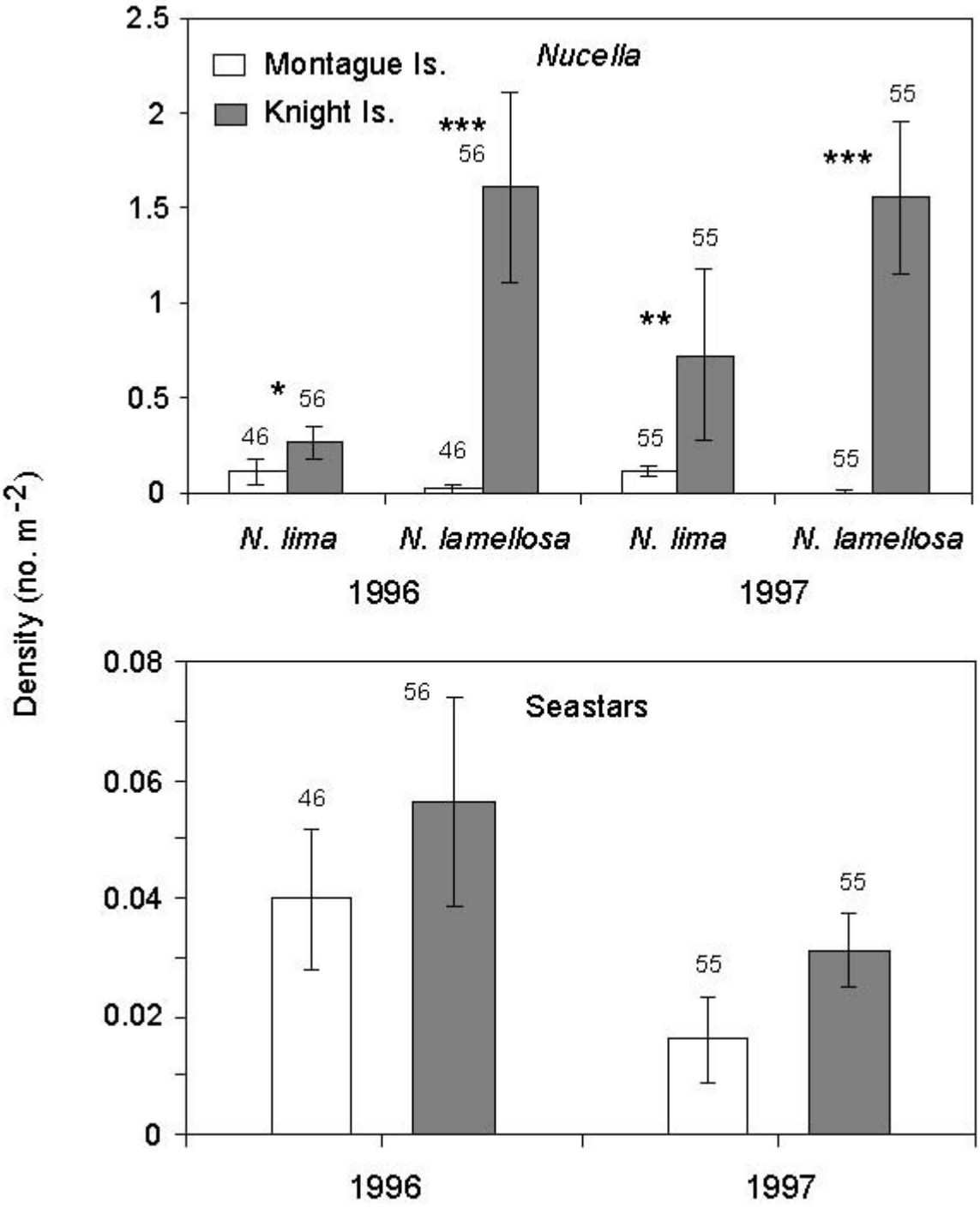

Figure 7. Mean density of *Nucella lamellosa* and *N. lima* and large seastars (*Evasterias troschelii*, *Pisaster ochraceus*, *Pycnopodia helianthoides*, and *Dermasterias imbricata*) at Montague Island and Knight Island in May-July 1996 and 1997. Error bars are one standard error of the mean. Numbers above the bars are sample sizes (no. of shore segments). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

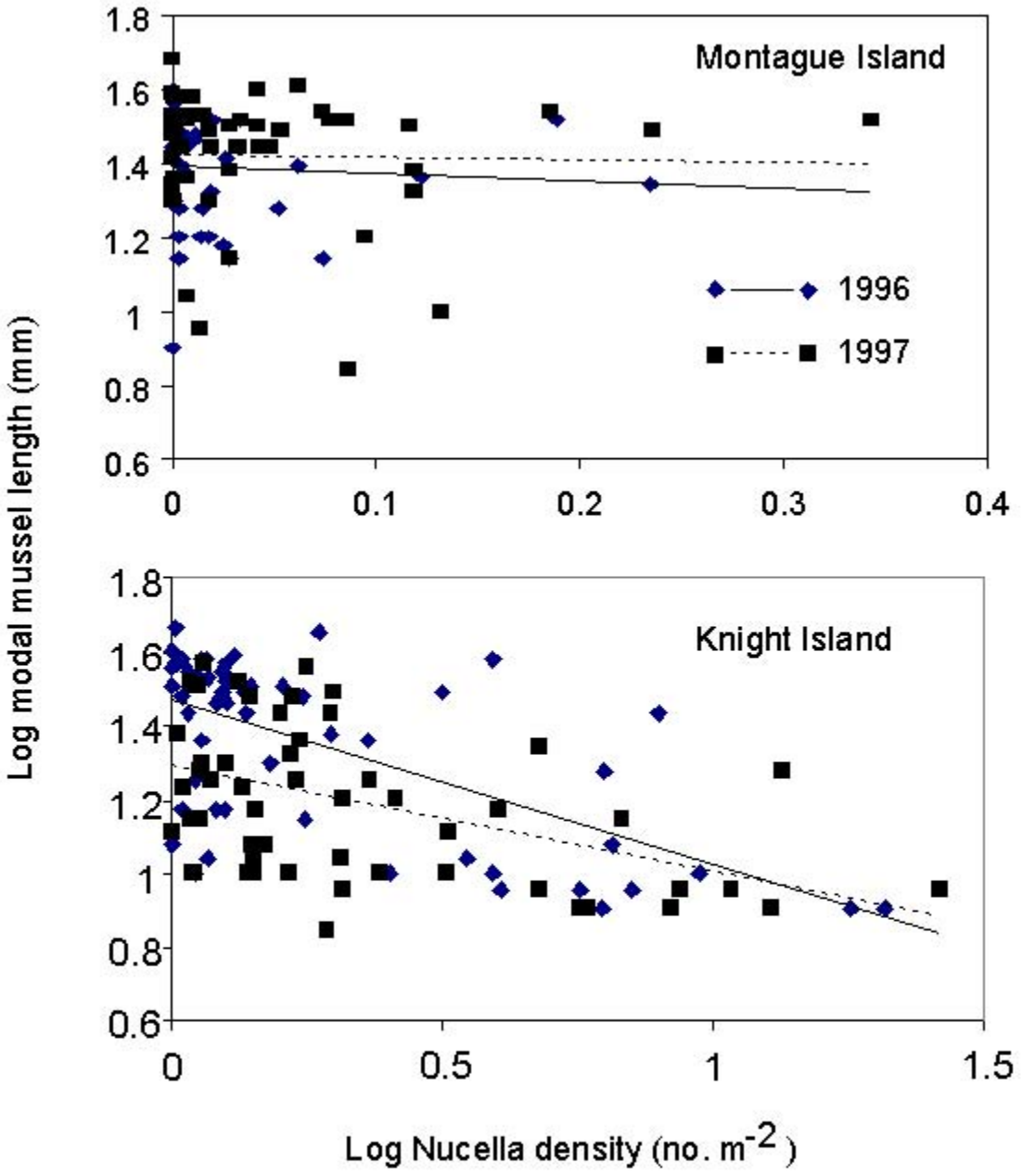

Figure 8. Correlation of the modal length of the mussel length-biomass distribution with the density of *Nucella lima* and *N. lamellosa* (grouped) at study sites on Montague Island and Knight Island in 1996 and 1997.

Appendix SO-06

Gage, T. K. 1998. Effects of invertebrate predators on clam populations in Prince William Sound, Alaska, with implications for the recovery of sea otters from the *Exxon Valdez* oil spill. M.S. Thesis, University of Washington, Seattle, Washington. (Copy available at Alaska Resources Library and Information Services, 3150 C Street, Suite 100, Anchorage, AK 99503 [907] 272-7547.)

Abstract: The abundance of sea otters (*Enhydra lutris*) in some areas of Prince William Sound, Alaska, has not yet recovered from the effects of the *Exxon Valdez* oil spill (EVOS). One possible explanation for the lack of sea otter recovery is the limited availability of food. I studied the effects of predatory benthic invertebrates (sea stars, snails, crabs) on the dynamics of clam populations, the primary prey of sea otters in Prince William Sound. I evaluated the hypothesis that high rates of clam consumption by predatory invertebrates are limiting the size of clam populations in oiled areas and, consequently, the local recovery of sea otters from EVOS. Field observations and laboratory studies were conducted to estimate the significance of clams in the diets of invertebrate predators.

I collected data on density, diet, and activity of predatory invertebrates intertidally and subtidally at 4–10 m depth in four bays. Two of the bays were oiled by EVOS and two were unoiled. I found the sea star *Pycnopodia helianthoides* to be the most abundant predatory benthic invertebrate in all four bays. Densities of *Pycnopodia* were not significantly different between oiled and unoiled areas. Published literature suggests broad overlap in diets of *Pycnopodia* and sea otters. My data, however, indicate that *Pycnopodia* in Prince William Sound have a diverse diet composed primarily of gastropods too small to be of significant nutritional value to sea otters. Clams were present in the diet of *Pycnopodia*, but at very low numbers in all areas. Clam species and size categories typically consumed by sea otters in Prince William Sound were poorly represented in the sampled *Pycnopodia* diet.

Laboratory studies were conducted to determine feeding times of *Pycnopodia* on the clam *Protothaca staminea*. *Protothaca* and other venerid bivalves are common in Prince William Sound and are preferred prey of sea otters. Separate studies were conducted with small (15–25 mm) and large (40–50 mm) clams. Studies showed that *Pycnopodia* less than 10 cm in radius were unable to consume large clams. Feeding times of larger *Pycnopodia* on large clams decreased as the size of *Pycnopodia* increased. Feeding times of *Pycnopodia* on small clams are variable. Density and dietary data collected in Prince William Sound were combined with laboratory feeding times to estimate feeding rates of *Pycnopodia* in Prince William Sound. *Pycnopodia* were estimated to consume less than 7.7% and 2% per year of the available large and small clams in Prince William Sound, respectively.

I conclude from the field and laboratory data that predatory invertebrates are not consuming clams at high rates in Prince William Sound. Therefore, invertebrate predators do not appear to be limiting the local recovery of sea otters from damage caused by EVOS by competing for the same resource.

Appendix SO-07

Dean, T. A., J. L. Bodkin, S. C. Jewett, D. H. Monson, and D. Jung. 2000. Changes in sea urchins and kelp following a reduction in sea otter density as a result of the *Exxon Valdez* oil spill. Marine Ecology Progress Series 199:281–291.

Abstract: Interactions between sea otters (*Enhydra lutris*), sea urchins (*Strongylocentrotus droebachiensis*), and kelp were investigated following the reduction in sea otter density in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill in 1989. At northern Knight Island, a heavily oiled portion of the Sound, sea otter abundance was reduced by a minimum of 50% by the oil spill, and from 1995 through 1998 remained at an estimated 66% lower than in 1973. Where sea otter densities were reduced, there were proportionally more large sea urchins. However, except in some widely scattered aggregations, both density and biomass of sea urchins were similar in an area of reduced sea otter density compared with an area where sea otters remained about 10 times more abundant. Furthermore, there was no change in kelp abundance in the area of reduced sea otter density. This is in contrast to greatly increased biomass of sea urchins and greatly reduced kelp density observed following an approximate 90% decline in sea otter abundance in the western Aleutian Islands. The variation in community response to a reduction in sea otters may be related to the magnitude of the reduction and the non-linear response by sea urchins to changes in predator abundance. The number of surviving sea otter may have been high enough to suppress sea urchin populations in Prince William Sound, but not in the Aleutians. Alternatively, differences in response may have been due to differences in the frequency or magnitude of sea urchin recruitment. Densities of small sea urchins were much higher in the Aleutian system even prior to the reduction in sea otters, suggesting a higher rate of recruitment.

Harlequin Duck (*Histrionicus histrionicus*) Appendices

(HD)

APPENDIX HD-01

CORRELATES OF HARLEQUIN DUCK DENSITIES DURING WINTER IN PRINCE WILLIAM SOUND, ALASKA: HABITAT ATTRIBUTES, HISTORY OF OIL CONTAMINATION, AND FOOD[1]

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ABSTRACT

We assessed sources of variation in Harlequin Duck (*Histrionicus histrionicus*) densities during winters 1995-1997 in areas of Prince William Sound, Alaska contaminated by the 1989 *Exxon Valdez* oil spill and in a nearby unoiled area. Habitat attributes that explained variation in duck densities included distance to streams and reefs, the degree of exposure to wind and wave action, and the dominant substrate type. We suggest that, on a broad scale, winter habitats of harlequin ducks are linked to their life history requirements for predictable environments to ensure high survival and adequate foods to meet high energetic demands. Finer scale habitat use presumably reflects an optimization process balancing costs and benefits of specific habitat features. After accounting for effects of habitat attributes, densities were consistently lower in oiled areas than unoiled, suggesting that population recovery from the oil spill was not complete, due either to lack of recovery from initial oil spill effects or continuing deleterious effects. Prey density and abundance were not strongly related to duck densities after accounting for habitat and area effects, although prey may influence harlequin duck densities as mediated through relationships of habitat attributes and prey density or abundance. Also, prey density and prey availability per duck were similar between oiled and unoiled areas, suggesting that food was not limiting harlequin duck population recovery from the oil spill, although we had low power to detect differences. Harlequin duck life history traits suggest that winter food availability is unlikely to limit populations under natural conditions. High levels of winter site fidelity, a reflection of the predictable environments of wintering harlequin ducks, likely affected our

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[1]Published: 2000. Condor 102:920–926.

results. High philopatry may be related to lower densities than expected in oiled areas due to chronic, residual oil spill effects on the same local aggregations of birds or due to low rates of immigration to enhance wintering groups with depressed numbers due to past oil spill effects.

Key Words: density, *Exxon Valdez* oil spill, food, habitat, Harlequin Duck, *Histrionicus histrionicus*, population recovery.

INTRODUCTION

Within a species, densities of birds vary among locations at every scale from biomes to microhabitats, in large part in response to variation in biotic and abiotic attributes of the environment. Use of habitats is thought to reflect an optimization process, in which birds select combinations of habitat attributes that lead to maximized fitness (MacArthur and Pianka 1966, Rosenzweig 1985). This process presumably seeks to balance benefits (e.g., energy intake) against risks (e.g., from predation or weather). Site fidelity also may influence bird distribution (Robertson and Cooke 1999) and individuals may return to a particular area despite changes in habitat quality (Hilden 1965, Cooch et al. 1993).

During winter, waterfowl must meet a variety of costs (e.g., maintenance, feather synthesis, courtship, mate defense, acquisition of nutrient reserves for reproduction), while balancing mortality risk. Most members of the seaducks tribe (Mergini) winter in the harsh, but relatively stable, nearshore marine environments of north temperate and subarctic latitudes. Seaducks typically exhibit life histories in which annual productivity is low and variable and reproductive life spans are long (Goudie et al. 1994). This strategy requires low rates of mortality during nonbreeding periods (Stearns 1992, Sæther et al. 1996) and, therefore, these species must select winter habitats that meet immediate demands and also confer a high likelihood of survival.

Within their holarctic distribution, Harlequin Ducks (*Histrionicus histrionicus*) are inextricably linked to nearshore marine environments during the nonbreeding portion of the annual cycle (Robertson and Goudie 1999). Adults leave coastal areas only for a few summer months, when they migrate to fast-moving streams to nest and raise broods (Robertson 1997; Cooke et al. 2000). Despite the importance of nearshore areas for Harlequin Duck populations, finer scale quantifications of winter habitat associations have rarely been conducted (Goudie and Ankney 1988).

In March 1989, the *Exxon Valdez* ran aground, spilling nearly 42 million L of oil into Prince William Sound, the wintering area for approximately 14,000 Harlequin Ducks (Lance et al. 1999). As much as 40% of the spilled oil was deposited in intertidal and subtidal zones of Prince William Sound (Galt et al. 1991, Wolfe et al. 1994), the areas used by Harlequin Ducks. Although much of the oil degraded and dissipated within a few years of the spill, some residual oil was still present in these areas during the course of our study (Hayes and Michel 1999). Immediate bird mortality from the *Exxon Valdez* oil spill was high (Piatt et al. 1990) and more than 1,000 Harlequin Ducks were estimated to have died as a direct result of the immediate effects of the spill (John Piatt, pers. comm.). Further, there have been concerns about continued effects of the *Exxon Valdez* oil spill on Harlequin Duck populations and lack of full population recovery (*Exxon Valdez* Oil Spill Trustee Council 1999).

We studied Harlequin Duck habitat associations in Prince William Sound during winter to: (1) identify environmental variables that correspond to high Harlequin Duck densities during winter, to better understand the habitat requirements of the species in light of life history requirements for high survival; and (2) to assess the status of Harlequin Duck population recovery from the *Exxon Valdez* oil spill. Evaluation of Harlequin Duck population recovery from the oil spill has been constrained by a paucity of prespill data from winter, the most important period for Harlequin Ducks in Prince William Sound and the "core" subpopulations

from a population structure perspective (Cooke et al. 2000). For this aspect of the study, we adopted a control-impact study design to assess potential oil spill effects, in which we compared densities of Harlequin Ducks between oiled and unoiled areas, recognizing the need to control for intrinsic area differences (Wiens and Parker 1995). Habitats within Prince William Sound are diverse, making it necessary to segregate effects of history of oil contamination from other environmental factors. Lower densities than expected on oiled areas (after accounting for other factors) could result from either failure to recover from immediate population impacts or from continuing deleterious effects of the spill; either case would lead to an interpretation of lack of full population recovery.

METHODS

Study Area

Located on the southcentral coast of Alaska, Prince William Sound encompasses about 39,000 km^2 including 4,800 km of shoreline. A temperate rainforest dominated by Sitka spruce (*Picea sitchensis*) and western hemlock (*Tsuga heterophylla*) covered most of the convoluted shoreline and islands in Prince William Sound. Temperatures seldom dropped below -20° C and, except for a few coves and lagoons, most areas remained ice-free during winter. Tidal amplitude averaged about 4 m. Predominant nearshore vegetation included rockweed (*Fucus distichus*), eelgrass (*Zostera marina*), and scattered offshore bull kelp (*Nereocyctis luetkeana*). All areas were undeveloped and human disturbance was low. Hunting was negligible for Harlequin Ducks in Prince William Sound.

Study locations were within oiled and unoiled areas of Prince William Sound (Figure 1). The oiled study area included 2 bays on Knight Island, Herring Bay and Bay of Isles, which were heavily oiled by the *Exxon Valdez* spill. The unoiled area was in the Stockdale Harbor and Port Chalmers region of northwestern Montague Island, which was selected because of the close proximity to the oil spill zone, thus minimizing area differences beyond habitat attributes (e.g., climate).

Harlequin Duck Surveys

We conducted surveys of Harlequin Duck numbers and distribution during 4-12 December 1995, 12-24 February 1996, 4-14 December 1996, and 14-23 February 1997, completing 5 replicates on Knight Island and 7 on Montague Island. Surveys involved a census of the area within 200 m from shore. Survey craft were staffed by a 2-3 person team consisting of a boat operator/observer and at least one observer/data recorder. We mapped locations and flock sizes of all Harlequin Ducks on mylar overlays of aerial photos. Locations were digitized using a geographic information system (GIS).

To generate average Harlequin Duck densities associated with each site at which habitat variables were measured (see below), we calculated the number of ducks detected during shoreline censuses within 200 m linear shoreline distance of the midpoint of each sampling site using GIS. Duck densities were expressed as the average number of birds within the designated shoreline segment over the replicate surveys.

Habitat Attributes

To select sites for sampling habitat attributes, the shoreline of each study area was divided into 200 m sampling units. From randomly selected start points, 216 sites (113 on Knight Island and 103 on Montague Island) were systematically selected for sampling during summers of 1996 and 1997.

For each site, a number of habitat variables were documented that we felt could be related to winter Harlequin Duck densities, including: *exposure* - a description of wind and wave action, categorized as full exposure, partial exposure, and not exposed; *dominant strata* - substrate type, categorized as rocky (bedrock and boulder areas) and mixed (unconsolidated or various mixtures of sand, pebbles, and cobble); *distance to stream mouth* - straight line distance from the midpoint of the sampling site to nearest stream mouth measured by GIS and categorized as <200 m, 200-500 m, 500-1000 m, and > 1000 m; *distance to reef* - straight line distance from the midpoint of the sampling site to the nearest offshore reef measured by GIS and categorized as 200-500 m, 500-1000 m, and > 1000 m; and *intertidal slope* - the average slope (in degrees) of the mussel zone. Observations with missing data for a habitat variable were excluded from habitat association models including that variable.

Habitat Association Models

We conducted general linear model analyses using SAS (SAS Institute Inc., Cary, North Carolina, USA) to assess relationships of habitat attributes (explanatory variables) to average Harlequin Duck densities (the response variable), using each sampling site as an observation. In examination of scatterplots of Harlequin Duck densities by habitat and food variables, we found that the distributions violated the assumption of linearity; by conducting a square root transformation of Harlequin Duck densities, the assumption of linearity was met, therefore we used the square root of average Harlequin Duck densities in all subsequent regression analyses. Categorical variables were included as a set of 1/0 indicator variables, with one level of each variable designated as the reference level and, thus, not included in model selection procedures (Ramsey and Schafer 1997).

We took two approaches to describing habitat relationships to duck densities and then assessing effects of oiling history after accounting for effects of habitat features. Under the first approach (Option A), we first analyzed data from each area independently to determine habitat attributes related to Harlequin Duck densities. For each area, we used Mallow's C_p values to direct model selection in data-based model selection context (Burnham and Anderson 1998). This method contrasts a number of models and uses the principle of parsimony to determine which model is best fit by the data (Hilborn and Mangel 1997), avoiding assumptions and biases of traditional stepping (i.e., forward, backward, and stepwise) model selection procedures (Anderson et al. 1994, Flack and Chang 1987). We then selected models that best explained variation in duck densities for both areas combined, using any variable that was included (i.e., explained significant variation) in the best-fitting model for either area along with interaction terms for each variable and area. Finally, an area (oiling history) term was added to each of the best-fitting models for both areas combined to determine whether oiling history explained additional variation in the data, i.e., variation beyond that already explained by the habitat

variables. We believe this approach was conservative with regard to assessing an effect of history of oiling because it accounted for any possible intrinsic area differences prior to assessment of oil history effects and, in fact, could have attributed some variation due to oiling history to other habitat attributes if these attributes differed by area.

In the second approach (Option B), areas were not analyzed separately and area (oiling history) was included during initial model selection procedures. Explanatory variables included in the analysis included all habitat parameters, their interactions with area, and an area (oiling history) term. This approach was simpler than the first analysis but allowed less control over confounding effects of area differences in Harlequin Duck densities related to habitat differences between areas and area differences due to history of oil contamination.

The Role of Food

Diets of Harlequin Ducks in marine areas consist primarily of intertidal and shallow subtidal benthic invertebrates, in particular amphipods, limpets, snails, chitons, and mussels (Vermeer 1983, Goudie and Ankney 1986, Gaines and Fitzner 1987, Goudie and Ryan 1991, Patten et al. 1998). Because prey abundance or density may influence seaduck distribution (e.g., Stott and Olson 1973, Guillemette et al. 1993), we estimated these parameters within each study area to assess relationships of prey availability to duck densities and compare measures of prey availability between areas.

To sample intertidal blue mussels (*Mytilus trossulus*), we established 10 transects perpendicular to the shoreline at 20 m intervals within each sampling site. We removed all mussels from within a 500 cm^2 quadrat placed at a randomly selected location along each transect and recorded the width of the mussel zone. Mussels were sorted by size class and counted. Lengths of mussels >5 mm were measured. Ash-free dry mass of mussels were determined using a muffle furnace.

Sampling for Harlequin Duck foods other than mussels was conducted at a subset of 15 of the systematically selected shoreline sites in each area. Because of generally low densities of Harlequin Ducks on Knight Island, 4 additional sites with relatively higher Harlequin Duck densities were selected to ensure that sampling represented the full range of Harlequin Duck densities. Similarly, 4 sites with moderate to low duck densities were added on Montague Island. Nonrandom sites were used in general linear model analyses, but not for characterization of the study areas. Samples were obtained at 3 locations at each of 2 depths (0.5 to -0.5, -0.5 to -1.5 mean lower low water [MLLW]) along each of the 200 m shoreline sites. At each location, divers collected and bagged all algae or eelgrass and scraped all visible epifauna from the substrate and airlifted them into a mesh bag. Epifauna were later scraped from algae and eelgrass, combined with epifauna from substrate, sorted, and identified to 7 prey types (limpets, chitons, lacunid snails, littorine snails, other snails, crustaceans, and amphipods). Samples from all locations within a site were pooled. Ash-free dry weights of prey were determined using a muffle furnace. Only ash-free dry weights of foods <25 mm were included in estimates of biomass, as these are the size-classes of prey likely taken by Harlequin Ducks.

For data analyses, prey data were included in 4 forms: *total food density* - the combined average densities (g/100 m^2) of mussels in the mussel zone and other prey items in the 0.5 to -1.5 m MLLW zone; *total food abundance* - an estimate of the biomass (kg ash-free dry mass) of

all food types within the 200 m sampling site, based on expansion of food densities to the areas of the mussel zone for mussels and the 0.5 to -1.5 m MLLW zone for other prey; *food density without mussels* - because biomass estimates of mussels were considerably higher (usually more than an order of magnitude) than other prey types, yet they constitute a relatively small part of the diet of Harlequin Ducks, we also used density estimates excluding mussels; *food abundance without mussels* - similarly, we used prey abundance estimates excluding mussels.

To examine effects of prey density and abundance on Harlequin Duck distributions, we assessed additional variation in duck densities related to food variables after accounting for habitat and area effects as determined by habitat association modeling (see above). We regressed residuals (observed Harlequin Duck densities - predicted densities) from the 5 best-fitting habitat association models of Options A and B against the 4 measures of prey abundance and density. This was not a powerful, direct test of the effects of food, as much of the variation related to food was likely accounted for by habitat attributes, as prey abundance and habitat were likely correlated. We took this approach, however, because sample sizes for sites with habitat measures only were several times higher than those for sites that included prey data and, thus, provided the best data set for examining correlates of Harlequin Duck density variation. Under this approach, relationships between food density or abundance and duck densities (after accounting for other effects) would suggest that food has an influence beyond that explained by correlations of prey abundance or density and other habitat attributes.

We also compared measures of prey density and abundance between areas to assess the potential role of food limitation to population recovery from the oil spill. Food limitation could constrain population recovery if the oil spill reduced prey availability, either through direct effects or indirectly through alterations of trophic web structure (Peterson 2001). Higher densities of prey or more prey (on a per duck basis; see below) on oiled areas would indicate that food limitation was unlikely. Conversely, higher densities or more prey on unoiled areas would indicate that food limitation might be involved in lack of population recovery. Similar prey density and abundance would be equivocal; however, these interpretations should be viewed with caution, as no studies have directly tested whether Harlequin Duck carrying capacity during winter is set by food availability.

Average food density per site was compared between areas using a t-test. Food abundance was compared in relation to duck abundance, under the premise that assessments of density dependent population limitation require per capita resource availability. We calculated food abundance per duck for each area as the average food abundance per site (for those sites where all prey were sampled) divided by the average duck abundance per site (calculated over all sites). Duck abundance for this calculation was the density over 400 m (as described above) divided by 2, so that both food and duck abundances were on the same scale. Variance was calculated for a ratio of 2 independent estimates (Seber 1973) and 2-tailed *Z* scores were calculated to compare areas (Snedecor and Cochran 1980).

RESULTS

Average Harlequin Duck numbers per site, excluding nonrandomly selected sites, were considerably higher at our unoiled study area than at the oiled area (Table 1) and our intent was to determine whether this was related to habitat differences, differences in history of oil

contamination, or some combination of differences in habitat attributes and oil spill effects. Some aspects of the habitat were distinctly different between areas (Table 1), particularly intertidal slope and dominant strata, with smaller differences apparent for exposure and distance to stream parameters. On both areas, Harlequin Ducks were almost always observed very close to shore, in intertidal and shallow subtidal habitats.

Habitat Association Models

On unoiled Montague Island, Harlequin Duck densities were related to a number of habitat attributes (Table 2). In all 5 of the best-fitting models, densities were positively related with having a reef within 500 m and a stream within 200 m; in 4 of the 5 models, positive associations were described between duck densities and occurrence of a stream within 200 - 500 m. In all models, densities on partially exposed sites were lower than those on unexposed or fully exposed sites and, in 2 of the 5 models, densities were higher on fully exposed sites than unexposed. Intertidal slope and dominant strata did not have consistent, strong effects.

Habitat association modeling using data from Knight Island only resulted in patterns similar to Montague Island (Table 2). For example, in all 5 best-fitting models, duck densities were positively related to having a reef in the 200 - 500 m interval. Also, on Knight Island, 4 of the 5 best models described positive associations between duck densities and occurrence of a stream within 200 m. Full exposure was associated with higher Harlequin Duck densities than for either partially exposed or nonexposed sites. Like on Montague Island, intertidal slope and dominant strata on Knight Island were not strongly or consistently related to Harlequin Duck densities. Similarities in patterns of results between Montague and Knight Islands, in the absence of consideration of area effects, suggest that similar habitat attributes are selected by Harlequin Ducks in both areas, despite overall area differences in the relative occurrence of the habitat attributes (Table 1) and differences in Harlequin Duck abundance (see intercepts; Table 2).

Under option A for both areas combined (Table 3), in which habitat parameters and area interactions were included in model selection prior to inclusion of an area term, there were unambiguous positive correlations in all 5 best-fitting models between Harlequin Duck densities and the closest stream and reef categories, consistent with results for each area independently. Full exposure was positively related to duck densities in all 5 models, although the negative interaction term indicated that the effect of full exposure was primarily expressed on Montague Island; further, negative interactions of area by partial exposure suggested that this habitat attribute was negatively related to duck densities on Knight Island. Mixed strata was positively associated with duck densities, but the stronger negative interaction terms suggested that the relationship was positive on Montague Island and negative on Knight Island. Results for habitat attributes within Option A should be viewed with some caution because, as mentioned in methods, this approach may have resulted in oiling history effects being attributed to habitat parameters due to the lack of an area term in the model selection process.

Relationships of habitat attributes and duck densities for both areas combined were more simply interpreted under Option B (Table 3), in which an area term was included during model selection. Again, the categories for closest reef and stream distances were consistently correlated

with higher Harlequin Duck densities. Full exposure also was related to higher duck densities for all 5 models and, under this Option, there were no associated negative interaction terms, suggesting that the positive relationship with full exposure was expressed on both areas. Negative effects of partial exposure were inconsistent (3 of 5 models) and relatively small. Positive parameter estimates for mixed strata in all models, in association with consistently stronger interaction terms, suggested positive associations of duck densities and mixed strata on Montague Island and negative associations on Knight Island.

Effects of History of Oil Contamination

Irrespective of the Option used for data analysis, area terms were consistently (and significantly under the Option A approach) negative (Table 3). In other words, duck densities were lower on Knight Island than Montague Island after accounting for effects of habitat attributes and differences in these attributes between areas, which we interpret as evidence that history of oil contamination was related to Harlequin Duck densities.

The Role of Food

Regressions of duck density residuals from habitat association models against 4 measures of food density and abundance gave exactly consistent results from all 5 best-fitting models of both Options A and B; therefore, we present results using residuals from Option A, Model 1 as representative of patterns in our data. Duck density residuals were not related to total food abundance (R^2 = 0.0011, F = 0.0338, df = 30, P = 0.8553), total food density (R^2 = 0.0004, F = 0.0114, df = 31, P = 0.9157), or food abundance without mussels (R^2 = 0.0450, F = 1.6945, df = 36, P = 0.2013; Figure 2). Food density without mussels was positively correlated with duck density residuals (R^2 = 0.1902, F = 8.6922, df = 37, P = 0.0055). However, this positive relationship was highly influenced by a single observation (Figure 3), a site on oiled Knight Island that was nonrandomly selected to represent high duck densities and which also had high densities of subtidal foods (especially snails and amphipods); without this observation, the relationship was nonsignificant (R^2 = 0.0734, F = 2.8513 df = 36, P = 0.0999). Taken together, these analyses suggest that variation in food data explained little variation in duck densities beyond that explained by habitat attributes. Food abundance or density may be important determinants of duck densities but, if this is the case, correlations between habitat attributes and food measures were sufficient to explain most of this variation.

Estimates of prey density and prey abundance per duck were similar between Montague and Knight Islands (Table 4); no statistical differences were detected between areas, although variation around these estimates was high and, thus, power to detect biologically meaningful differences was low. These data are somewhat equivocal with regard to assessing the role of food limitation on population recovery, particularly given the associated broad confidence intervals, although the similarities between areas for point estimates of all measures suggests that food availability on Knight Island was not dramatically lower as a result of the oil spill.

DISCUSSION

Habitat Relations to Harlequin Duck Densities

We found that winter Harlequin Duck densities were related to a number of the habitat attributes that we measured. We assume that habitat associations that we observed were related to habitat profitability and reflected, to some degree, solutions to the optimization process of balancing benefits of habitats against detrimental aspects (Abrahams and Dill 1989, Guillemette et al. 1993). This balance is influenced by structural and functional characteristics of the species (Hilden 1965), such as the life history requirement for high winter survival, as well as high levels of philopatry (see below), in the case of Harlequin Ducks.

Occurrence of a stream within 200 m was consistently and positively related to Harlequin Duck densities, both for each area independently and for both areas combined. Presence of a stream may influence prey distribution and, also, may provide freshwater to reduce osmotic stress for birds that ingest salts while feeding on marine invertebrates (Nyström and Pehrsson 1988). Similarly, a nearby reef was always positively associated with Harlequin Duck densities for both areas separately and when areas were combined. Reefs serve as safe haul-out sites and also offer intertidal foraging opportunities. Fully exposed sites tended to have higher Harlequin Duck densities than partially exposed or unexposed sites. Fully exposed sites may have higher productivity, and hence higher prey abundance, than less exposed sites, although birds at these sites also may be more vulnerable to deleterious weather effects. We were unable to conduct surveys during foul weather when birds may have moved to less exposed sites.

Relations of Harlequin Duck densities to strata type varied by area, with tendencies of positive associations of duck densities with mixed strata on Montague Island but negative associations on Knight Island, suggesting that substrate type is not an important aspect of harlequin duck winter habitat. Intertidal slope was not related to Harlequin Duck densities in most models; we had predicted negative associations resulting from duck responses to increased foraging areas in areas with shallower slopes. Lack of a relationship suggested that more foraging area does not correspond to higher numbers of ducks; in turn, this may indicate that food abundance does not limit harlequin duck populations (see below).

Few other studies have quantified winter Harlequin Duck habitat associations. Goudie and Ankney (1988) documented that Harlequin Ducks were closer to shore and used reefs more than other seaduck species in Newfoundland. Harlequin Duck winter habitats have been qualitatively characterized by a number of authors and have been consistently described as being very close to shore and in a varied mix of substrates (Fleishner 1983, Gaines and Fitzner 1983, Hirsch 1980, Vermeer 1983), in agreement with our findings.

Harlequin Duck habitat use and life history are inextricably linked. Among ducks, Harlequin Ducks are long-lived and have low and variable annual productivity (Goudie et al. 1994), a life history that requires high survival. High survival, in turn, depends on selection of stable and predictable habitats (Stearns 1992). On a broad scale, coastal habitats are thought to offer more stable wintering environments for waterfowl than inland sites (Diefenbach et al. 1988). Within coastal habitats, Harlequin Ducks occupy the narrow intertidal and shallow subtidal zone, a productive part of the marine environment. Goudie and Ankney (1986) described Harlequin Ducks as living near an energetic threshold as a result of their small body

size and relatively harsh wintering environments. As a result, Harlequin Ducks must forage nearly continuously during daylight hours of winter (Fischer 1998, Goudie and Ankney 1986) and, thus, require habitats with readily available and predictable food. Although benthic intertidal communities can vary in species composition over time, invertebrate biomass in intertidal and subtidal zones is generally high and stable. The generalist diet of Harlequin Ducks is thought to reflect their need for continuous foraging and, hence, reduced latitude for prey selectivity (Goudie and Ankney 1986). Harlequin Duck foraging strategy is reflected in their habitat use. Use of shallow water reduces dive and search times for more time efficient foraging (Guillemette et al. 1993). Some selected habitat attributes may correspond to higher prey density or quality (e.g., fully exposed sites). Use of areas near streams and reefs may reduce energetic costs and time of transit between foraging areas and other resources (e.g., fresher water, haul-out sites). In summary, Harlequin Ducks must use habitats that predictably allow them to meet daily energy costs within their time-limited foraging regime, while minimizing risk of mortality in concordance with their life history requirement for high survival probabilities.

Effects of History of Oil Contamination

We found that after accounting for effects of habitat attributes, history of oil contamination from the *Exxon Valdez* spill was related to duck densities, with densities lower on oiled Knight Island than would be predicted based on the habitat attributes that we measured. Our data were consistent with a hypothesis that Harlequin Duck populations were not fully recovered from the oil spill. We acknowledge that area differences also could be related to factors that we did not measure, although our intent at the onset of this study was to measure all intrinsic attributes that we suspected could be related to Harlequin Duck densities. We are unaware of other factors that we did not include that might be important and that are not closely correlated with the attributes that we measured.

Evidence from other studies is consistent with a hypothesis that Harlequin Duck populations were experiencing continued effects of the *Exxon Valdez* oil spill during the course of this study. Trust et al. (2000) concluded that Harlequin Ducks and the ecologically similar Barrow's goldeneye (*Bucephala islandica*) continued to be exposed to oil through 1998, based on higher induction of cytochrome P450 1A in oiled areas than unoiled. Also, Harlequin Duck adult female survival during winters 1995-1998 was lower on oiled areas than unoiled (Esler et al., unpubl. ms.) and lab studies support logical links between reduced survival rates and oil exposure (Holmes et al. 1978, 1979). Because population dynamics of birds with life histories like Harlequin Ducks are particularly sensitive to variation in adult female survival (Goudie et al. 1994, Schmutz et al. 1997), lower survival on oiled areas may have led to population declines (Rosenberg and Petrula 1998) and hence lower densities on oiled areas than predicted, as found in this study. Harlequin Duck populations likely have relatively low intrinsic growth rates, so full recovery (i.e., duck densities at levels predicted from intrinsic habitat attributes) likely will not occur until long after deleterious effects of the oil spill have ceased.

Day et al. (1997) conducted an avian habitat use study in Prince William Sound in the period immediately following the *Exxon Valdez* spill (1989 - 1991). They concluded that Harlequin Ducks showed negative relationships to oiling intensity during summer through 1990 but recovery by summer 1991. Of more relevance for comparison to this study, Day et al. (1997)

found no oil spill effects during winter. Why were our results different from those of Day et al. (1997)? First, because deleterious effects of the oil spill continued through the period of our study and until at least 1998 (Trust et al. 2000, Esler et al., unpubl. ms., Rosenberg and Petrula 1998), differences in Harlequin Duck abundance relative to history of oil contamination may have been more pronounced during our study than during the studies of Day et al. (1997). Also, Day et al. (1997), presumably by necessity due to their broader study question to look at all marine birds over a wider geographic area, used bays as sampling units and characterized habitats at the scale of the entire bay. Our study demonstrated that Harlequin Ducks respond to much smaller scale variations in habitat attributes. Also, Harlequin Ducks exhibit high fidelity to specific shoreline segments (Robertson et al. 1999, Cooke et al. 2000). Therefore, we were able to account for differences in environmental attributes at the scale that Harlequin Ducks select habitats before testing for relationships to history of oil contamination, allowing for a finer scale and presumably more powerful test.

The Role of Food

Food has been suggested to be related to distribution and abundance of some seaducks (e.g., Nilsson 1972, Stott and Olson 1973, Guillemette et al. 1993). In our study, food did not explain additional variation in duck densities beyond habitat attributes; however, because habitat and prey distribution were likely related, our approach limited our ability to directly assess effects of food on harlequin duck densities. Also, because of logistical difficulties associated with quantification of subtidal prey, the number of sites sampled was relatively small and, in association with high variability in food measures, resulted in low power for detecting effects of food.

Characteristics of Harlequin Ducks suggest that they may be more time-limited than food-limited. Energetic requirements of this small-bodied seaduck result in nearly continuous feeding during daylight hours of winter and a generalist diet that includes many common benthic invertebrates (Goudie and Ankney 1986). This foraging strategy, particularly in association with high levels of winter site fidelity (see below), suggests that food may be abundant overall, and the crux for Harlequin Ducks is to maximize energy intake during a short daily foraging period. Under this scenario, Goudie and Ankney (1986) predicted that Harlequin Ducks would preferentially take prey with high energy densities but would maximize intake by limiting search times and consuming any prey they encounter. We also would predict that Harlequin Ducks would forage in shallow areas with high prey densities, as these attributes would increase habitat profitability by reducing time spent searching during dives (Guillemette et al. 1993). Our data are somewhat consistent with this prediction, as we found that Harlequin Ducks foraged in very shallow water and food density was slightly, positively related to duck densities after accounting for habitat effects (Figure 2); again, habitat effects may have accounted for considerable variation in prey density prior to this analysis. Some authors (Nilsson 1972) have found that food exploitation by wintering diving ducks was small relative to standing crop; we suggest that this is likely the case for Harlequin Ducks, given their foraging requirements.

We found no strong evidence that food availability was limiting Harlequin Duck population recovery from the *Exxon Valdez* oil spill (Table 4). We recognize that we had low power to detect differences between areas. However, as described above, Harlequin Duck life

history and foraging strategy suggest that winter food likely is not limiting under natural conditions. In light of this, similar point estimates and variability in food abundance and density between oiled and unoiled areas supports our conclusion of lack of food limitation in oiled areas.

Significance of philopatry

Seaducks have a general pattern of high philopatry throughout their annual cycle (e.g., Savard and Eadie 1989) and a growing body of data suggests that this is true for Harlequin Ducks (Cooke et al. 2000). High philopatry can be an adaptive behavioral strategy in predictable environments (Robertson and Cooke 1999). Harlequin Duck winter habitat use is likely influenced by high levels of philopatry (Cooke et al. 2000), which reflects high stability of nearshore environments coupled with advantages of philopatry, including site familiarity and interannual pair reunion (Robertson and Cooke 1999). High philopatry, in association with life history requirements for high survival, also suggests that winter food is predictably and adequately abundant.

From the perspective of oil spill recovery, high levels of winter site philopatry suggest: (1) if residual oil spill damages exist, birds from oiled areas are vulnerable to spill effects as they return to those areas annually (i.e., these birds are affected disproportionately and are subject to cumulative effects), and (2) if dispersal and movements among areas are limited, recovery of groups of birds in oiled areas can occur only through demographic processes specific to that group (i.e., numbers are not bolstered through immigration from other areas). Lower densities than expected on oiled areas detected in this study may be a result of one or both of these processes.

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Table 1. Summary of winter harlequin duck densities and habitat attributes at sampling sites within Prince William Sound, Alaska, 1995-1997. Data for categorical variables are shown as number of sites and, parenthetically, percentage of sites within each category.

Parameter	Montague Island (Unoiled)	Knight Island (Oiled)
Average (± SE) Harlequin Ducks (ducks/400 m)	2.99 (±0.22); n=103	0.59 (±0.08); n=113
Average (± SE) Intertidal Slope (degrees)	5.80 (±0.38); n=103	25.46 (±1.69); n=112
Exposure		
Full	25 (24.3%)	15 (13.3%)
Partial	35 (34.0%)	49 (43.4%)
None	43 (41.7%)	49 (43.4%)
Dominant Strata		
Rocky	39 (37.9%)	83 (73.5%)
Mixed	64 (62.1%)	30 (26.5%)
Distance to Stream Mouth		
0-200 m	10 (9.7%)	12 (10.6%)
200-500 m	10 (9.7%)	19 (16.8%)
500-1000 m	14 (13.6%)	22 (19.5%)
> 1000 m	69 (67.0%)	60 (53.1%)
Distance to Reef		
200-500 m	10 (9.7%)	8 (7.1%)
500-1000 m	18 (17.5%)	22 (19.5%)
> 1000 m	75 (72.8%)	83 (73.5%)

Table 2. Top 5 models describing relationships of habitat attributes and winter (1996-1998) harlequin duck densities (square root transformed) on Knight Island, which was oiled during the *Exxon Valdez* spill, and unoiled Montague Island, Prince William Sound, Alaska.

		Habitat Model Parameter Estimates[a]									
	Mallow's		Intertidal	Exposure		Strata	Stream Distance (m)			Reef Distance (m)	
Model	C_p	Intercept	Slope	Full	Partial	Mixed	0-200	200-500	500-1000	200-500	500-1000
Montague Island (Unoiled)											
1	4.9062	1.5563	-------[b]	-------	-0.3827	-------	0.4254	0.3770	-------	0.5635	-------
2	5.5172	1.4174	0.0215	-------	-0.3890	-------	0.5220	0.4364	-------	0.5765	-------
3	5.6628	1.4853	-------	0.1930	-0.3144	-------	0.4745	0.3917	-------	0.5097	-------
4	6.0325	1.5891	-------	-------	-0.3659	-------	0.3817	-------	-------	0.6055	-------
5	6.1322	1.2824	-------	0.3749	-0.2320	0.2264	0.4191	0.3407	-------	0.4995	-------
Knight Island (Oiled)											
1	4.5194	0.5013	-------	0.3451	-------	-0.1772	0.3320	-------	-------	0.5022	-------
2	4.5479	0.4546	-------	0.3860	-------	-------	0.2806	-------	-------	0.4971	-------
3	4.7895	0.6406	-0.0044	0.2921	-------	-0.2458	0.2971	-------	-------	0.4614	-------
4	5.0939	0.4229	-------	0.3738	-------	-------	0.2730	-------	-------	0.5351	0.1618
5	5.3358	0.4879	-------	0.3669	-------	-------	-------	-------	-------	0.5035	-------

[a] Reference values for categorical model parameters were: exposure = none; strata = rocky; stream distance = >1000 m; and reef distance = >1000 m.

[b] ------- indicates that the parameter was not selected for inclusion in the model.

Table 3. Top 5 models describing variation in winter (1996-1998) harlequin duck densities (square root transformed) in relation to habitat attributes, interactions of habitat attributes and areas (Montague and Knight Islands, Prince William Sound, Alaska), and area, which was interpreted as an indication of the effect of oil contamination on Knight Island, after having accounted for intrinsic habitat differences between areas. Results of 2 analysis approaches are presented: one in which the area term was added after model selection of habitat terms and habitat by area interactions (Option A) and one in which the area term was included in model selection procedures (Option B).

		Habitat Model Parameter Estimates[a]										Interaction Terms (Area by Parameter)[b,c]					
	Mallow's		Intertidal	Exposure		Strata	Stream Distance (m)			Reef Distance (m)		Partial	Full	Mixed	Stream	Reef	
Model	C_p	Intercept	Slope	Full	Partial	Mixed	0-200	200-500	500-1000	200-500	500-1000	Exposure	Exposure	Strata	200-500 m	500-1000 m	Area[c,d]
Option A																	
1	6.1865	0.7451	-------[e]	0.8761	-------	0.7245	0.2951	-------	-------	0.5168	-------	-0.2431	-0.7677	-1.0521	-------	-------	-0.5652(0.1704);0.0011
2	6.4185	0.7663	-------	0.8422	-------	0.6654	0.2943	0.3084	-------	0.4930	-------	-0.2487	-0.7192	-0.9680	-0.4988	-------	-0.5387(0.1733);0.0021
3	6.6935	0.7713	-------	0.8534	-------	0.7032	0.2716	-------	-------	0.5123	-------	-0.2543	-0.7378	-1.0043	-0.1947	-------	-0.5442(0.1737);0.0020
4	6.8275	0.7255	-------	0.8469	-------	0.7265	0.2874	-------	-------	0.5500	0.1313	-0.2646	-0.7557	-1.0401	-------	-------	-0.5616(0.1703);0.0011
5	7.5336	0.7151	-------	0.9081	0.1001	0.7185	0.3001	-------	-------	0.5112	-------	-0.3171	-0.7697	-1.0262	-------	-------	-0.7320(0.2094);0.0006
Option B																	
1	5.9305	1.1663	-------	0.4459	-------	0.3159	0.3433	-------	-------	0.5095	-------	-------	-------	-0.4784	-------	-------	-0.6861
2	6.1194	1.2644	-------	0.3589	-0.1292	0.2672	0.3298	-------	-------	0.5210	-------	-------	-------	-0.4593	-------	-------	-0.7077
3	6.2173	1.2502	-------	0.3244	-0.1535	0.2693	0.3211	-------	-------	0.5608	0.1518	-------	-------	-0.4482	-------	-------	-0.7131
4	6.6900	1.1404	-------	0.4315	-------	0.3249	0.3385	-------	-------	0.5393	0.1206	-------	-------	-0.4724	-------	-------	-0.6871
5	6.8285	1.2766	-------	0.3443	-0.1459	0.2615	0.3195	-------	-------	0.5410	-------	-------	-------	-0.4340	-------	0.1671	-0.7487

[a] Reference values for categorical model parameters were: exposure = none; strata = rocky; stream distance = >1000 m; and reef distance = >1000 m.
[b] Interactions of area with other habitat attributes were not selected in any model.
[c] Reference value for area = unoiled Montague Island.
[d] Results of Area term for Option A are presented as the parameter estimate (SE); *P* value.
[e] ------- indicates that the parameter was not selected for inclusion in the model.

Table 4. Average (±SE) density and abundance per duck of harlequin duck prey (amphipods, chitons, limpets, snails, and mussels < 25 mm) at sampling sites within Prince William Sound, Alaska, 1997.

Parameter	Montague Island (Unoiled)	Knight Island (Oiled)	*P*
Density (g AFDW[a]/100 m^2)	2030.76 (± 2077.18)	1964.13 (± 2474.37)	0.94 (t = 0.08)
Abundance (kg AFDW/duck)	51.75 (± 61.43)	100.48 (± 194.71)	0.81 (Z = 0.24)
Density w/o mussels (g AFDW/100 m^2)	45.89 (± 39.14)	42.80 (± 29.22)	0.80 (t = 0.251)
Abundance w/o mussels (kg AFDW/duck)	3.84 (± 4.71)	3.23 (± 5.72)	0.94 (Z = 0.08)

[a]Ash free dry weight.

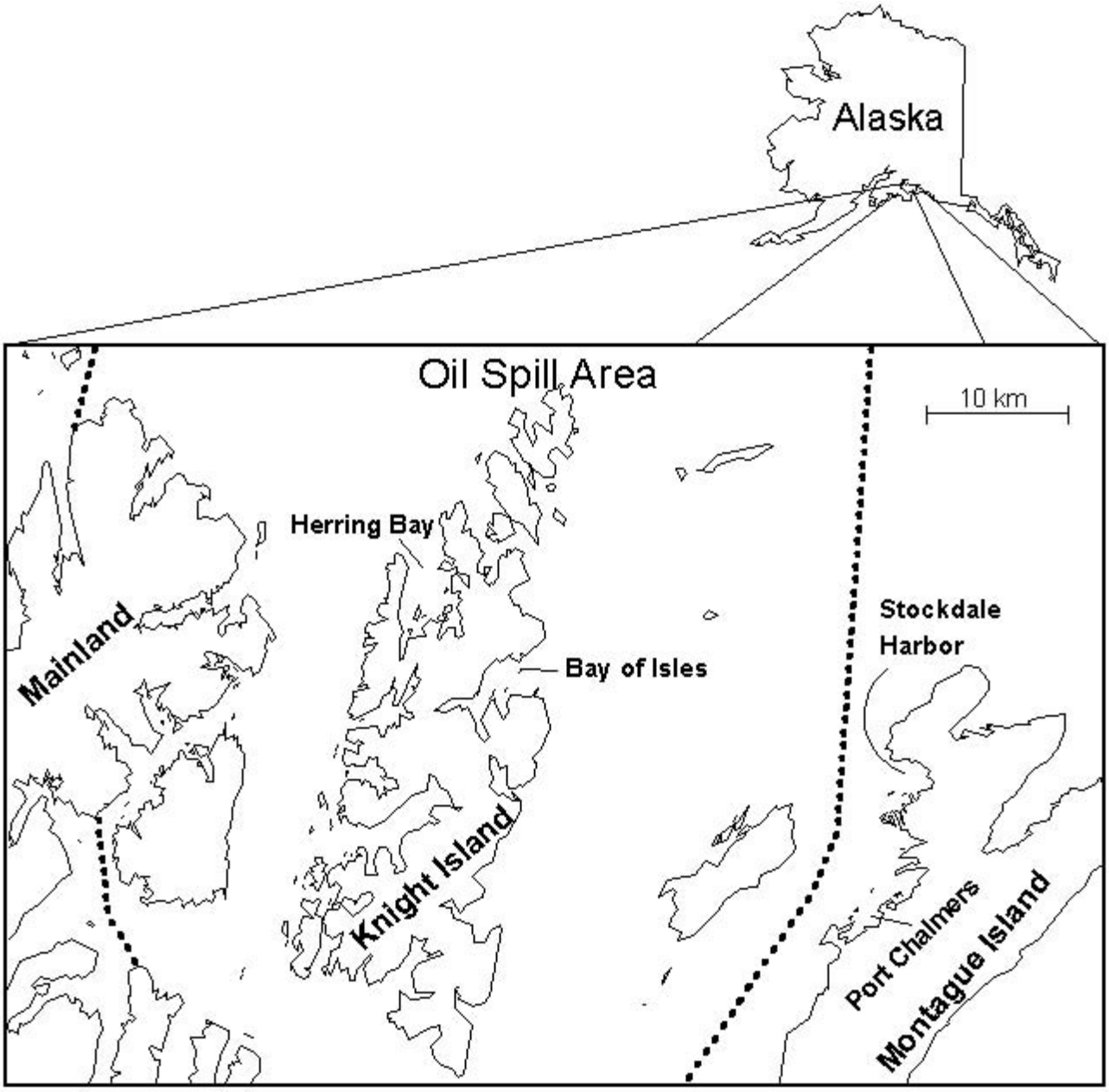

Figure 1. Study areas for assessing variation in winter harlequin duck densities, Prince William Sound, Alaska.

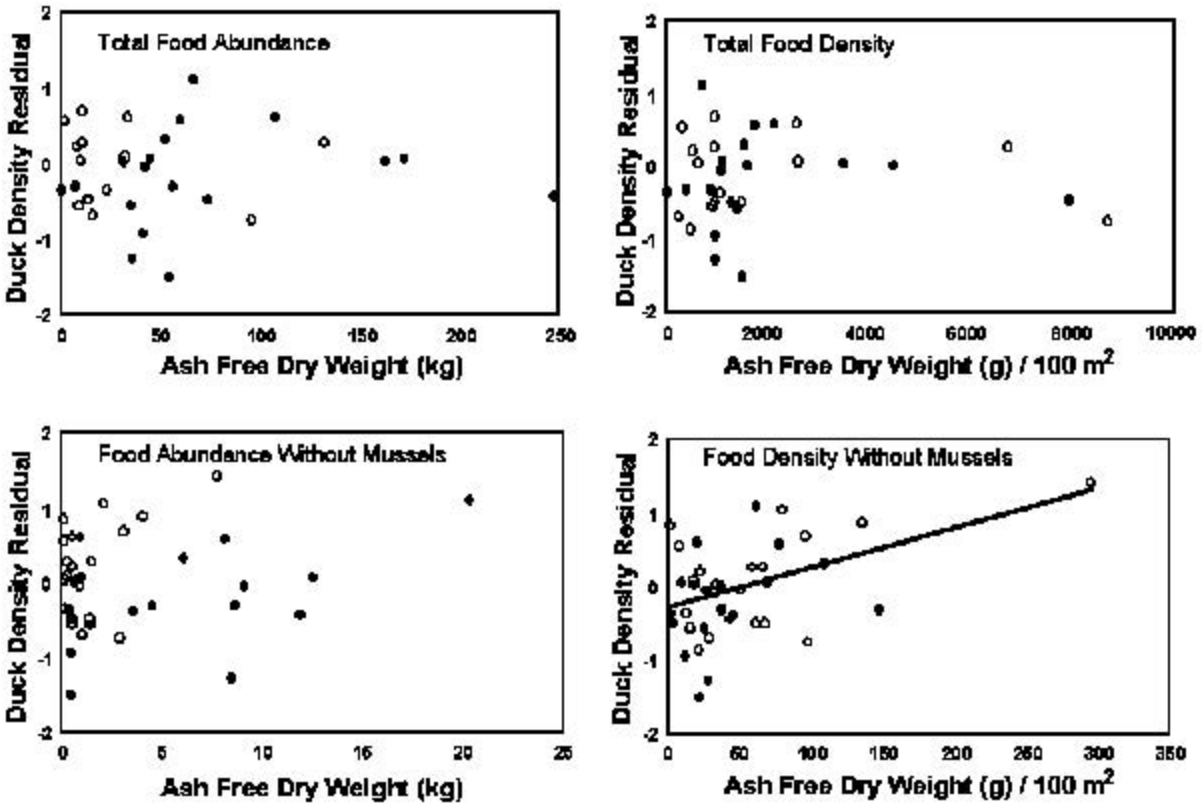

Figure 2. Linear relationships of residuals of harlequin duck densities (ducks/400 m shoreline; square root transformed) from a general linear model of habitat associations (Option A, Model 1, including area term) against measures of prey abundance and density. Open circles represent Knight Island (oiled) study sites and closed circles represent Montague Island (unoiled) sites.

APPENDIX HD-02

WINTER SURVIVAL OF ADULT FEMALE HARLEQUIN DUCKS IN RELATION TO HISTORY OF CONTAMINATION BY THE *EXXON VALDEZ* OIL SPILL[1]

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Abstract: Harlequin duck (*Histrionicus histrionicus*) life history characteristics make their populations particularly vulnerable to perturbations during nonbreeding periods. The 1989 *Exxon Valdez* oil spill was a significant perturbation to harlequin duck nonbreeding habitats in Prince William Sound, Alaska, which resulted in population injury. We used radiotelemetry to examine survival of adult female harlequin ducks during winters of 1995-1996, 1996-1997, and 1997-1998, to assess the progress of population recovery from the oil spill, and to evaluate potential continuing constraints to full recovery. We implanted 294 harlequin ducks (154 and 140 in oiled and unoiled areas, respectively) with transmitters and tracked their signals by airplane from October through March. We examined variation in survival rates relative to area and season (early, mid, and late winter). The 3 best models, as determined by comparisons of Akaike's information criterion (AIC_c) values, all indicated that survival of birds in oiled areas was lower than that in unoiled areas. Inclusion of body mass in the 3 best models did not improve their fit. In the model that best fit our data, survival was high in early winter for both areas, was lower during mid and late winter seasons, and was lowest in oiled areas during mid winter. Cumulative winter survival estimated from this model was 78.0% (SE = 3.3%) in oiled areas and 83.7% (SE = 2.9%) in unoiled areas. To assess whether area differences in survival were more likely related to oiling history versus geographic differences unrelated to oiling history, we contrasted a model with our original data set to a similarly structured model in which ducks (n = 75) from Green Island, an oiled site near our unoiled study area with similar intrinsic attributes, were recoded as being from the unoiled area. This alternate model fit less well (ΔAIC_c = 3.94), suggesting that oiling history was related to survival. Based on a demographic model, area differences in survival offer a likely mechanism for observed declines in populations on oiled areas. Other studies have found that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil as much as 9 years after the spill. We speculate that oil exposure, mortality, and population dynamics are linked and conclude that continuing effects of the oil spill restrict recovery of harlequin duck populations.

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Introduction

Harlequin ducks spend most of their annual cycle in nearshore marine environments, with breeding age birds leaving only for a few summer months to nest and raise broods on fast-moving streams (Robertson and Goudie 1999). Populations of harlequin ducks may be particularly sensitive to perturbations to their nonbreeding habitats. Harlequin ducks, like many seaducks, exhibit a life history in which variable and generally low annual reproductive effort is compensated by relatively high adult survival and, thus, long reproductive life spans (Goudie et al. 1994). This type of life history evolves under conditions of predictable and stable nonbreeding environments (Stearns 1992). Further, Goudie and Ankney (1986) described harlequin ducks, which are small-bodied relative to most other seaducks, as existing near an energetic threshold during winter, with little flexibility for increasing caloric intake or relying on stored reserves. While this strategy may be tenable under predictable and stable conditions, it does not accommodate perturbations that result in either decreases in energy acquisition or increases in metabolic costs.

The release of 11 million gallons of crude oil into the waters of Prince William Sound as a result of the March 1989 grounding of the *Exxon Valdez* was a significant perturbation to the nonbreeding habitat of harlequin ducks. As much as 40% of the spilled oil was deposited in intertidal and subtidal zones of Prince William Sound (Galt et al. 1991, Wolfe et al. 1994), the habitats used by harlequin ducks, and some residual oil was still present in these areas during the course of our study (Hayes and Michel 1999). Immediate bird mortality from the *Exxon Valdez* oil spill was high (Piatt et al. 1990) and more than 1,000 harlequin ducks were estimated to have died as a direct result of the immediate effects of the spill (John Piatt, U. S. Geological Survey, pers. comm.). Further, there are concerns that there may be continued, longer-term effects on harlequin duck populations in oil spill-affected areas (*Exxon Valdez* Oil Spill Trustee Council 1999).

This study was part of a program to assess population recovery of harlequin ducks from the *Exxon Valdez* oil spill in Prince William Sound. We focused on adult female survival during winter because (1) population dynamics of long-lived waterfowl species are particularly sensitive to changes in adult female survival (Goudie et al. 1994, Schmutz et al. 1997); (2) harlequin duck populations are likely sensitive to perturbations on wintering areas; and (3) Prince William Sound is primarily used by harlequin ducks during nonbreeding life stages. Paine et al. (1996), in a critique of studies immediately following the *Exxon Valdez* oil spill, recommended that demographic measures likely provide a better assessment of injury than species occurrence or abundance. We agree, and suggest that demographic studies not only serve to assess injury or recovery status, but also can lend insight into the processes and mechanisms underlying any constraints to full recovery.

Methods

As described by Paine et al. (1996), the *Exxon Valdez* oil spill was an imperfect experiment - a one-time perturbation without replication and, as in the case of wintering harlequin ducks, with little prespill data for comparison. Under these conditions, our approach was to compare oiled and unoiled areas, while attempting to minimize or account for differences

between areas that might confound interpretation of oil spill effects (Wiens and Parker 1995). We recognize that our statistical inference is to areas only, and that assessment of oil spill effects is subject to interpretation. We present ancillary data relevant for this interpretation.

Data Collection

This study was conducted in Prince William Sound (60°N, 148°W), the area most affected by the oil spill, during winters of 1995-1996, 1996-1997, and 1997-1998. We used radio telemetry to estimate survival of adult female harlequin ducks captured throughout the oil spill zone and on nearby unoiled Montague Island (Fig. 1).

Harlequin ducks, unlike most waterfowl, undergo wing molt on their marine wintering areas (Robertson and Goudie 1999). We herded flocks of flightless birds into funnel traps using sea kayaks during 20 August to 17 September, 1995-1997, the dates of peak wing molt by adult females. Captured harlequin ducks were removed from the trap, placed in holding pens, and transported by skiff to a larger vessel for processing. All birds were banded with unique U.S. Fish and Wildlife Service aluminum bands. We identified sex based on plumage characteristics and estimated age class by probing bursal depth (Mather and Esler 1999). Body mass ($\pm$ 1 g) was measured on an electronic balance.

Radio transmitters were surgically implanted into adult (after third year) female harlequin ducks using modifications (Mulcahy and Esler 1999) of the procedure described by Korschgen et al. (1996). Surgeries were conducted by veterinarians experienced in avian implant surgeries. Implanted transmitters have been successfully used in waterfowl studies (e.g., Olsen et al. 1992, Haramis et al. 1993), and an increasing body of literature suggests that radio transmitters implanted into wild waterfowl are less disruptive than external methods of attachment (see Esler et al., unpubl. ms.). Specifications of transmitters used in this study were described by Mulcahy and Esler (1999). Birds recovered from anesthesia for at least one hour before being released at the sites of their capture.

Radioed harlequin ducks were monitored approximately weekly from an airplane to determine mortality status and location. Monitoring flights began after the first birds were radioed and continued through the last week of March. Transmitters were equipped with mortality sensors that indicated death of a bird by a doubling of the transmitter pulse rate. Indicated mortalities were confirmed either by recovery of the radio or location of the radio signal in upland habitats, which harlequin ducks do not use during the nonbreeding season. Monitoring of radios for which signals were lost continued through the end of March.

Data Analysis

Unbiased survival estimation using telemetry requires meeting several critical assumptions (Pollock et al. 1989a), including: (1) radioed animals are representative of the population of interest; (2) survival is independent among individuals; (3) radio-marking does not affect survival during the study period; and (4) censoring of animals for which signals are lost is independent of the fate of those animals (i.e., missing animals are no more or less likely to be dead than animals for which fate is known). We felt that the first 2 assumptions were met based on our capture technique and marking regime. We perceived little chance of a systematically

biased sample based on susceptibility to capture, as we often were able to catch most birds within a given shoreline segment. Also, because we were marking only adult females, we felt that survival among individuals was independent beyond shared area effects, e.g., we were not marking both members of a pair or a mother and her offspring. We explicitly tested assumptions 3 and 4 (Esler et al., unpubl. ms.) and found that these were met for our sample.

For each week's sample of relocations, we counted mortalities and numbers of harlequin ducks at risk of mortality, following procedures outlined in Pollock et al. (1989a, 1989b) and Bunck et al. (1995). We used 1 October as the beginning of the data analysis period to ensure that all birds in the sample had survived a 14-day post-surgery censor period (Mulcahy and Esler 1999) and had completed wing molt. We made an *a priori* decision to combine data from all years to assure adequate power for detecting biologically meaningful differences between areas. A small number of birds (n = 6) moved between oiled and unoiled areas during winter; if a bird was detected in a different area for $\geq$ 2 consecutive observations, we included those observations in the at-risk data set for the newly occupied area.

We defined seasons as early winter, midwinter, and late winter, corresponding to the first 9 weeks of data collection, the middle 8 weeks, and the final 9 weeks. Our most general survival model contained 52 parameters, 26 for each area, and corresponded to the Kaplan-Meier method (Pollock et al. 1989a) of computing binomial estimates of survival. Variance estimates for this model were calculated using Greenwood's formula (Pollock 1989a). We examined the effects of season, area, and several season by area interactions on survival by comparing a series of reduced (fewer parameters) models. For all such model comparisons, we constrained survival to be equal among weeks within each season and area. The best model was that with the lowest Akaike's information criterion, adjusted for small sample size (AIC_c) (Burnham and Anderson 1998). The AIC_c balances the goodness-of-fit of the model (from the maximum likelihood) with the number of parameters to be estimated. Survival estimates and variances were calculated by iterative solution of the likelihood using program MARK (White and Burnham 1999).

We also assessed effects of body mass on survival by adding standardized body mass to the best-fitting models as determined above. A reduction in AIC_c value would indicate that the addition of the body mass term resulted in a more parsimonious model and thus that body mass was related to winter survival. Body mass was standardized to account for annual, geographic, and molt stage variation unrelated to our hypothesis of interest by using residuals around a general linear model (Esler et al., unpubl. ms.) as the body mass parameter. Body mass residuals could not be calculated for 12 of the radioed birds, thus model comparisons were conducted excluding these individuals.

Results

At 1 October, the beginning of the survival monitoring period, 294 radio-marked adult female harlequin ducks were included in the sample (154 from oiled areas and 140 from unoiled areas). Kaplan-Meier estimates of cumulative winter survival were 76.6% (SE = 4.0%) on oiled areas and 86.6% (SE = 3.2%) on unoiled (Fig. 2).

We contrasted 11 different models with various area and season combinations (Table 1). The best fitting model (Model 1) was one in which survival varied by season, with estimates higher in early winter than other seasons and lower in oiled than unoiled areas during midwinter

(Table 2). Cumulative winter survival estimated from this model was 78.0% (SE = 3.3%) in oiled areas and 83.7% (SE = 2.9%) in unoiled areas. Two other models had AIC_c values <2 units higher than Model 1. In Model 2, survival varied by season and was lower in oiled areas than unoiled during midwinter (Table 2). In Model 3, survival was high in the fall for both areas, lower and constant during mid and late winter on the unoiled area, and lower on oiled areas than unoiled during mid and late winter, particularly during midwinter (Table 2). These 3 best models all included an area effect, with survival on oiled areas lower than on unoiled areas (Fig. 2). The sum of AIC_c weights for models without an area effect was < 0.05, indicating that area effects were strongly supported by the data. Similarly, seasonal effects were well supported by the data, with survival during early winter consistently higher than in mid and late winter in the top 3 models. Inclusion of body mass increased AIC_c values of Models 1, 2, and 3 ($\Delta AIC_c \geq 0.69$), indicating that mass during wing molt was not strongly related to survival.

A difficulty of this study design was determining whether survival differences between oiled and unoiled areas were more likely related to intrinsic differences (such as habitat, disease, climate, social influences, or predator densities) rather than history of oil contamination. To address this, we looked more closely at data for birds (n = 75) from the Green Island area. Although Green Island was in the oil spill area, it was closer to unoiled Montague Island than to other oiled sites (Fig. 1). Also, habitats and duck densities (Esler, unpubl. data) were similar to the Montague Island study area. We found that the Kaplan-Meier estimate of cumulative survival of birds captured at Green Island (76.8%; SE = 5.7%) was more similar to that for all oiled areas combined than to unoiled Montague Island. We also contrasted a general season by area model (modified Model 8, Table 1; 3 areas = Green Island, other oiled areas, and unoiled Montague Island) to 2 models each with 2 areas (1 model with Green Island pooled with other oiled areas and 1 model with Green Island pooled with Montague Island). The AIC_c for the model with Green Island pooled with other oiled areas was $\geq$ 3.94 units lower than either of the other 2 models, suggesting that oiling history better explains differences in survival between areas than do intrinsic area differences.

Discussion

Winter survival of adult female harlequin ducks was lower on oiled areas than unoiled areas, primarily due to poorer survival during the midwinter period. In both areas, survival during early winter was higher than during mid or late winter. To understand how these estimates of survival might influence population dynamics, we incorporated the overall cumulative winter survival estimates for each area from Model 1 into a harlequin duck population model (Robertson 1997), holding all other parameters constant. The estimate of annual population change (λ) was 0.9464 for oiled areas (i.e., annual population declines of about 5.4%). For unoiled areas, λ = 1.0054, suggesting a relatively stable population. These estimates are consistent with trends estimated from population surveys conducted during fall 1995-1997 (Rosenberg and Petrula 1998). Differences in adult female survival offer a likely mechanism for differences in population trends between areas and, further, poor survival on oiled areas may be responsible for population declines.

Our data suggest that area differences in winter survival are more likely due to history of oil contamination than intrinsic area differences. For oiling history to affect survival

probabilities, and subsequent population trends, there must be some mechanism by which birds from oiled areas are compromised. One potential mechanism is that the immediate effects of the spill or subsequent effects of residual oil resulted in reductions of harlequin duck prey. However, during the period of this study, densities of harlequin duck prey were similar between oiled Knight Island and unoiled Montague Island and winter body mass of female harlequin ducks was similar between oiled and unoiled areas (Esler, unpubl. data), suggesting that differential food abundance did not explain differences in survival between areas.

Exposure to residual *Exxon Valdez* oil is another potential mechanism by which harlequin duck survival could be affected, as oil exposure is known to have deleterious toxic (Leighton 1993) and metabolic (Jenssen 1994) consequences. To determine if harlequin ducks in Prince William Sound were still being exposed to residual oil, Trust et al. (unpubl. ms.) measured induction of cytochrome P4501A (P450), which can indicate exposure to polycyclic aromatic hydrocarbon constituents of crude oil, in harlequin ducks captured during winter 1998 in both oiled and unoiled areas. P450 induction was much higher in harlequin ducks from oiled areas than those from unoiled areas, and Trust et al. (unpubl. ms.) concluded that this was almost certainly due to exposure to residual *Exxon Valdez* oil, as background hydrocarbon levels were negligible in intertidal areas of Prince William Sound prior to the oil spill (Short and Babcock 1996) and PCB levels were low and similar between areas (Trust et al., unpubl. ms.). Further, some residual oil was documented in nearshore habitats contemporary to our study (Hayes and Michel 1999). Finally, P450 results from harlequin ducks are consistent with those from several other nearshore vertebrates from oiled areas (Brenda Ballachey, U.S. Geological Survey, unpubl. data).

Could exposure to residual *Exxon Valdez* oil result in lower survival probabilities and concomitant population declines? Most lab studies have shown that mallards (*Anas platyrhynchos*) are tolerant of internal ingestion of oil, with toxic effects not evident until very high doses. These studies have been used to suggest that harlequin ducks should be unaffected by residual *Exxon Valdez* oil (Stubblefield et al. 1995, Boehm et al. 1996). However, other studies have found that the addition of other stressors such as cold temperatures caused oiled ducks in the lab to suffer considerably higher mortality than unoiled birds (Holmes et al. 1978, 1979). This compounding effect of environmental stress and oil exposure seems to be a more appropriate analog for wild harlequin ducks, which exist under relatively harsh winter conditions with little flexibility for accommodating additive stresses (Goudie and Ankney 1986). Our data indicate that mid and late winter may be stressful periods in the annual cycle of harlequin ducks even under unperturbed conditions, as survival on unoiled areas was lower during these seasons than during early winter.

The divergence of survival probabilities between oiled and unoiled areas during midwinter (Fig. 2) is consistent with a hypothesis of additive effects of oil in the presence of other stressors. Harlequin ducks are sight feeders and, during midwinter when day length is shortest, they spend most of their time foraging (Goudie and Ankney 1986, Fischer 1998). Prince William Sound is one of the farthest north wintering areas for harlequin ducks (Robertson and Goudie 1999), thus day light available for foraging is particularly limited. Because harlequin ducks have little flexibility for accommodating increased energy demands during winter (Goudie and Ankney 1986) that could result from either toxic insults or plumage oiling, they may not be able to handle additive effects of the oil spill, even if relatively small. We speculate that

differences in survival and populations trends may be related to documented differences (Trust et al., unpubl. ms.) in contaminant exposure.

Management Implications

Although populations of some animals may be unaffected or recover rapidly from oil spill effects (Wiens et al. 1996), others, such as harlequin ducks, have characteristics that make them vulnerable to population-level effects of oil spills for years following the event. For harlequin ducks, these characteristics include a life history requiring high adult survival, occurrence in habitats most affected by oil spills and which may hold residual oil for years, adaptation to stable and predictable marine environments, and high site fidelity. These traits also make harlequin ducks, and similar species, vulnerable to chronic, low-level oil pollution (Clark 1984). In the cases of either oil spills or chronic oil pollution, of course, the primary management recommendation is prevention; oil that does not go into the water does not threaten marine bird populations. Unfortunately, for harlequin ducks in the spill-affected area, there is little direct management action that now can improve winter survival. Hunter harvest of harlequin ducks is negligible in Prince William Sound and bag limits were already reduced following the oil spill. The extent of the *Exxon Valdez* oil spill zone is too large to recommend intensive habitat restoration; also, residual oil may be deeply buried in sediment (Hayes and Michel 1999) and oil removal efforts could result in significant disruption of intertidal habitats. Therefore, harlequin duck population recovery in Prince William Sound will depend largely on natural dispersal of residual oil and intrinsic population growth.

Factors that affect wintering aggregations likely are influencing subpopulations that are largely distinct demographic units (Cooke et al. 2000). Winter site fidelity of harlequin ducks is high (Robertson 1997, Cooke et al. 2000) and pair formation occurs on the wintering areas (Gowans et al. 1997, Robertson et al. 1998). Fortunately, levels of dispersal are high enough that subpopulations within the oil spill zone were not genetically distinct (Lanctot et al. 1999), i.e., the oil spill did not threaten a unique genetic resource. However, levels of dispersal are likely low and recovery of groups of birds in oiled areas must occur primarily through demographic processes specific to that group (i.e., numbers are not enhanced through immigration from other areas). Population recovery will require not only time for demographic processes to operate, but also elimination of continuing deleterious oil spill effects. Our data suggest that deleterious effects of the *Exxon Valdez* oil spill were evident as many as 9 years following the spill. Managers must recognize that, while oil spill effects may be short-lived for some species, full population recovery for species like harlequin ducks may require decades. In a broader context, the characteristics of harlequin ducks that make them vulnerable to oil spill effects also make them susceptible to population level consequences of other perturbations during nonbreeding periods, including human disturbance, habitat deterioration, and local overharvest.

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Table 1. Models used to estimate winter survival rates of adult female harlequin ducks in Prince William Sound, Alaska, using various combinations of season (early, mid, and late winter) and area (oiled and unoiled). The best model is that with the lowest Akaike information criterion, adjusted for small sample size (AIC_c; Burnham and Anderson 1998).

Models	Model description[a]	Number of parameters in model	AIC_c weight	AIC_c
1	EWO=EWU, MWO, MWU=LWO=LWU; survival varies between early winter and other seasons, areas differ during midwinter	3	0.314	480.7
2	EWO=EWU, MWO, MWU, LWO=LWU; survival varies among all seasons, areas differ during midwinter	4	0.199	481.7
3	EWO=EWU, MWO, MWU=LWU, LWO; survival varies between early winter and other seasons, areas differ during mid and late winter	4	0.144	482.3
4	EWO=EWU=MWU=LWU, MWO, LWO; survival does not vary seasonally in unoiled areas, areas differ during mid and late winter	3	0.100	483.0
5	EWO=EWU=MWU=LWO=LWU, MWO; survival varies between midwinter on oiled areas and all other season and area combinations	2	0.088	483.3
6	EW, MW, LW, O<>U; survival varies among seasons, with a constant area difference	4	0.074	483.6
7	EWO=EWU, MWO=MWU, LWO=LWU; survival varies by seasons	3	0.046	484.6
8	EWO, EWU, MWO, MWU, LWO, LWU; survival varies by all season and area combinations	6	0.028	485.6
9	EWO=MWO=LWO, EWU=MWU=LWU; survival varies by areas	2	0.004	489.3
10	EWO=EWU=MWO=MWU=LWO=LWU; survival does not vary by season or area	1	0.003	490.1
11	general model; estimates generated for each week and area	52	0.000	516.1

[a]EWO = early winter in oiled areas, EWU = early winter in unoiled areas, MWO = midwinter in oiled areas, MWU = midwinter in unoiled areas, LWO = late winter in oiled areas, and LWU = late winter in unoiled areas.

Table 2. Parameter estimates (SE) for the top 3 models describing adult female harlequin duck survival during winter in Prince William Sound, Alaska. See Table 1 for model descriptions.

Season[a]	Oiled Areas	Unoiled Areas
Model 1		
Early Winter	0.969 (0.012)	0.969 (0.012)
Mid Winter	0.870 (0.031)	0.934 (0.014)
Late Winter	0.925 (0.016)	0.925 (0.016)
Overall	0.780 (0.033)	0.837 (0.029)
Model 2		
Early Winter	0.969 (0.012)	0.969 (0.012)
Mid Winter	0.870 (0.031)	0.953 (0.020)
Late Winter	0.914 (0.021)	0.914 (0.021)
Overall	0.770 (0.034)	0.843 (0.029)
Model 3		
Early Winter	0.969 (0.012)	0.969 (0.012)
Mid Winter	0.870 (0.031)	0.940 (0.017)
Late Winter	0.910 (0.030)	0.933 (0.019)
Overall	0.767 (0.039)	0.850 (0.034)

[a]Seasons are of differing lengths (Early = 9 weeks, Mid = 8 weeks, and Late = 9 weeks).

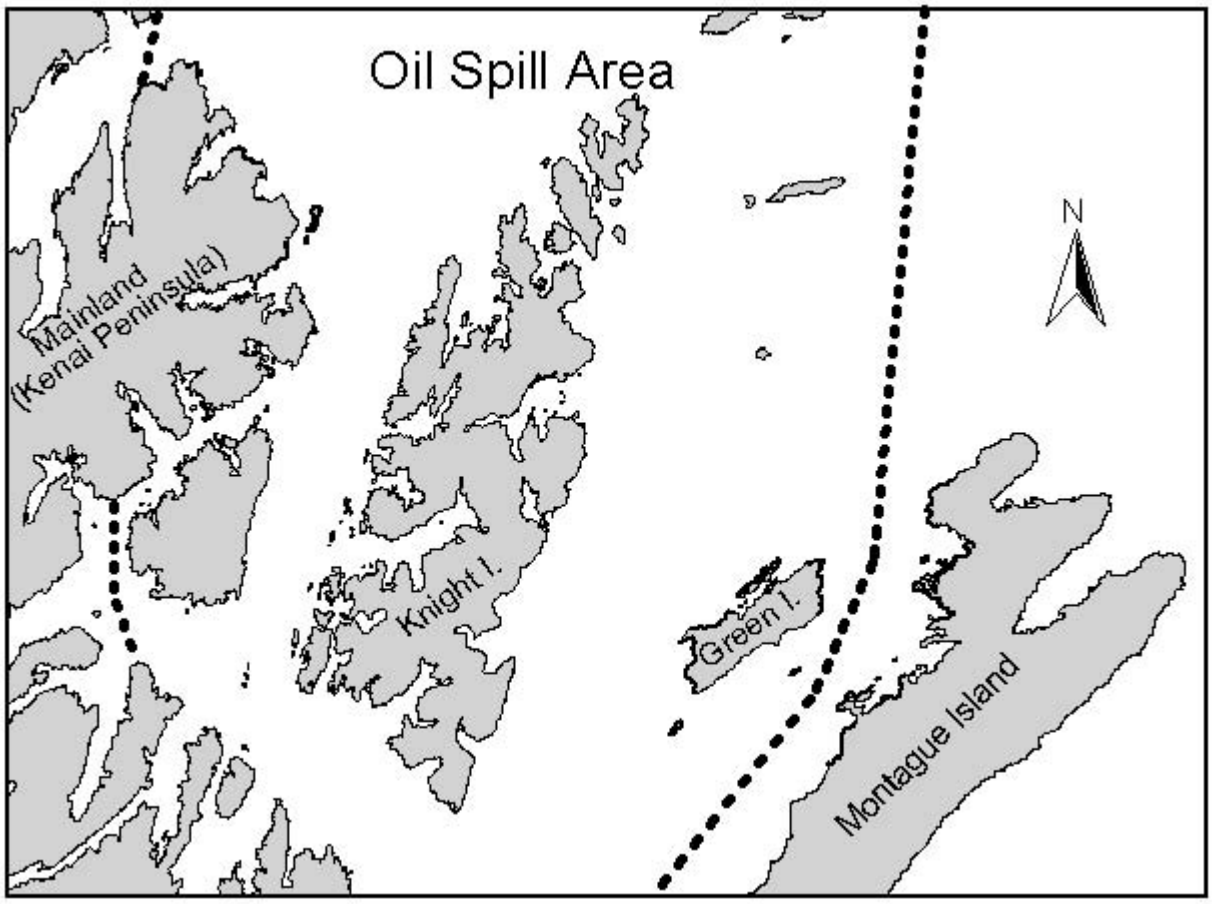

Figure 1. Study sites for estimating survival of adult female harlequin ducks in Prince William Sound, Alaska. Shorelines described by bold lines represent capture areas. The oil spill area is bounded by dashed lines.

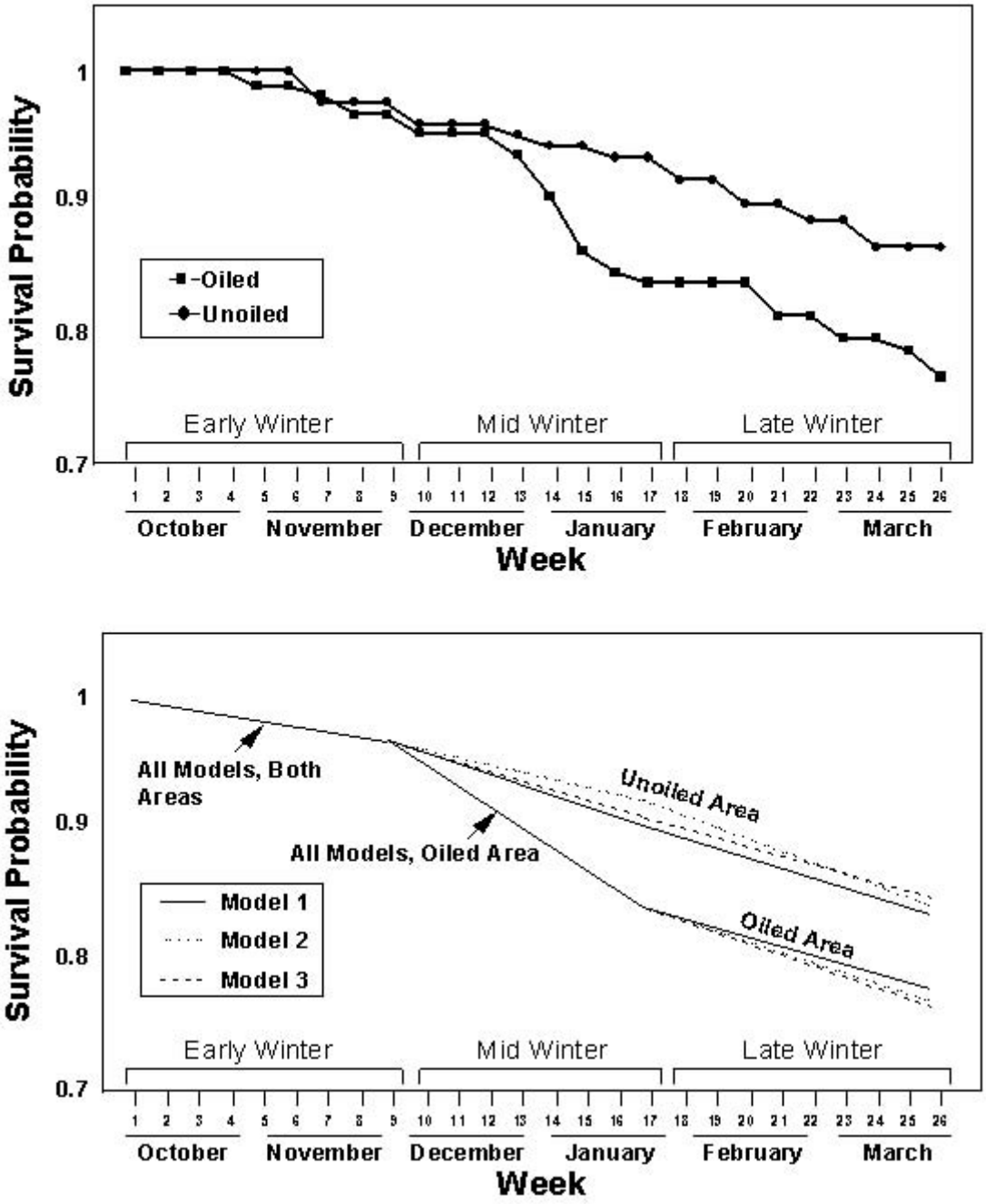

Figure 2. Winter survival probabilities for harlequin ducks in Prince William Sound, Alaska, based on Kaplan-Meier estimates (top) and the 3 best-fitting (see Table 1) reduced models (bottom).

APPENDIX HD-03

EVALUATION OF BURSAL DEPTH AS AN INDICATOR OF AGE CLASS OF HARLEQUIN DUCKS[1]

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Abstract

We contrasted the estimated age class of recaptured Harlequin Ducks (Histrionicus histrionicus) (n=255) based on bursal depth with expected age class based on bursal depth at first capture and time since first capture. Although neither estimated nor expected ages can be assumed to be correct, rates of discrepancies between the two for within-year recaptures indicate sampling error while between-year recaptures test assumptions about rates of bursal involution. Within-year, between-year, and overall discrepancy rates were 10%, 24%, and 18%, respectively. Most (86%) between-year discrepancies occurred for birds expected to be after third year (ATY) but estimated to be third year (TY). Of these ATY-TY discrepancies, 22 of 25 (88%) birds had bursal depths of 2 or 3 mm. Further, five of six between-year recaptures that were known to be ATY but estimated to be TY had 2 mm bursas. Reclassifying birds with 2 or 3 mm bursas as ATY resulted in reductions in between-year (24% to 10%) and overall (18% to 11%) discrepancy rates. We conclude that age determination of Harlequin Ducks based on bursal depth, particularly using our modified criteria, is a relatively consistent and reliable technique.

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Introduction

The bursa of Fabricius (hereafter bursa) has a long history of use for age assessment of wild waterfowl (e.g., Hochbaum 1942, Hanson 1949), although reliability estimates for the method rarely have been reported (Hohman and Cypher 1986, Esler and Grand 1994). The bursa is an immunosuppressive organ that forms as a sac on the dorsal side of the proctodeal region of the cloaca (Glick 1983). The bursa is present in juveniles, regresses as the bird matures, and eventually disappears in adults (Hochbaum 1942, Ward and Middleton 1971). Although the bursa has been used for age determination of ducks in spring (Anderson et al. 1969, LaGrange and Dinsmore 1988, Ankney and Alisauskas 1991,Young 1993), bursal involution may occur before or during an individual's first reproductive cycle, rendering it unreliable during that period (Hohman and Cypher 1986, Esler and Grand 1994). However, reliability may be higher during non-breeding seasons (Peterson and Ellarson 1978, Hohman and Cypher 1986). For birds that do not breed for two or more seasons following hatching (e.g., Canada Geese [Branta canadensis; Hanson 1949, Hochbaum 1942], Oldsquaws [Clangula hymelis; Peterson and Ellarson 1978], and other seaducks [Goudie et al. 1994]), the degree of bursal involution may be useful for differentiating age classes.

Our objective was to assess the utility of bursal characteristics for estimating age classes of Harlequin Ducks (Histrionicus histrionicus) by examining rates of discrepancies in age class designations of individuals over two or more capture events. In the absence of a known age sample, discrepancy rates provide a useful measure of reliability of bursal depth as an indicator of age class. Recaptures within-year provide an estimate of sampling error and recaptures between-year test assumptions about changes in bursal depth through time.

Methods

We captured flightless Harlequin Ducks during August and September of 1995-1997 in western Prince William Sound, AK using methods similar to Clarkson and Goudie (1994). We marked individuals with United States Fish and Wildlife Service leg bands. Age classes of all birds were estimated using internal bursal depth at each capture event. The bursa was exposed and a metal probe was inserted into the bursal sac to measure depth ($\pm$ 1mm). If the bursa was absent or $\leq$ 1 mm the birds were initially classified as after-third-year (ATY). Birds with bursal depths of >10 mm were classified as second-year (SY) and those with intermediate depths (2-10 mm) were classified as third-year (TY). Age classes are based on calendar year, thus, SY birds were approximately 14 months old, TY birds approximately 26 months, and ATY birds 38 months or older. Criteria for these initial classifications were based on those used in other studies of Harlequin Ducks (e.g., Goudie 1996) and the assumption that bursal involution should be complete after the third year when Harlequin Ducks reach breeding age (Hohman and Cypher 1986, Esler and Grand 1994, Goudie et al. 1994). Bursal depth for SY and younger birds is consistently >10 mm (Linduska 1945, Peterson and Ellarson 1978, Hohman and Cypher 1986, Henny et al. 1991). We assumed that bursal depth of TY birds would be intermediate as involution progressed (Ward and Middleton 1971). The age class criteria described above have been used to estimate age classes of harlequin ducks in other studies (e.g., Goudie 1996). Hatch year (HY) birds distinguished from older birds on the basis of size, presence of down, and

notched tail feathers; bursal depth of HY birds was not measured. These criteria were used to assign age classes throughout the course of the study. Bursal depths (mm), in contrast to age classification only, were recorded for birds estimated to be TY and ATY during the 1997 field season and late in 1996. Age class designations of recaptured birds were made without knowledge of age class estimates from previous captures.

Because of the lack of known-age birds, the accuracy of using bursal depth to determine age could not be tested directly. Instead, we used records from multiple captures to determine whether individuals could be consistently classified. Consistent classification (i.e., low discrepancy rates) for within-year capture events would suggest low measurement error. Consistent classification for between-year captures (i.e., an increase of one year in age class for every year between capture events) would support the original age class criteria and assumptions about the rates and timing of bursal involution.

To document discrepancy rates, we compared estimated to expected age classifications for each individual for each recapture event. Estimated age was the age classification based on bursal depth at the time of initial capture or recapture. An expected age class designation was generated at the time of recapture, based on previous age class designations and the time elapsed between capture events. Neither estimated nor expected ages were assumed to be correct; we simply contrasted the two to determine if there was a discrepancy or consistency. Discrepancies occurred when estimated age differed from expected age (i.e., within-year recaptures with different estimated age classes or between-year recaptures that differed from a pattern of one increase in age class estimate per year). We calculated frequency of discrepancies and identified classes of discrepancies that occurred. We compared frequencies of discrepancies among groups using chi-square goodness of fit tests.

Results

We recaptured 217 individuals one or more times for a total of 255 recaptures; 104 occurred within-year and 151 occurred between-years. Overall, estimated age classes of 82% (209) of recaptured ducks were consistent with expected age based on previous captures (Table 1). Of the recaptures, 176 were female, 79 were male. Proportions of consistencies and discrepancies did not differ between sexes (χ^2= 0.070, df = 1, P = 0.79).

Of within-year recaptures, 90% of estimated and expected ages were consistent (Table 1). This suggests that at least one of the age class estimates resulted from measurement error in 10% of cases, under the assumption that bursal depth would not change within a capture season.

Discrepancies between estimated and expected age classes occurred in 24% of between-year recaptures (Table 1), which is higher than would be expected if errors resulted only from measurement error (10%; see above). Most (86%) between-year discrepancies occurred when age class was expected to be ATY but estimated to be TY (Table 1). Bursal depths were recorded for 25 of 31 birds classified as between-year ATY-TY discrepancies. Of these ATY-TY birds, 22 (88%) had bursal depths of 2 or 3 mm. Five ATY-TY discrepancies were known to be ATY (see below) and had bursal depths of 2 mm. These results suggest that harlequin ducks with bursal depths of 2 or 3 mm should be classified as ATY. By reclassifying these birds as ATY, the overall proportion of discrepancies decreased from 18% to 11% (χ^2 = 5.77, df = 1, P = 0.02), the proportion of between-year discrepancies decreased from 24%

to 10% (χ^2 = 10.404, df = 1, $\underline{P} < 0.01$), and the proportion of within-year discrepancies did not change (χ^2 = 0.203, df = 1, $\underline{P}$ = 0.65). These results are consistent with the 10% measurement error predicted from within-year recaptures.

We had one instance in which a within-year discrepancy was associated with a between-year capture event. This individual was originally captured in 1995 and classified as an ATY; in 1997 the bird was captured twice and classified as an ATY once and a TY (2 mm bursa) once. As we were certain that the bird was an ATY in 1997 (see below), we classified the between-year recapture as a consistency and the within-year recapture as a discrepancy based on the original criteria. Using modified criteria, age class designations at all captures were consistent.

We had a subset of 45 individuals known to be ATY. Thirty-six birds were captured twice, first in 1995 and again in 1997, and nine were captured all three years. Because no HY birds were recaptured, all birds originally captured in 1995 and recaptured in 1997 definitely belonged in the ATY age class. Our between-year discrepancy rate for these known ATY birds was 13% using the original classification criteria. Five of the 6 discrepancies (83%) detected in this group were birds with bursa depths of 2 mm. By reclassifying these birds as ATY, our known ATY bird discrepancy rate dropped to 2% (χ^2 = 3.873, df = 1, $\underline{P}$ = 0.049).

Discussion

Based on discrepancy rates, we found that bursal depth enabled classification to relative age class, particularly after adoption of modified criteria for age class designation. Our estimate of measurement error rate (10% within-year discrepancy rate) for bursal age determination is comparable to error rates reported for some other age determination techniques. For example, age classes of 93% of female American Wigeon (Anas americana) (Wishart 1981) and 87.5% of Northern Pintails (Anas acuta) (Duncan 1985) were determined accurately using wing feather characteristics of known-age samples.

Measurement error can result from observer error; we attempted to minimize this source of error in our study by having only four trained observers measure bursas. We recommend similar cautions for other studies. Another potential source of measurement error may result from damage to the bursa while probing. Improper or prolonged probing may abrade the bursa and, as a result, bursal depth may be altered during the healing process, resulting in an inaccurate age class designation upon recapture. It may also be possible to puncture the bursa by probing too hard. Hanson (1949) found that with a small amount of added pressure a recently closed bursa may be pierced.

Our data strongly suggest that our original age class criteria, for TY and ATY birds, were inappropriate. Classifying birds with bursas $\leq$ 3 mm as ATY, 4-10 mm as TY, and > 10 mm as SY resulted in significantly lower between-year discrepancy rates than the original criteria. After reclassification, many of the remaining discrepancies likely were due to measurement error at one or all of the captures. The results from our known ATY sample corroborate our conclusions.

The ability to determine age classes of waterfowl accurately is essential for understanding the effect of age on many aspects of population ecology (e.g., Johnson et al. 1992). Adoption of age determination methods, without indications of their accuracy or reliability, could lead to erroneous conclusions about the ecological significance of age. While our data suggest that bursal age determination of harlequin ducks is relatively reliable, we stress that investigators be

aware that errors in age class designation are likely to occur when using this, or any other, technique.

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Table 1. Age classifications of recaptured harlequin ducks based on bursal characteristics.

Age-Class		Frequency	
Expected age	Estimated age	Between years	Within years
Discrepancies			
ATY	SY	1	1
TY	SY	0	4
ATY	TY	31	4
SY	TY	0	1
TY	ATY	4	0
SY	ATY	0	0
Consistencies			
SY	SY	0	16
TY	TY	4	47
ATY	ATY	111	31
Total recaptures		151	104

APPENDIX HD-04

SURGICAL AND IMMEDIATE POST-RELEASE MORTALITY OF HARLEQUIN DUCKS (HISTRIONICUS HISTRIONICUS) IMPLANTED WITH ABDOMINAL RADIO TRANSMITTERS WITH PERCUTANEOUS ANTENNAE[1]

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Abstract

Radiotelemetry is an essential tool in the study of free-ranging bird populations and a variety of transmitter attachment methods has been developed. A promising new method is abdominal implantation of a transmitter with a percutaneous antenna. Researchers using this technique should be concerned about and aware of mortality during surgery and the immediate post-release period (the 14 d period following surgery). Of 307 radio implant surgeries done in 1995 through 1997 in Harlequin Ducks (Histrionicus histrionicus), we documented 7 (2.3%) deaths during surgery and anesthetic recovery. Of 295 birds released with implanted radios, 10 (3.4%) died during the immediate post-release period. Modifications to anesthetic procedures used in the 204 surgeries done in 1996 and 1997 reduced mortality to 1.5% during surgery and 1.5% during the immediate post-release period. Anesthetic modifications included: intubation of all birds, placing birds on an elevated platform that allowed the bird's head to rest at a level lower than that of its body during surgery, use of a heated water blanket under the birds during surgery, monitoring of body temperature, and use of a heart monitor in addition to Doppler ultrasound to monitor heart rates and arrhythmias. Low levels of mortality associated with abdominal implantation of radio transmitters may be unavoidable, but mortality can be minimized with adaptive adjustments to anesthetic technique.

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Introduction

Radiotelemetry has become an essential tool for studying aspects of wild bird populations. Transmitters externally attached to birds, e.g., using backpack harnesses,[3] can cause adverse effects on behavior,[7,9] breeding and reproduction,[5,15,20] flight speed and metabolism,[6,16,17] and survival and return rates.[4,8,15,22,23] External transmitters attached by glue or subcutaneous anchors can be lost at a high rate.[9,21,24] In an effort to reduce adverse attachment effects and to increase transmitter retention rates, researchers have developed methods for abdominal implantation of radio transmitters.[11,10]

Recently, an abdominal implantation technique incorporating a percutaneous antenna has been successfully used for satellite transmitters.[18] Because such techniques are relatively new, there have been few reports of mortality experienced during surgery and in the period immediately following release, when mortality directly related to surgery and anesthesia could occur. Surgery-related causes of post-release mortality could include infection, dehiscence of the incision, hypothermia or other metabolic alteration, and an increased susceptibility to predation. In this paper, we describe the surgical and immediate post-release mortality experienced during a project involving the implantation into Harlequin Ducks (Histrionicus histrionicus) of a large number of conventional, VHF radio transmitters with percutaneous antennas.

Methods

From 1995 through 1997, we surgically implanted approximately 100 VHF radio transmitters each year into Harlequin Ducks in Prince William Sound, Alaska as part of a study to assess over-winter survival of these birds following the 1989 M/V Exxon Valdez oil spill. Only free-ranging female Harlequin Ducks greater than 3 yr of age, based upon cloacal examination of bursal involution,[12] were implanted with transmitters. Capture of ducks and surgeries were done at the same time each year (last week of August through the third week in September) during the annual wing molt. Ducks were captured while flightless by herding them into traps.[2] Each bird was banded with a unique U. S. Fish and Wildlife Service (USFWS) aluminum leg band.

A standard procedure was used to surgically implant transmitters.[10] Most of the surgeries were done by one of the authors (D.M.M.) with the remainder done by other veterinarians, all with previous experience implanting radio transmitters into birds. Briefly, anesthesia of the birds was induced with isoflurane (Aerrane, Ohmeda PPD, Inc., Liberty Corner, New Jersey 07938, USA) delivered by a cone with a vaporizer setting of 4.0 to 5.0% and was maintained at vaporizer settings ranging from 1.5 to 4.0%. Oxygen flow rate was maintained at 1 L/min. Following pre-surgical preparation, a midline incision was made into the abdomen and the right abdominal air sac. The antenna was passed through a trochar inserted from outside the bird as dorsally as possible at the intersection of the right pubic bone and the synsacrum. The transmitter was fitted into the right abdominal air sac and the incision was closed with 3-0 vicryl suture (Ethicon, Somerville, NJ 08876). The sole attachment of the transmitter to the body of the duck consisted of a single interrupted suture of 3-0 vicryl through the skin, body wall, and the catheter collar at the base of the antenna. Birds were allowed to recover from anesthesia for at least 1 hr before being released at their capture sites. Surgeries were done in a covered but

unheated workspace on the aft deck of a chartered motor vessel. Anesthesia was administered primarily by two biologists given training in the procedure or by veterinarians.

Birds were not intubated during the first half of the fieldwork in 1995. During the last half of 1995, and during both 1996 and 1997, the birds were intubated with uncuffed endotracheal tubes and were placed on foam pads with one end formed into a sloping ramp. The foam pads were designed so that the bird's head, when enclosed in the induction cone or when the bird was intubated, rested below the level of its body. Anesthetic monitoring in 1995 consisted of a Doppler ultrasound monitor (Parks Medical Electronics Inc., Aloha, Oregon 97007, USA) placed on the left cranial tibial artery. The anesthetist monitored anesthetic depth and respiration. In 1996 and 1997, body temperatures were monitored using a temperature sensor (Electro-Therm TM99A, Cooper Instrument, Middlefield, Connecticut 06455, USA) inserted into the cloaca. Also, in 1996 and 1997, we used a heart monitor with leads placed in standard positions and the birds were placed on a constant temperature water circulating pad (Gaymar T/Pump TP 400, Gaymar Industries Inc., Orchard Park, New York 14127, USA) to help maintain body temperature.

In 1995, transmitters (ATS, Isanti, Minnesota 55040, USA) weighed 15 g and were roughly spherical in shape (1.7-2.4 cm diameter), due to embedding in resin. In 1996 and 1997, transmitters (Holohil Corporation, Carp, Ontario K0A1L0, Canada) weighed 17.5 g, and were housed in brass cylinders measuring 4.0 cm by 1.5 cm, coated with a bio-compatible compound. A custom antenna collar (CBD-1, Vascath Corporation, Missisauga, Ontario L5A3V3, Canada) was added to the base of the antenna of each transmitter and was sealed with silicon adhesive. All transmitters had wire whip antennas. Rubber reinforcing was added to the basal 4 cm of the antennas in 1996 and 1997. The transmitters used in 1995 had a mortality switch activated by temperatures <27°C. In 1996 and 1997, transmitters were equipped with motion-sensitive mortality sensors activated by immobility of the transmitter for more than 12 hr. In all years, activation of the mortality switch doubled the transmitter pulse rate.

Radio tracking was conducted from fixed-wing aircraft. The first radio tracking flight occurred an average of 3.7 days (range: 3-5 days; $\underline{n}$=3) after the first radio was deployed each year. Intervals between flights during the period when any individual bird was within 14 days of surgery averaged 6.4 days (range: 3-12 days; $\underline{n}$=17). On each flight, mortality status and general location were noted for each bird. When a mortality signal was detected within 14 days of surgery, we tried to locate and recover the transmitter and carcass in order to examine the remains for the cause of death. In 1995 and 1996, gross necropsies were done on carcasses, but their use for unrelated analyses prevented histopathological examination. We used two-tailed Fischer's exact tests to assess difference ($\underline{P}$=0.05) in proportions of mortality between years. Proportions of interrupted surgeries and surgery or recovery deaths were calculated from total surgeries initiated, whereas proportions of post-release deaths were calculated from the numbers of birds released with transmitters and radio-tracked.

Results

A total of 307 surgeries were done on Harlequin Ducks during the 3 yr of the study, resulting in the release of 295 birds implanted with conventional radio transmitters equipped with percutaneous antennas (Table 1). Five surgeries were interrupted without implantation of a

transmitter, seven birds died during surgery or recovery, and 10 birds died within 14 days of release. Losses of birds during these periods decreased over the 3 yr course of the study (Table 1).

Seven birds died during the 307 surgeries and anesthetic recoveries, for an overall mortality rate of 2.3% (Table 1). Four of the deaths occurred in the first half of 1995, prior to the introduction of routine intubation of birds, the use of a foam ramp which placed the head of the bird lower than the body, and improved monitoring of body temperature and heart rate. In 1995, two birds died suddenly during surgery and could not be revived, and two birds experienced anesthetic difficulties after the abdominal incisions were made and the surgeries were continued but the birds died during recovery. Following the anesthesia modifications, the combined mortality rate for 1996 and 1997 was 1.5% (three surgery or recovery deaths of 204 total surgeries), compared to 3.9% (four deaths in 103 surgeries) in 1995 ($P=0.229$). In 1996, no surgery deaths occurred, but two birds died during anesthetic recovery. In 1997, one bird died during anesthetic recovery.

Mortality in the 14 day post-release period over all 3 yr was 3.4% (Table 1). Seven of the 10 post-release deaths occurred in 1995 during which the post-release mortality rate was 7.2% (Table 1). Four of the seven birds, and possibly a fifth, died within a few days of release; aspiration of fluids during surgery may have contributed directly or indirectly to death. Following introduction of the anesthesia modifications in 1995, there were only two post-release deaths of birds; both occurred in the second week after release. In 1996, post-release mortality was limited to three birds (3.1%), of which only one could have occurred within a few days of surgery. In 1997, no birds died in the immediate post-release period. When we pooled the 1996 and 1997 data (three post-release deaths of 198 radioed birds) to compare to the 1995 data, there was a decline in the post-release mortality rate to 1.5% ($P=0.017$).

Determination of an exact time of death in the post-release period was usually not possible because identifying death of a bird depended on detection of a mortality signal from the transmitter. Times of death were assigned to ranges of days due to intermittent scheduling of tracking flights. Of the 10 birds that died after release, all or parts of the carcasses were recovered from nine birds. Seven birds were subjected to predation or scavenging by bald eagles (Haliaeetus leucocephalus), or a mink (Mustela vison), or an unknown mammal, possibly a river otter (Lutra canadensis). The cause of death was not determined for one bird, despite recovery of an intact carcass, and one carcass was not recovered. In 1995, one bird that had died from an infection at the site of the transmitter implantation was recovered less than two days after surgery. This case of air sacculitis was the only known post-surgical infection in any of the implanted birds. Overall mortality rates (surgery, recovery, and post-release, combined) were significantly ($P<0.008$) lower in 1996 and 1997 (6 of 204; 2.9%) than in 1995 (11 of 103; 10.7%).

Surgeries were interrupted on five occasions over all 3 yr. For one bird in 1996 and one bird in 1997 anesthetic difficulties (severe, or repeated episodes of apnea) occurred before the abdominal incision was made (Table 1). Accidental incision of an abdominal organ (small intestine or ventriculus) occurred twice in 1995 and once in 1996. The incised organs were repaired and the birds were released without radios.

Discussion

Few reports have been published detailing mortality of wild waterfowl during surgery to implant transmitters or reporting the deaths of birds shortly after release. No deaths from surgeries occurred when 10 mallards (Anas platyrhynchos) were implanted with transmitters with percutaneous antennas and held for 28 days after surgery.[10] About 2% of 253 canvasbacks (Aythya valisneria) implanted with transmitters with internal antennas died during surgery and recovery.[14] An 8.2% mortality rate occurred when 49 mallards were implanted with transmitters with internal antennas.[4] No direct mortality occurred within 1-2 days of release of 12 spectacled eiders (Somateria fischeri) implanted with satellite transmitters with percutaneous antennas.[18] After adjustments to anesthetic technique, our surgical mortality (1.5%) was comparable or lower than the rates experienced in other studies. Mortality in 1996 and 1997 was reduced compared to 1995, which we attributed to improvements made to anesthetic technique. In 1996 and 1997, all birds were intubated and placed on foam ramps so that their heads were lower than their bodies which reduced the chance of aspirating fluids draining from the upper gastrointestinal tract and allowed for assisted breathing. Aspiration of fluids could have weakened the birds and made them more susceptible to predation. We had not previously used endotracheal tubes because of the short duration of the surgery (10 to 12 min) and of the total anesthetic period (18 to 24 min). Considering only 1996 and 1997, when anesthetic modifications were used for all surgeries, the surgery/recovery and immediate post-release mortality rates were each 1.5%. Although the effectiveness of heated water blankets for maintaining avian body temperature during extended surgery has recently been questioned,[19] we felt they were useful given the short duration of anesthesia and the low ambient temperatures (<10 °C).

Two syndromes causing mortality during surgery and anesthetic recovery were experienced: sudden, irreversible cardiopulmonary arrest that occurred during surgery and death of birds that failed to fully recover from anesthesia. The latter deaths typically occurred within about an hour of the completion of surgery. A similar syndrome of death during anesthetic recovery of rock doves (Columba livia) was attributed to putative hypothermia[1], but cloacal temperatures were normal in the Harlequin Ducks that died in 1996 and 1997 in our study. One or both of these syndromes have caused mortality during transmitter implantation surgery into spectacled eiders, common and thick-billed murres (Uria aalge and U. lomvia), and greater white-front geese (Anser albifrons) (D. M. Mulcahy, unpublished data).

The use of biologists to administer inhalant anesthesia to birds was a matter of practicality, but may have increased mortality. The use of technicians trained and experienced in anesthesia potentially could further reduce the mortality rate because of their ability to recognize anesthetic complications at an earlier stage.

Surgical sequelae must be differentiated from implantation effects. Complications (infection, dehiscence of the incision) directly related to the implantation surgery itself likely would occur within one week of the implantation; adverse effects of adjusting to an implanted radio (e.g., increased susceptibility to predation, effects on reproduction, adverse metabolic effects) might occur in the first week or later. Implantation of satellite transmitters altered nesting behavior of common and thick-billed murres.[13] We cannot assume deaths or disappearances of birds with transmitters within the first two weeks to be surgically related, as, without recovery and necropsy of the carcass, surgically related deaths cannot be distinguished

from birds killed by predators, for example. A 14 d period of data censoring following transmitter implantation has become standard.

The results of our study point out the importance of using the best possible anesthetic and surgical techniques. Although occasional deaths had occurred during transmitter implantation surgery of other species of birds, the rates were very low. At the beginning of our research on Harlequin Ducks research in 1995, mortality was high, until modifications in the anesthetic technique were introduced. This result suggests that species of birds may differ in the anesthetic and surgical obstacles they present when using an identical technique. Investigators using this technique of transmitter implantation must recognize that deaths are probably unavoidable, but can be minimized by careful attention and adaptive adjustment to anesthetic and surgical techniques.

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Table 1. Numbers of interrupted surgeries and deaths during surgery, the anesthetic recovery period, and the immediate post-release period (14 d) experienced during 3 yr of radio transmitter implants in Harlequin Ducks. Percentages are given in parentheses. The percentages of interrupted surgeries and surgery/recovery deaths are calculated on the number of total surgeries. The percentages of post-release deaths are calculated on the number of birds released with transmitters (1995, n=97; 1996, n=98; 1997, n=100; total for all years, n=295).

			Deaths (%)	
Year	Total Surgeries	Interrupted Surgeries	Surgery/Recovery	Post-Release
1995	103	2 (1.9)	4 (3.9)	7 (7.9)
1996	102	2 (2.0)	2 (2.0)	3 (3.1)
1997	102	1 (1.0)	1 (1.0)	0 (0.0)
Totals	307	5 (1.7)	7 (2.3)	10 (3.4)

APPENDIX HD-05

TESTING ASSUMPTIONS FOR UNBIASED ESTIMATION OF SURVIVAL OF RADIO-MARKED HARLEQUIN DUCKS[1]

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Abstract: Unbiased estimates of survival based on individuals outfitted with radio transmitters require meeting the assumptions that (1) radios do not affect survival and (2) animals for which the radio signal is lost have the same survival probability as those for which fate is known. In most survival studies, researchers have made these assumptions without being able to test their validity. We tested these assumptions by comparing interannual recapture rates (and, by inference, survival) between radioed and unradioed adult female harlequin ducks (*Histrionicus histrionicus*) and, for radioed females, between right-censored (i.e., missing) birds and birds with known fates. We found that recapture rates were not lower ($P = 0.585$) for birds equipped with implanted radio transmitters with external antennas (21.6 $\pm$ 3.0% [$\pm$ standard error]) than unradioed birds (21.7 $\pm$ 8.6%), evidence that radios did not affect survival. Recapture rates also were similar ($P = 0.486$) between right-censored (20.6 $\pm$ 5.1%) and known-fate individuals (22.1 $\pm$ 3.8%), suggesting that missing birds were not subject to differential mortality. We also determined that capture and handling resulted in short-term loss of body mass for both radioed and unradioed females ($P \leq 0.001$) and that this was more pronounced for radioed birds (15.4 $\pm$ 7.1 g; $P = 0.034$). However, after a year, body mass of recaptured females with radios was not different from expected ($P = 0.123$) under a hypothesis of no radio effect. Our study indicates that implanted radios are an unbiased method for estimating survival of harlequin ducks and likely other species under similar circumstances.

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Introduction

Radio telemetry has been used widely in studies of wildlife survival (White and Garrott 1990). Unbiased survival estimation using telemetry requires meeting several critical assumptions (Pollock et al. 1989), including: (1) radioed animals are representative of the population of interest; (2) survival is independent among individuals; (3) radio-marking does not affect survival during the study period; and (4) censoring of animals for which signals are lost is independent of the fate of those animals (i.e., missing animals are no more or less likely to be dead than animals for which fate is known). The first 2 assumptions often can be met through application of an appropriate experimental design, whereas the latter 2 are under less control by researchers and can not necessarily be assured by *a priori* planning. In most studies, investigators must make these latter 2 assumptions without being able to test their validity. In this study, we tested assumptions about radio effects and censored individuals for adult female harlequin ducks outfitted with implanted radio transmitters with external antennas.

A considerable body of literature exists describing effects of radio transmitters on wildlife species. In birds, deleterious effects of externally mounted transmitters (particularly those attached with backpack harnesses) have been documented in numerous studies, including changes in behavior (Massey et al. 1988, Pietz et al. 1993), reduced reproductive effort (Pietz et al. 1993, Rotella et al. 1993, Paquette et al. 1997, Garrettson and Rohwer 1998), and reductions in survival or return rates (Marks and Marks 1987, Burger et al. 1991, Cotter and Gratto 1995, Ward and Flint 1995, Dzus and Clark 1996). Although not all studies have shown negative effects of external transmitters (e.g., Hines and Zwickel 1985, Foster et al. 1992), the broad occurrence of documented deleterious effects clearly raises concern about generating unbiased survival estimates using externally mounted transmitters. Surgical implantation of transmitters into the abdominal cavity (Korschgen et al. 1984, 1996; Olsen et al. 1992) offers a promising alternative. In direct comparisons, implanted transmitters have consistently shown fewer deleterious effects than externally-attached radios (Rotella et al. 1993, Dzus and Clark 1996, Paquette et al. 1997), although no previous studies have contrasted long-term survival of birds with internal radios to unmarked individuals.

Survival estimates from radioed animals are generated based on the assumption that the probability of detecting animals is independent of their mortality status (Bunck et al. 1995), an assumption that is critically important for animals for which radio signals are lost and remain undetected through the rest of the telemetry monitoring period (i.e., right-censored). Recognizing potential violation of this assumption, some investigators (e.g., Conroy et al. 1989) have presented results that include maximum survival estimates, where all right-censored animals are assumed to have lived through the study period, and minimum estimates, where they are assumed to have died. Most investigators, however, produce survival estimates under the assumption that mortality rates of undetected animals are the same as detected animals. We are not aware of any studies that have directly addressed this assumption. Two studies (Miller et al. 1995, Cox et al. 1998) have reported returns of failed radios from hunter-killed northern pintails (*Anas acuta*), documenting that some right-censored birds were alive and in the study site during telemetry monitoring; however, the proportional frequencies of returns of known fate and right-censored birds were not compared.

Our study offered a unique opportunity to test assumptions of survival estimation of radio-marked animals. Harlequin ducks have high fidelity to molt sites (Robertson 1997), high annual survival (Goudie et al. 1994), and are susceptible to capture during wing molt. These traits, in conjunction with deployment of relatively large numbers of radios, allowed for good sample sizes to compare recapture rates and, by extension, survival differences among groups of birds. To test the assumption of a lack of radio effects on survival, we compared recapture rates of radioed and unradioed birds. We also compared recapture rates of radioed birds of known fate with those that were right-censored due to a lost radio signal to test the assumption of similar survival probabilities between these groups. Recapture probability of an individual is the product of between-year fidelity to the study site, capture probability if the bird is on the study site, and survival between capture events. Because site fidelity and capture probability of previously captured birds should not be related to radio status, we assumed that differences in recapture rates among groups of birds would reflect survival differences. We recognize that previously captured birds may exhibit trap shyness; however, because all birds included in this study, irrespective of radio status, were subjected to similar capture methods, handling, and holding time upon their original capture, we assume that the degree of trap shyness would not vary based on radio status. Also, we examined body mass changes of both radioed and unradioed individuals recaptured within- and between-years to assess potential short- and long-term effects of radio transmitters on body mass. Body mass has been positively related to survival probability for some waterfowl species (Conroy et al. 1989, Longcore et al. 1991, Bergan and Smith 1993) and, thus, is important to assess as a potential mechanism affecting survival of birds with radio transmitters.

Methods

Harlequin ducks were captured in Prince William Sound, Alaska as part of efforts to examine winter survival probabilities in relation to history of contamination by the *Exxon Valdez* oil spill. Captures occurred during 20 August to 17 September, 1995-97, the period of peak wing molt by adult females. Harlequin ducks were captured by using sea kayaks to herd molting, flightless birds into a funnel trap along shore. Once captured, birds were transported by boat to the main vessel for processing. Each bird was leg-banded with a unique U. S. Fish and Wildlife Service aluminum band, which was used to identify recaptured individuals. Sex was identified based on plumage characteristics and age class was estimated by probing bursal depth (Mather and Esler 1999). Body mass ($\pm$ 1 g) was measured on an electronic balance; estimated mass of radio transmitters was subtracted from measured masses of birds recaptured with implanted radios.

Radio transmitters were surgically implanted into adult (after-third-year) female harlequin ducks. In 1995, transmitters (ATS, Isanti, MN) weighed 15 g and were roughly spherical in shape (1.7-2.4 cm diameter), due to embedding in resin. In 1996, transmitters (Holohil, Carp, Ontario) weighed 17.5 g, and were formed as brass cylinders measuring 4.0 cm by 1.5 cm and were coated with a bio-compatible compound. All transmitters had wire whip antennas with a dacron-covered silastic sleeve glued to the base of the antenna. To deter birds from breaking antennas, a rubber reinforcement was added to the basal 4 cm of the antennas in

1996, which extended 3 cm outside of the duck's body when implanted. Expected battery life was $\geq$ 7 months for 1995 radios and $\geq$ 18 months for 1996 radios.

A modification of the procedure described by Korschgen et al. (1996) was used to surgically implant transmitters (Mulcahy and Esler 1999). Briefly, anesthesia of the birds was induced and maintained with isoflurane (Aerrane, Ohmeda, Liberty Corner, NJ). Following pre-surgical preparation, a midline incision was made into the abdomen and the right abdominal air sac was breached. The antenna was passed through a trochar inserted from outside the bird and placed as dorsally as possible at the intersection of the right pubic bone and the synsacrum. The transmitter was fitted into the right abdominal air sac and the incision was closed with absorbable sutures. The sole attachment of the transmitter to the body of the duck consisted of a single interrupted suture through the skin, body wall, and the collar at the base of the antenna. Birds recovered from anesthesia for at least 1 hour before being released at the sites of their capture.

Radioed harlequin ducks were monitored approximately weekly from an airplane to determine mortality status, location, and radio signal strength. Monitoring flights began after the first birds were radioed and continued until the last week of March. Transmitters were equipped with mortality sensors that indicated death of a bird by a doubling of the transmitter pulse rate. The mortality sensor was activated by temperatures < 27°C for 1995 radios and by immobility for > 12 hr for 1996 radios. Indicated mortalities were confirmed either by recovery of the radio or location of the radio signal in upland habitats, which harlequin ducks do not use during the nonbreeding season. Monitoring of radios for which signals were lost continued through the end of the monitoring period.

We used a one-tailed Fisher's Exact Test (Ramsey and Schafer 1997:548) to test the null hypothesis that recapture rates (proportions of birds recaptured) were not lower for radioed adult females than unradioed. Recaptures were defined as the capture of an individual in the year subsequent to previous marking or handling. We also estimated the proportional reduction in recapture rates of radioed to unradioed birds, and the associated variance, following methods for double ratio estimation (Cochran 1977:183); values near or above 1 would be consistent with no radio effect. No unradioed adult females were released in 1995, therefore we compared recapture rates of unradioed birds released in 1996 to both recapture rates of radioed birds from 1995 and 1996 combined, and 1996 only in case there were annual differences in recapture rates of radioed birds that might influence the results. Four birds were captured and radioed in 1995 and not recaptured again until 1997; these were not included in our analyses, as unradioed birds with comparable capture histories were not available. Animals captured in all three years were represented by 2 recapture events. The sample of radioed birds included only those known to have survived the 14-day period following radio implant surgery, a censor interval designed to eliminate effects of surgery or handling (Mulcahy and Esler 1999).

To test whether survival differed between birds with known fates (i.e., known to have survived or died during the monitoring period) and birds for which radio signals were lost during the monitoring period, we compared recapture rates of these groups following the methods described above for radioed to unradioed comparisons. Our null hypothesis for the one-tailed Fisher's Exact Test was that the recapture rate of right-censored birds was not lower than that of birds of known fate. Again, we calculated proportional differences in recapture rates with values near 1 indicating no differences between groups.

To examine differences in body mass between recaptured birds with radios and those without, we first standardized mass to account for seasonal, annual, geographic, and individual variation unrelated to our hypotheses of interest. We used residuals around a general linear model as our measure of standardized body mass. The model was generated from body mass data from molting females captured during our studies (n = 607), including all birds used in subsequent analyses. We used only data for first captures of females within a year to generate the model. The best-fitting model was determined by comparison of Mallow's C_p values of all possible combinations of main effects in a data-based model selection context (Burnham and Anderson 1998). Main effects included in the model selection process were: Area (an indicator variable in which unoiled Montague Island = 0 and capture sites in oiled areas = 1); Year (1/0 indicator variables for 1996 and 1997, with 1995 set as the reference value); Age (1/0 indicator variables for juvenile and subadult age classes, with the adult age class set as the reference value); and Ninth Primary Length (a continuous variable indexing the stage of wing molt). The model with the lowest C_p value was of the form:

Mass = 606.18 - 9.61*Area - 18.64*Year 1996 - 15.06*Juvenile Age Class -
0.19*Ninth Primary Length.

Because subadult and adult age classes did not differ in body mass variation during wing molt (i.e., the Subadult Age Class variable was not included in the best-fitting model), we used birds of both age classes for subsequent analyses of body mass changes. For an individual, the difference in body mass residuals between the original capture and subsequent recapture reflects the relative change in body mass after accounting for variation due to other factors. Differences in body mass residuals could not be calculated for a small number of birds that, at $\geq$ 1 of their captures, had not shed their old primaries and therefore molt stage (Ninth Primary Length) could not be determined and the general linear model could not be applied.

To examine whether body mass was affected by radio implantation after a full year, we compared the average between-year change in residuals between recaptured birds that were radioed and those that were unradioed using a t-test. We also compared the average change in residuals to zero, the expected result under a null hypothesis of no effect.

We assessed the effects of radio status and duration between captures on short-term changes in body mass using a general linear model. The dependent variable was the change in body mass residuals between within-year capture events of individuals and independent variables were radio status and the number of days between capture events.

Results

Twenty-three adult female harlequin ducks were captured, banded, and released without radio transmitters during 1996; of those, 5 (21.7 $\pm$ 8.6% [$\pm$ standard error]) were recaptured in 1997. Of 185 adult females implanted with radio transmitters in 1995 and 1996 that survived the 14-day postsurgery period, 40 were recaptured, a rate (21.6 $\pm$ 3.0%) not lower (P = 0.585) than that of unradioed birds. Similarly, when considering only 1996 radioed birds, 23 of 95 were recaptured, a rate (24.2 $\pm$ 4.3%) comparable to our unradioed sample (P = 0.691). The proportional difference in recapture rates (radioed recapture rate/unradioed recapture rate) was

0.995 (± 0.417) when including all radioed birds and was 1.114 (± 0.485) when considering only 1996 radioed birds; these results suggest no reduction from 1, i.e., no evidence for a radio effect on recapture rate.

Radio signals were permanently lost during the monitoring period (right-censored) for 63 birds transmittered during 1995 and 1996. Thirteen (20.6 ± 5.1%) of the right-censored birds were subsequently recaptured, which was similar ($P = 0.486$) to the recapture rate of birds with known fates during the monitoring period (27 of 122; 22.1 ± 3.8%). The proportional difference in recapture rates was 0.932 (± 0.280). Dates of right-censoring occurred throughout the monitoring period (Fig. 1). The number of undetected radios increased during the final 4 weeks of the monitoring period, probably due to battery exhaustion of 1995 transmitters. We compared recapture rates of right-censored birds and birds with known fates, excluding those with signals lost during the final 4 weeks, to determine whether mechanisms resulting in signal loss other than battery failure could be related to survival. We found that the recapture rate (9 of 40; 22.5 ± 6.6%) of birds right-censored during the first 5 months of the monitoring period was not lower ($P = 0.613$) than that for birds with known fates reported above. Also, the proportional difference between groups (1.017 ± 0.345) was near 1. Most lost signals occurred during the winter following 1995 captures (Fig. 1). Of 17 radioed birds recaptured in 1996, 13 had broken off their antenna at or near the skin surface (Mulcahy et al. 1999), likely explaining some signal loss; however, we also recaptured some individuals with intact antennas that were right-censored, perhaps as a result of other types of radio failure.

Body mass residuals of unradioed adult and subadult females ($n = 42$) averaged 5.0 (± .3) g higher in the year of recapture than the previous year, a result not different from zero ($t_{41} = 1.176$, $P = 0.246$). For radioed adult females ($n = 34$), body mass residuals averaged 7.4 (± .7) g lower upon their recapture than in the year of their first capture, not different ($t_{33} = 1.584$, $P = 0.123$) from the expected value of zero under a hypothesis of no radio effect. The 12.5 (± 6.4) g difference between groups was marginally significant ($t_{74} = 1.961$, $P = 0.054$). Taken together, these results do not suggest a strong radio effect on body mass after a year.

For within-year recaptures, the number of days between capture events did not explain variation in the change in body mass residuals between capture events ($t_{50} = 0.031$, $P = 0.975$) within a general linear model including a radio status term. Also, average number of days between capture events did not differ ($t_{51} = 0.368$, $P = 0.714$) between radioed (13.0 ± 0.9) and unradioed (13.3 ± 0.6) birds. Therefore, the analysis reduced to t-test comparisons. Body mass residuals of unradioed females ($n = 33$) declined an average of 15.0 (± 4.3) g between capture events, a result significantly lower than zero ($t_{32} = 3.480$, $P = 0.001$). Body mass residuals of radioed females ($n = 20$) declined 30.3 (± 5.7) g, also different from zero ($t_{19} = 5.349$, $P < 0.001$). The 15.4 (± 7.1) g difference in changes in body mass residuals between groups was marginally significant ($t_{51} = 2.178$, $P = 0.034$). These results suggest that capture and handling have short-term effects on body mass for both radioed and unradioed birds but that these effects were greater for those birds receiving radio transmitters.

Discussion

We found no evidence to suggest that adult female harlequin duck survival estimation was biased by either deleterious effects of implanted radio transmitters or differential survival

between known-fate and right-censored birds. Recapture rates invariably were quite similar between groups, building confidence for using these methods to test hypotheses related to survival.

This study is the first to compare interannual survival between birds with implanted radios and unradioed birds. Our finding that recapture rates were not reduced for harlequin ducks with implanted radios supports a growing body of evidence suggesting that implanted radios are less likely to result in biased survival estimates than externally attached radios. In comparisons of birds with implanted radio transmitters to others with externally attached transmitters, survival of birds with implanted transmitters was higher (Dzus and Clark 1996, Paquette et al. 1997). Other studies have documented lower survival or return rates for sharp-tailed grouse (*Tympanuchus phasianellus*; Marks and Marks 1987), black brant (*Branta bernicla nigricans*; Ward and Flint 1995), and rock ptarmigan (*Lagopus mutus*; Cotter and Gratto 1995) with external transmitters than for unradioed birds. However, no differences in survival were detected between externally-transmittered and unradioed spotted owls (*Strix occidentalis*; Foster et al. 1992) and blue grouse (*Dendragapus obscurus*; Hines and Zwickel 1985). We recommend that investigators be aware of potential bias using externally attached transmitters and consider the use of implanted transmitters as a potentially unbiased alternative.

Disadvantages of radio implantation include longer handling time and requirement of veterinary support for implant surgeries, although these are relatively minor compared to the desirability of obtaining unbiased estimates of survival and minimizing adverse effects on marked individuals. Schulz et al. (1998) reported elevated heterophil:lymphocyte ratios in captive mourning doves (*Zenaida macroura*) following abdominal implantation of radio transmitters, although postsurgery body mass and other blood chemistry parameters were not affected. Also, extrusion and loss of implanted radio transmitters with external antennas was documented (Mulcahy et al. 1999) for some of the harlequin ducks in this study. This could result in bias in survival estimation if extrusion and loss resulted in undetected mortality. However, recapture rates did not differ between a year without known extrusions and 1 with documented extrusions, the incidence of extrusion and loss was relatively low, recaptured birds that had lost their radios were apparently healthy, and radio loss occurred after the monitoring period (Mulcahy et al. 1999). Further, our results from this study show that recapture rates of radioed birds, including birds that lost radios, were similar to those of unradioed birds, corroborating the conclusion of Mulcahy et al. (1999) that extrusions did not affect health of birds. Radio extrusions can be avoided largely through attention to radio design and surgical technique (Mulcahy et al. 1999).

Short-term effects of transmitter implantation in birds have been detected, including reduced nesting effort (Meyers et al. 1998), surgical and postrelease mortality (Mulcahy and Esler 1999), and reductions in body mass documented in this study. However, biases to survival estimation can be avoided by censoring data during the period immediately following implantation when these effects occur. For our studies, 14 days was an appropriate censor interval. Ten mortalities of radioed harlequin ducks (out of 295 radioed and released during 1995 - 1997) were documented during the 14 days following radio implant surgery (Mulcahy and Esler 1999), compared to zero during the next 14 days. Also, the results from this study show no evidence of differential survival of radioed birds after the 14-day censor interval relative to unradioed birds.

One potential bias resulting from using radio telemetry to estimate survival is that deaths potentially related to the radio-tagging process (i.e., within the censor interval) may not be distributed at random within the sample of captured birds and, thus, the assumption that the radioed birds entering into the monitoring period are representative of the population of interest may be violated. In other words, the small number of deaths associated with radio-marking (Cox and Afton 1998, Mulcahy and Esler 1999) may occur in birds that had a different (presumably lower) survival probability had they not been captured than birds that survived the censor interval. In this case, one might predict higher recapture rates for radioed birds that survived the censor period than unradioed birds; we did not detect this, although we had little power to detect these presumably subtle effects. We believe that this potential bias had little effect on our survival estimates, as the incidence of deaths within the censor interval is relatively low (Cox and Afton 1998, Mulcahy and Esler 1999) and deaths were related more to procedural attributes than individual variation. We encourage investigators to minimize deaths due to radio-marking by adaptive modifications to capture and radio-marking techniques.

Loss of radio signals and the subsequent assumption that right-censored individuals have the same survival probability as individuals with known fates, is an issue that has been difficult to address in field studies. In many cases, undetected radios likely result from radio failure (Miller et al. 1995, Cox et al. 1998, this study), but other plausible scenarios of loss of a radio signal exist that are not independent of mortality status, e.g., a predator destroys the antenna or radio during the predation event. We demonstrated that this bias does not exist for our study of harlequin duck survival. Due to the paucity of data addressing this bias, however, we recommend other attempts to test this assumption.

Short-term body mass loss associated with radio-marking has been previously documented (Dugger et al. 1994) and we found short-term reductions in body mass, presumably related to capture and handling in both radioed and unradioed individuals. Body mass loss is a concern when estimating survival because of the documented relationship between body mass and subsequent mortality in some situations (Conroy et al. 1989, Longcore et al. 1991, Bergan and Smith 1993), although not others (Dugger et al. 1994, Migoya and Baldassarre 1995, Miller et al. 1995, Cox et al. 1998). However, because there were no strong radio effects on interannual body mass change and, particularly, because of our finding that interannual recapture rates did not differ between radioed and unradioed birds, we conclude that the short-term mass loss associated with radio transmitter implantation does not affect subsequent survival.

Management Implications

Survival is an important demographic parameter for understanding population status and predicting population trends, as well as for identifying environmental or anthropogenic factors that affect wildlife species. This is particularly true for species with life history traits similar to harlequin ducks, i.e., long-lived with relatively low investment in annual reproduction (Goudie et al. 1994, Schmutz et al. 1997). Thus, it is critical to use methods for measuring survival that result in unbiased estimates. Our results suggest that use of abdominally implanted radio transmitters for estimating harlequin duck survival does not violate assumptions of (1) no effect of radio transmitters and (2) no differential survival between right-censored and known-fate individuals. Based on our results, and those of studies contrasting external transmitters with

implanted transmitters, we suggest that implanted transmitters likely offer investigators a less biased method. Finally, we recommend that investigators attempt to quantitatively test assumptions of survival estimation for their particular species of interest and situation. Generation of survival rates in an unbiased manner is critically important for making subsequent management decisions for wildlife populations.

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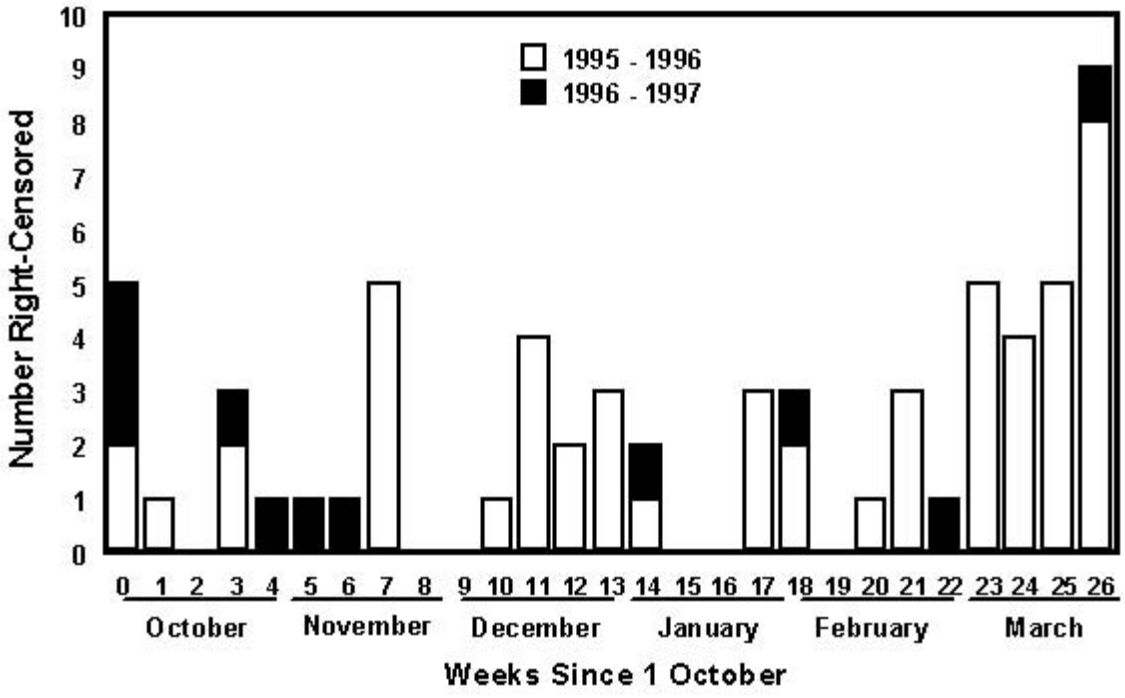

Figure 1. Distribution of dates of signal loss (right-censoring) of radio-marked adult female harlequin ducks in Prince William Sound, Alaska.

APPENDIX HD-06

LOSS FROM HARLEQUIN DUCKS OF ABDOMINALLY IMPLANTED RADIO TRANSMITTERS EQUIPPED WITH PERCUTANEOUS ANTENNAS[1]

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Abstract

We documented extrusion and loss of abdominally implanted radio transmitters with percutaneous antennas from adult female Harlequin Ducks (Histrionicus histrionicus). Birds were captured during wing molt (late August to mid-September) in 1995-1997. Of 44 Harlequin Ducks implanted with radios and recaptured, 7 (16%) had lost their transmitters and 5 (11%) had radios in the process of extruding. Most (11 of 12) extrusions and losses occurred in birds implanted with radios in 1996 and recaptured in 1997. We suggest that transmitter extrusions and losses were due largely to changes in transmitter design made between 1995 and 1996. Transmitters implanted in 1996 were cylindrical rather than spherical, had a flat end with an abrupt edge, and the lower portion of the antenna was reinforced. Radio losses occurred after the 7-mo monitoring period and caused no apparent harm to the birds. Investigators using implanted radios with percutaneous antennas for long-term projects should be aware of the potential for radio extrusion and should design minimize the problem by using transmitters that have no sharp edges and that are wide, rather than narrow.

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Introduction

Radio telemetry has been used widely in studies of wildlife survival, movements, habitat use, and breeding. An increasing body of literature suggests that radio transmitters surgically implanted into wild waterfowl are less disruptive than transmitters that are externally attached, based on differences in survival or return rates (Ward and Flint 1995, Dzus and Clark 1996), behavior (Pietz et al. 1993), and reproductive rates (Pietz et al. 1993, Rotella et al. 1993, Ward and Flint 1995, Paquette et al. 1997). The disadvantages of externally mounted transmitters stimulated the development of techniques for surgical implantation of transmitters (Korschgen et al. 1984, Olsen et al. 1992, Korschgen et al. 1996).

Waterfowl studies using implanted transmitters have reported high rates of success (e.g., Olsen et al. 1992, Haramis et al. 1993, Hohman et al. 1993, 1995). Loss of an internal transmitter has been documented only once, when a transmitter with an internal antenna was passed through the oviduct of a nesting female (Garrettson and Rohwer 1996). In this paper, we document the occurrence of extrusion and loss from Harlequin Ducks (Histrionicus histrionicus) of abdominally implanted radio transmitters with external antennas and we offer recommendations to minimize this problem.

Methods

We surgically implanted radio transmitters into adult female Harlequin Ducks from 1995-1997 as part of a study of their over-winter survival in Prince William Sound, Alaska. We captured birds and implanted transmitters each year during the last week of August through the third week of September during annual wing molt (when birds were flightless). Each bird was banded with a unique U. S. Fish and Wildlife Service (USFWS) aluminum leg band, which allowed identification of recaptured birds.

The procedure described by Korschgen et al. (1996) was used to surgically implant transmitters. Briefly, anesthesia was induced and maintained with isoflurane (Aerrane, Ohmeda, Liberty Corner, New Jersey). Following pre-surgical preparation, a midline incision was made into the abdomen and the right abdominal air sac was breached. The antenna was passed through a trochar inserted from outside the bird and placed as dorsally as possible at the intersection of the right pubic bone and the synsacrum. The transmitter was fitted into the right abdominal air sac and the incision was closed with absorbable sutures. The only attachment of the transmitter to the body of the duck consisted of a single interrupted suture through the skin, body wall and the collar at the base of the antenna. Birds recovered from anesthesia for at least 1 h before being released at the sites of their capture. Surgeries were done in a covered but unheated workspace on the aft deck of a chartered motor vessel.

The transmitters (ATS, Isanti, Minnesota) we used in 1995 weighed 15 g and were embedded in resin, which resulted in a roughly spherical shape (1.7-2.4 cm diameter). The transmitters (Holohil Systems, Carp, Ontario, Canada) we used in 1996 weighed 17.5 g and were enclosed in brass cylinders coated with a bio-compatible compound and measuring 4.0 cm by 1.5 cm. All transmitters had wire whip antennas with a dacron-covered sleeve glued to the base of the antenna. To deter birds from breaking antennas, rubber reinforcement was added to the basal 4 cm of the antennas in 1996, which extended 3 cm outside of the body.

In the second and third years of the study, we recaptured some birds that had been implanted with transmitters in a previous year. The presence of one of our leg bands and an external antenna or a visible antenna stump immediately identified a retained radio. We palpated the abdomens of all recaptured and transmitter-implanted birds to detect non-functioning radios that lacked a visible antenna stump and used a radio receiver, tuned to the proper frequency and placed immediately adjacent to the bird, to determine the presence of a functioning radio. In 1996 and 1997, most birds with non-functioning or missing radios were re-implanted with a new radio. After the abdomen was opened, a visual and tactile inspection was made of all accessible spaces in the abdomen and abdominal air sacs. We radiographed several birds in 1997 using a portable radiograph machine (Bowie Portable X-Ray Generator, Bowie Manufact., Lake City, Iowa) and instant film (Polaroid Transparent Radiographic Instant Film, Type TPX) to confirm that the transmitters had, indeed been lost, rather than migrated into the anterior thorax of the birds.

Results

We recaptured 44 ducks in 1996 and 1997 that had been implanted with radios in 1995 or 1996 (Table 1). Of the 40 ducks that were recaptured one year after radio implantation, 6 (15%) had lost their transmitters and 5 (13%) had radios that were in the process of extruding (Table 1). Of the 17 ducks implanted with spherical transmitters in 1995 and recaptured in 1996, 13 had broken off the antenna where it exited the skin, leaving either no stump or only 1-2 mm of antenna extending from the skin. None of the birds implanted in 1995 had lost the transmitter when recaptured in 1996. The transmitter was missing from one of the four birds implanted in 1995 and recaptured in 1997; the antennas were broken off of the transmitters in the other three birds (Table 1). We could palpate the transmitters in the caudal right quadrant of recaptured ducks as a firm mass of appropriate size and shape.

In 1997, we recaptured 23 ducks implanted with cylindrical transmitters in 1996. Of these, 12 (52%) had radios present, with no sign of extrusion; 2 of the 12 ducks had broken off the antennas at the end of the reinforced base (Table 1). Three birds (13%) had radios present internally, but with the dacron antenna collar pulled out through the body wall and skin, two birds (9%) had pulled both the dacron antenna collar and part of the transmitter out through the skin, and six birds (26%) had lost their radios entirely. In 1997, we confirmed by radiography for three ducks that the transmitters had been entirely lost from the body instead of having migrated to another location within the body.

We replaced 20 radios in recaptured birds 1-2 yr after the first implantation. During these surgeries, extensive adhesions were found involving intestines, air sac membranes, liver, and the ventral body wall. A thick (1-2 mm) fibrous sheath completely surrounded the entire transmitter body and had to be cut to remove the enclosed transmitter. The adhesion between the antenna collar and the body wall was broken by gently pulling on the transmitter body. Hemorrhage was minimal from the incised connective tissue sheath and the disrupted antenna collar attachment site.

We captured two birds in 1997 with the transmitters partially protruding through the body wall. In these birds, the arc of the caudal end of the transmitter body closest to the antenna attachment had been pulled first and farthest through the body wall, suggesting that a lever action

had been applied to the reinforced base of the antenna. The fit between the skin and the transmitter body was tight, preventing both the immediate loss of the transmitter and the leakage of water into the abdomen, which might have caused infectious peritonitis or air sacculitis. We removed both transmitters, after aseptic preparation of the site, by applying traction to the antenna base and gentle clearing of inflammatory debris from around the skin-transmitter interface, using the blunt end of a scalpel handle. We surgically reduced and closed the resulting fistulae.

Discussion

All attachments to wild animals, such as tags, bands, marks, and instrument packages, suffer a rate of loss specific to the species of animal, environmental conditions, type of attachment, and mechanism of attachment. We chose abdominal implantation of radio transmitters for this project because of the potential deleterious effects of externally mounted transmitters (Pietz et al. 1993, Rotella et al. 1993, Ward and Flint 1995, Dzus and Clark 1996, Paquette et al. 1997). Loss rates of surgically implanted transmitters are often assumed to be low (Zimmer 1997) compared to externally attached transmitters with only one documented loss of an implanted transmitter reported (Garrettson and Rohwer 1996). Because abdominally implanted transmitters equipped with internal coiled antennas suffer reduced signal strength due to the body mass of the animal, we used percutaneous antennas. The technique developed by Korschgen et al. (1996), utilizing a percutaneous antenna was used in one study without report of transmitter loss (Petersen et al. 1995).

We believe that the increased rate of loss of transmitters implanted in 1996 compared to those implanted in 1995 resulted from changes in dimensions and configurations of the transmitters. In 1995 the transmitters were rounder and wider than the cylindrical transmitters used in 1996. The 1995 transmitters were built by constructing the electronics around the partial circumference of a cylindrical battery and then embedding both in epoxy resin, resulting in a spherical shape. Only one bird that had been implanted in 1995 and recaptured in the 2 years following implantation had lost its transmitter. The 1996 transmitters and batteries were enclosed in a cylindrical brass case, lightly coated with a biocompatible material. The flat posterior end of the brass case met the curved side wall in an abrupt, 90 degree angle, which was inadequately blunted by the biocompatible coating. The antenna exited the side of the brass case adjacent to this sharp angle. A final change in design was the addition of rubber reinforcement around the initial 4 cm of antenna, in an effort to reduce the bird's ability to break the antenna at the level of the skin.

We speculate that the birds groom and manipulate the solid wire antenna, causing metal fatigue and failure where the antenna exits the skin, as documented with the 1995 transmitters. The additional reinforcement material added to the base of the antennas in 1996 reduced the antenna failure rate, although some birds still managed to break the antenna at the distal end of the reinforcement. The birds likely continued to groom and manipulate the remaining, reinforced antenna base until they pulled the antenna collar out through the body wall. Adhesions, contractures, and proliferative connective tissue prevented the transmitter from then falling back into the air sac and drawing the antenna back into the bird. The placement of the antenna on the side of the cylindrical transmitter body next to the flat end caused a lever action when the bird

pulled on the antenna base. This placed the abrupt edge of the transmitter's flat end adjacent to the body wall, which helped to enlarge the hole where the antenna exited. Eventually the entire transmitter was pulled through the fistula.

The smaller cross-section of the cylindrical transmitter used in 1996 was able to pass through a smaller fistula. Also, the narrower transmitter probably could pass more easily through the angle formed by the pubic bone and the synsacrum. To reduce this effect, the surgeon must assure that the antenna is passed through the body wall as dorsally as possible at the origin of the pubic bone on the synsacrum. The angle between these two anatomical structures narrows dorsally, and the intersecting soft tissues are thicker and stronger dorsally.

There have been few reports of long-term retention rates because abdominal implantation of transmitters in birds, especially those using percutaneous antennas, is a relatively new technique. The transmitter with a percutaneous antenna that we used is anchored in the short term by a suture through the antenna collar and body wall and in the long term by the body wall attachment to the antenna collar that occurs during healing. Therefore, there is little chance for the transintestinal expulsion of transmitters experienced in ictalurid and salmonid fishes (Summerfelt and Mosier 1984, Chisholm and Hubert 1985, Marty and Summerfelt 1986).

All of the ducks that had lost transmitters and were recaptured in fall 1997 had been located regularly from August-September 1996 through April 1997. Therefore, transmitters must have been lost between the end of April and the time the birds were recaptured in late August and September 1997. Loss of transmitters did not appear to affect the health of implanted ducks. Recaptured birds with lost or extruding transmitters appeared healthy. The fibrous sheath that developed around the transmitter body may have sealed the transmitter from the air sac and peritoneal cavity during extrusion of the transmitter through the skin. Recapture rates in the year following implantation were higher for birds implanted in 1996 (24%) than in 1995 (19%) suggesting that survival was not compromised by loss of the transmitter.

We believe that the loss of abdominally implanted transmitters can be reduced by designing wider transmitter bodies with no abrupt edges, careful placement of the percutaneous antenna as dorsally as possible and elimination of the reinforcement at the base of the antenna. We recommend that investigators using implanted transmitters with external antennas work closely with manufacturers to design a transmitter that is appropriate for their work and which minimizes risk of extrusion and loss. Loss of radios could result in erroneous conclusions in studies relying on radio telemetry.

Acknowledgments

These data were collected under studies supported by the *Exxon Valdez* Oil Spill Trustee Council. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council. Data were collected with the assistance of Bryan Baetsle, Rick Ballas, Brad Benter, Tim Bowman, Kathy Burek, Jennifer DeGroot, Bob Jarvis, Danielle Mather, Dan Monson, Julie Morse, Dan Ruthrauff, Dorcas Schaeffer, Kim Trust, and the crews of the motor vessels *Auklet*, *Julia Breeze*, *Kittiwake II*, and *Waters*. D.V. Derksen, J. B. Grand and T. Van Pelt reviewed the manuscript. Use of trade names does not imply product endorsement.

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TABLE 1. Fate of radio transmitters implanted into female Harlequin Ducks during wing molt, Prince William Sound, Alaska, 1995-1997.

Year Implanted/Recaptured	n	Radios lost	Radios retained		
			Undamaged	Antenna Broken	Radio Extruding
1995/1996	17	0	4	13	0
1995/1997	4	1	0	3	0
1996/1997	23	6	10	2	5
TOTALS	44	7	14	18	5

APPENDIX HD-07

HEMATOLOGY AND SERUM CHEMISTRY OF FREE RANGING, MOLTING, FEMALE HARLEQUIN DUCKS AND A COMPARISON OF VALUES BETWEEN DUCKS FROM THE OILED AND UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA[1]

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Abstract

Hematology and serum chemistry reference ranges were established for molting female harlequin ducks (Histrionicus histrionicus) sampled from unoiled areas of Prince William Sound, Alaska. Blood values of harlequin ducks from the oiled areas of Prince William Sound sampled at the same time significantly differed only in having lower red blood cell counts and higher sodium and glucose levels.

[1]In preparation for submission to Marine Pollution Bulletin.

Introduction

Ten years after the event, harlequin ducks in Prince William Sound, Alaska, have not recovered from the effects of the Exxon Valdez oil spill. Harlequin ducks are true seaducks, spending all but the nesting portion of their life cycle in the marine environment. This ethology makes them very sensitive to the long-term effects of an oil spill that occurs in coastal waters.

Hematology and serum chemistries are standard tests used in the diagnosis of disease in animals. Considerable efforts are made to establish reference intervals for a variety of birds, however, the emphasis has been to define such values for species that are held in captivity, especially psittacines, raptors, and poultry (Cambell 1994, 1995, Fudge 1997, Heidenreich 1997, Hochleitner 1994). It is a challenge to define reference intervals and to use clinical pathology as a tool to study free-ranging birds in environments far from the laboratory. Besides the difficulties sometimes encountered in obtaining a sufficient number of samples of free-ranging birds, handling, storage, and transportation of blood samples collected during field studies is less than optimal. In addition, samples are rarely available from birds of both genders, from all ages, and at all stages of their life cycle, important variables in clinical pathology.

We present hematology and serum chemistry reference intervals and intervals for free-ranging female harlequin ducks captured during wing molt in an unoiled area of Prince William Sound, Alaska. We then compare these intervals to similar data obtained from birds at the same stage of their life cycle, but captured from an area that had suffered contamination from the oil spill in 1989.

Methods

Birds were captured in 1995 and 1996 from areas in Prince William Sound, Alaska, designated as oiled and unoiled study sites following the M/V Exxon Valdez oil spill. Only free-ranging female Harlequin Ducks greater than 3 yr of age, based upon cloacal examination of bursal involution (Mather and Esler 1999), were sampled. Capture of ducks was done at the same time each year (last week of August through the third week in September) during the annual wing molt. Ducks were captured while flightless by herding them into traps (Clarkson and Goudie 1994). All sampling and preliminary sample processing (to the point of separating serum from whole blood) was done onboard a chartered boat.

Two ml of blood was taken from the jugular vein using a 3-ml syringe and 21 gauge 25-mm needles. Duplicate blood smears were made on microscope slides, 1 ml was placed into a plastic conical tube and the remainder of the blood was placed into glass tubes. Blood was allowed to clot at ambient temperature for 1-12 hr before being centrifuged to separate serum that was then frozen at -20 C. Samples were held in the boat's freezer for up to three days until they could be shipped via floatplane to Cordova, Alaska, from which site they were transported by commercial courier service to an avian clinical pathology laboratory in California. A portion of the collected serum was sent for determination of haptoglobin levels.

In the laboratory, differential blood cell counts were made from the prepared blood smears. The presence of hemoparasites was noted. Hematology variables measured or calculated were: white blood cell count ($10^3/\mu L$), red blood cell count ($10^6/\mu L$), packed cell volume (%), mean corpuscular volume (fL), hemoglobin (g/dL), mean corpuscular hemoglobin

concentration (g/dL), azurophils (%), bands (%), heterophils (%), lymphocytes (%), monocytes (%), eosinophils (%), and basophils (%). The presence of a buffy coat, thrombocytes, reactive lymphocytes, and polychromasia and anisocytosis of erythrocytes was noted. Serum chemistry variables measured were: sodium (mEq/L), potassium (mEq/L), gamma glutamyltransferase (IU/L), alkaline phosphatase (IU/L), calcium (mg/dL), creatine phosphokinase (U/L), glucose (mg/dL), lactic dehydogenase (IU/L), phosphorus (mg/dL), aspartate aminotransferase (IU/L), total protein (g/dL), and uric acid (mg/dL). Sodium, potassium, gamma glutamyltransferase, and alkaline phosphatase were measured only in samples taken in 1996. Samples reported by the laboratory as hemolyzed were not included in any analysis. Within a year, some parameters could not be measured in some samples.

Reference intervals for blood parameters for molting female harlequin ducks were determined following recommendations of the National Committee for Clinical Laboratory Standards (1995). Only blood samples from ducks captured on the unoiled side of Prince William Sound were used to calculate reference intervals. In order to minimize the influence of potential mishandling, we eliminated samples for which there was an indication of suboptimal processing. We did not use samples with values of less than 2.0 mEq/mL for potassium or less than 155 mg/dL for glucose. We identified and eliminated outliers by calculating the ratio D/R where D was the absolute difference between an extreme observation (large or small) and the next largest or smallest observation, and R was the interval of all observations (Dixon 1953). Calculations of $D/R \geq 1/3$ were used as cut-off values (Reed 1971). Normality testing indicated that the distributions of most of the blood variables violated assumptions of normality or equal variance. Common transformations converted some but not all of the variables to normal distributions. A nonparametric procedure on the data without transformation was used to determine the reference intervals (National Committee for Clinical Laboratory Standards 1995). The lower reference limit, $\underline{r}_1$ (the 2.5^{th} percentile) was the observation corresponding to $\underline{r} = 0.025 (\underline{n} + 1)$ and the upper reference limit, $\underline{r}_2$ (the 97.5^{th} percentile), as the observation corresponding to $\underline{r} = 0.975 (\underline{n} + 1)$. We used the term reference interval rather than reference range (Dybkær and Solberg 1987)

We used the nonparametric, Mann-Whitney rank sum test to compare hematology and serum chemistry values obtained from female molting harlequin ducks captured from unoiled areas to values obtained from ducks captured from oiled areas of Prince William Sound using an experiment-wide error rate of 0.05. We used a sequential Bonferroni adjustment (Rice 1989) for $\alpha = 0.002$ for $\underline{n} = 27$, the total number of hematology and serum chemistry parameters compared. Statistical tests were done using commercial software (Sigmastat, Jandel Scientific Software, San Rafael, California, USA).

Results

The analytical laboratory reported erythrocyte polychromasia and anisocytosis and slightly degranulated heterocytes in all samples. Hemoparasites were observed in three samples: two leucocytozoon and one plasmodium; data from these samples were not used in determining reference intervals. Descriptions of blood cell types were not available from the laboratory.

Table 1 summarizes the hematological and biochemical values for the molting female harlequin ducks sampled from the unoiled area of Prince William Sound and includes the median

value, the 2.75 and 97.5 percentiles and the extreme values. Table 2 compares the hematological and biochemical values between blood from molting females in the unoiled areas to blood from female ducks in the oiled areas of Prince William Sound. Significant differences were found between the two groups of ducks for total red blood cell count, sodium, and glucose. Using the derived reference intervals, there were 43 (23 low, 20 high) individual values outside of the reference interval from birds in the unoiled areas and 130 (64 low, 66 high) individual values outside the reference interval from birds in the oiled areas of Prince William Sound.

Discussion

Tests for normality and equal variance showed that many of the blood and serum chemistry data that we collected were not normally distributed. Efforts to transform non-normally distributed data improved some but not all variables. Therefore, we chose to use nonparametric statistical methods to describe the reference intervals and to make our comparisons. When data are not normally distributed, nonparametric estimates of reference intervals, including rank tests such as the one we used, are more accurate than parametric methods (Potvin and Roff 1993, Reed et al. 1971). Although the majority of papers dealing with blood and serum chemistry data use parametric statistics and present reference intervals as means ±2 SD, many authors fail to test for normality and to adjust the use of parametric or nonparametric statistics accordingly. Others transform their data for the statistical comparisons, but present means ±2 SD without making clear whether these values were calculated from transformed or nontransformed data.

The large number of parameters that can be measured from a single blood sample also requires caution for analysis. Rice (1989) showed that there was a 95% chance of finding a significant difference between one of 12 pairs in a table of statistical parameters using a 0.05 level of significance. This led Work (1996), in his study of free-ranging seabirds, to use Rice's suggestion for a sequential Bonferroni adjustment for an experiment-wide error of 0.05. In comparing 26 pairs of blood and serum variables from ducks taken from unoiled area to ducks taken from oiled areas, our use of the same method reduced the number of significant differences from 10 to 3 (red blood cell count, sodium, and glucose). Although the red blood cell count was significantly lower in birds in the oiled areas of Prince William Sound, there was no report of Heinz-body anemia, as frequently occurs during acute exposure to oil (Leighton 1983, Yamato 1996). Also, the packed cell volume and red blood cell indices (mean corpuscular volume, hemoglobin, and mean corpuscular hemoglobin concentration) did not differ significantly. Sodium and glucose concentrations were significantly but moderately higher in birds from the oiled areas. Sodium and glucose levels could reflect a higher level of stress in birds living in oiled areas, or could be a result of different durations of capture chases, handling times, or effects of recent adverse weather prior to capture.

There was considerable variation in blood and serum chemistry variables in the ducks sampled from both the oiled and the unoiled areas. All the blood samples were analyzed at the same laboratory. However, there was no way to control for variations in the individual histories of each of the birds. Similarly, the facts that our study was done in a remote area and the samples had to be picked up and flown to a different state for analysis presented special problems. Also, our collection and preliminary processing of blood samples was done as an

adjunct to the major effort being made to surgically implant radio transmitters into harlequin ducks. Thus, there were inevitable variations in sample handling and processing and serum storage times. We feel that these problems are inherent in studies on free-ranging wildlife that are done in remote areas, with minimal equipment and facilities and with competing demands for space, time, and personnel.

Harlequin ducks are one of several species whose populations in Prince William Sound have not recovered from the effects of the 1989 Exxon Valdez oil spill. We used two approaches to determine if harlequin ducks living in areas of Prince William Sound that had been contaminated with oil in 1989 showed alterations in hematology or serum chemistry. First, we used blood samples from harlequin ducks living in an area of Prince William Sound that had never been oiled to establish normal reference intervals, and then compared values from samples taken from ducks in areas that had been oiled to the reference values. Second, we directly compared blood values from ducks taken from oiled areas to ducks from unoiled areas. The former approach represented a classical evaluation of blood samples from an experimental population. The latter approach helped control for variations, such as chase and holding times, and individual variations in short-term history because all samples from both areas were included.

Although oil spills in the marine environment occur relatively frequently and often involve considerable numbers of birds, most investigations of oiling in seabirds has focused on the acute effects and on the techniques for rehabilitation of oiled seabirds (Degernes 1995, Gibson and White 1990, Holcomb and White 1990, Leighton 1985, Leighton 1986, Leighton 1993, Tseng 1999, Tully et al. 1996, Yamato et al. 1996). Rehabilitation of acutely oiled seabirds is popular, but the results of cleansing and releasing oiled seabirds is not certain (Sharp 1996, Clark 1978). Because of the complexity of the question, relatively little research has been done on the long-term sequelae of acute exposure to oil or to chronic exposure to low levels of petroleum hydrocarbons. The primary targets of oil toxicity in birds, including seaducks, are the peripheral erythrocytes, with a resulting Heinz-body hemolytic anemia (Leighton 1983, Yamato 1996). The effect of low level, chronic exposure to hydrocarbons is undoubtedly complex, and may involve secondary effects, such as additive stress, suppressed immunity, and decreased reproduction (Leighton 1993). Rocke et al. (1984) found that ingestion of crude oil caused decreased resistance to infection by Pasteurella multocida caused by an impaired cellular response. A higher than expected prevalence of Plasmodium infection was found in oil-contaminated common murres (Uria aalgae); the involvement of the oil in the expression of the infection was not certain (Roertgen 1990).

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Table 1. Normal reference intervals and extreme observations for hematology and serum chemistry values of free ranging, molting female harlequin ducks taken from unoiled areas of eastern Prince William Sound, Alaska.

				Fractiles			
Parameter	Units	n	Median	0.275	0.975	Extremes	
Hematology							
White blood cell count	$10^3/\mu L$	72	14.0	6.60	30.0	6.60	32.0
Red blood cell count	$10^6/\mu L$	45	3.17	2.15	4.74	2.15	4.74
Packed cell volume	%	71	54	44	64	42	65
Mean corpuscular volume	fL	45	175	105	223	105	223
Hemoglobin	g/dL	45	15.7	8.6	20.1	8.6	20.1
Mean corpuscular hemoglobin concentration	g/dL	45	28.0	17.0	38.0	17.0	38
Azurophils	%	72	0	0	0	0	0
Bands	%	72	0	0	0	0	0
Heterophils	%	72	77	54	90	51	91
Lymphocytes	%	72	21	10	45	6	47
Monocytes	%	72	9	0	0	0	9
Eosinophils	%	71	1	0	7	0	9
Basophils	%	72	1	0	5	0	5
Serum Chemistry							
Sodium	mEq/L	30	155	138	202	138	202
Potassium	mEq/L	31	2.8	2.0	5.0	2.0	5.0
Gamma glutamyltransferase	IU/L	31	9	0	18	0	18
Alkaline phosphatase	IU/L	31	372	88	1020	88	1020
Calcium	mg/dL	72	10.7	8.50	14.2	8	14.6
Creatine phosphokinase	U/L	72	1121	366	4252	136	4968
Glucose	mg/dL	72	339	267	486	263	497
Lactic dehydogenase	IU/L	71	359	146	902	123	1000
Phosphorus	mg/dL	30	4.8	2.0	8.4	2.0	8.4
Aspartate aminotransferase	IU/L	71	64	16	163	12	195
Total protein	g/dL	72	3.65	2.60	5.40	2.50	6.40
Uric acid	mg/dL	72	9.7	4.0	16.8	4.0	16.9
Haptoglobin		68	90.5	34.9	235	24.4	257

Table 2. Comparison of hematology and serum chemistry variables of harlequin ducks captured from the unoiled and oiled areas of Prince William Sound, Alaska.

	Unoiled Area				Oiled Area			
Parameter	n	Median	Extremes		n	Median	Extremes	
Hematology								
Total white blood cell count ($10^3/\mu L$)	80	13.7	6.60	32.0	87	12	5	32
Total red blood cell count ($10^6/\mu L$)	48	3.17	2.15	4.74	51	2.85[a]	2.01	3.68
Packed cell volume (%)	79	54	42	64	86	55	5.0	69
Mean corpuscular volume (fL)	48	172.5	105	223	51	177	149	233
Hemoglobin (g/dL)	48	15.80	8.60	19.9	50	14.20	7.5	20.3
Mean corpuscular hemoglobin concentration (g/dL)	48	28.50	17	38	50	26.50	15	48
Azurophils (%)	80	0	0	0	88	0	0	0
Bands (%)	80	0	0	0	88	0	0	0
Heterophils (%)	80	76.5	51	91	87	77	47	93
Lymphocytes (%)	80	22	6.0	47	87	17	4	52
Monocytes (%)	80	0	0	9	87	0	0	4
Eosinophils (%)	79	1	0	14	87	2	0	24
Basophils (%)	79	1	0	5	87	1	0	6
Serum Chemistry								
Sodium (mEq/L)	37	155	135	202	42	161.5[a]	146	388
Potassium (mEq/L)	39	2.70	1.10	5	44	2.45	1.10	7.90
Gamma glutamyltransferase (IU/L)	39	9.0	0	18	44	11	3.00	19
Alkaline phosphatase (IU/L)	39	316	88	1200	44	305.5	16	1592
Calcium (mg/dL)	80	10.85	8	14.80	88	10.55	7.60	14.20
Creatine phosphokinase (U/L)	80	1074	136	4968	88	925.5	235	5556
Glucose (mg/dL)	80	336.5	153	497	88	376.5[a]	162	496
Lactic dehydogenase (IU/L)	80	363.5	123	1550	88	349	126	1916
Phosphorus (mg/dL)	39	4.80	1	15.60	44	4.80	2.4	11.6
Aspartate aminotransferase (IU/L)	80	65.5	12	379	88	58	17	522
Total protein (g/dL)	80	3.60	2.30	6.40	88	3.8	2.4	13.3
Uric acid (mg/dL)	80	9.75	4.0	16.9	88	10.5	0.9	21.2
Haptoglobin	76	97	24.4	257	83	102.8	21.6	236

[a] Value significantly different than value for same parameter among harlequin ducks from the unoiled areas ($P < 0.002$).

APPENDIX HD-08

CYTOCHROME P450 1A INDUCTION IN SeaduCKS INHABITING NEARSHORE AREAS OF PRINCE WILLIAM SOUND, ALASKA[1]

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Abstract

Following the *Exxon Valdez* oil spill, hepatic rates of EROD activity and thus, P450 1A expression were significantly higher in harlequin ducks (*Histrionicus histrionicus*) and Barrow's goldeneyes (*Bucephala islandica*) from oiled areas of Prince William Sound, Alaska, when compared to birds from unoiled sites. Polychlorinated biphenyl exposure did not account for area differences in P450 1A induction in harlequin ducks. Background hydrocarbon levels in Prince William Sound were negligible prior to the 1989 oil spill, but remnant *Exxon Valdez* oil was still present in nearshore habitats of the spill zone coincident with our study. We conclude that P450 1A induction in seaducks from areas oiled during the *Exxon Valdez* oil spill was likely due to exposure to residual oil. We speculate that biochemical and physiological changes in individuals chronically exposed to oil may be constraining population recovery of some seaduck species affected by the spill.

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Introduction

In 1989, the *Exxon Valdez* ran aground on Bligh Reef, spilling 11,000,000 gallons of crude oil into Prince William Sound (PWS), Alaska. Subsequent wind and ocean currents spread the oil southwest through western PWS and along the Kenai and Alaska peninsulas and the Kodiak Archipelago. As much as 40% of the spilled oil was deposited in intertidal and subtidal habitats of PWS (Galt et al., 1991; Wolfe et al., 1994), and some residual oil was still present in these habitats during the course of our study (Hayes and Michel, 1999). These nearshore environments are important for large numbers of vertebrates including molting and wintering waterfowl (Lance et al., 1999). Immediate postspill oil contamination caused acute mortalities of thousands of birds (Piatt et al., 1990), and concerns that continuing long-term oil exposure could be affecting avian populations remain. Exposure to oil through contaminated sediments or prey items could potentially elicit adverse physiological changes in birds (Leighton, 1993; Jenssen, 1994), which in turn, could have demographic consequences (e.g., Holmes et al., 1978, 1979) for the population. Populations of some species of birds, including harlequin ducks (*Histrionicus histrionicus*), have not fully recovered in areas of PWS affected by the oil spill (*Exxon Valdez* Oil Spill Trustee Council, 1999). Individuals may continue to be exposed to residual oil, and making that determination is important in understanding mechanisms constraining full recovery of bird populations.

Directly measuring oil constituents in bird tissues does not accurately reflect exposure to xenobiotic parent compounds (Lee et al., 1985). Polycyclic aromatic hydrocarbons (PAHs) are constituents of oil that, upon ingestion, are rapidly metabolized, thereby, making it difficult to determine the chemical structure of the original compound. One of the most sensitive and specific biochemical measurements for assessing exposure to PAHs is the induction of cytochrome P450 (P450), mixed-function oxygenase (MFO) systems (Woodin et al., 1997). Certain PAHs induce P450 responses, therefore measuring resultant enzyme production or activity can indirectly indicate exposure to oil constituents. For example, Woodin et al. (1997) measured P450 induction (specifically, the CYP 1A gene family) in intertidal fish collected from the field and from cages at various sites in PWS one year after the *Exxon Valdez* oil spill. They determined that P450 1A induction in fish from sites impacted by oil was significantly higher when compared to fish from areas unaffected by oil.

In this study, we measured P450 1A responses in harlequin ducks and Barrow's goldeneye (*Bucephala islandica*) from oiled and unoiled areas of PWS, eight to nine years after the spill, to assess potential continuing exposure of these seaduck species to *Exxon Valdez* oil. Due to their occurrence in nearshore habitats and consumption of benthic invertebrate prey, harlequin ducks and Barrow's goldeneyes are particularly susceptible to continued exposure to residual *Exxon Valdez* oil and, thus, are potentially vulnerable to subsequent physiological and population-level effects. In addition to oil-derived PAHs, certain polychlorinated biphenyl (PCB) congeners can induce cytochrome P450 systems. Therefore, we also measured congener-specific PCB concentrations in plasma from harlequin ducks overwintering in PWS to compare with P450 1A enzyme activity.

Methods

Field Collections

Barrow's goldeneyes and harlequin ducks were sampled from oiled and unoiled parts of PWS (Fig. 1) from 1996 through 1998. Samples from oiled sites were collected throughout the spill area. Samples also were collected from Montague Island, which was selected as an unoiled study site due to its proximity to the spill zone, thus limiting any geographic effects not related to the *Exxon Valdez* oil spill.

Barrow's goldeneyes were collected during December 1996 and February 1997 by shotgun from oiled Knight Island (Bay of Isles and Herring Bay) and unoiled Montague Island study areas (Fig. 1). Liver samples were collected to assess P450 induction by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity. Immediately upon retrieval of each carcass (within 10 minutes), approximately one gram of liver was dissected, wrapped in aluminum foil, and placed into liquid nitrogen.

Harlequin ducks were captured during March and April 1998, using a modified floating mist net trap (Kaiser et al., 1995) at Montague Island and (oiled) Crafton Island and Main Bay study sites (Fig. 1). Captured birds were placed under Isoflourane® anesthesia and livers were surgically biopsied to obtain a small (0.07 - 0.22 g, mean= 0.11 g) sample for EROD analysis. Immediately following biopsy, liver samples were placed in a cryogenic vial and frozen in liquid nitrogen. Following recovery from surgery, animals were released.

Three ml blood samples were collected into sodium heparinized glass evacuated tubes from each harlequin duck prior to surgery using 23 gauge, 1" needles and 5 cc syringes. Blood samples were centrifuged at approximately 1500 x g for 5 min, and plasma was decanted into 2 ml polypropylene microcentrifuge tubes. Plasma was frozen for biochemical and PCB congener analyses.

Laboratory Analyses

<u>EROD</u> <u>Activity</u>

Liver samples frozen in liquid nitrogen were shipped to Woods Hole for subsequent preparation and analysis. Individual liver pieces were homogenized in 7 ml final volume homogenizing buffer (0.05 M Tris, 0.15 M KCl, pH 7.4), and microsomes were sedimented by differential centrifugation as described previously (Stegeman et al., 1979). Microsomes were resuspended in approximately 2 ml per g tissue with resuspension buffer (0.05 M Tris, 0.1 mM EDTA, 1 mM DTT, 20% v/v glycerol, pH 7.4). Protein was determined in a 96 well plate using the micro-procedure of Smith et al. (1985).

7-Ethoxyresorufin-O-deethylase, the catalytic function of hydrocarbon-inducible CYP 1A, was measured using a kinetic modification of the plate-based assay of Kennedy et al. (1993). EROD activity was determined in duplicate in a 48 well plate at 20^0 C using a Cytofluor® fluorescent plate reader (Millipore, Bedford, MA). Each well contained 200 µl consisting of 1 µl of microsomes (4-15 µg protein), 2 µM 7-ethoxy resorufin in 50 mM Tris buffer, 0.1 M NaCl, pH = 7.8. Catalytic activity was initiated by the addition of NADPH in buffer to a final 1.67 mM

concentration. Fluorescence was determined at 1 min intervals over 6 min, and the linear slope (fluorescence per minute) was divided by the slope of the resorufin product standard curve (fluorescence per pmol) determined under the same conditions to yield pmol per minute per mg protein catalytic rates.

PCB Analysis

Harlequin duck plasma samples were analyzed for total PCB concentration and congener-specific concentrations of 93 congeners, including 12 known to induce P450 1A. To achieve a minimum sample volume of 0.5 ml, some samples were pooled based upon EROD values and capture sites (Table 1).

Plasma samples were prepared and analyzed using modified methods of Shoda (1997). Approximately 0.5 ml plasma was mixed with 5 ml hexane:diethylether (1:1) and shaken briefly. Two ml methanol was added, and the combined sample was mixed, shaken vigorously (by hand) and centrifuged for approximately 10 min. The extraction was repeated two more times with 5 ml hexane:diethylether. The combined hexane:diethylether extracts were concentrated to approximately 1 ml under a gentle stream of nitrogen. Sample clean-up was performed by passing the extract through a pasture pipette column containing (from bottom to top) glass wool, sand, silica gel, alumina and anhydrous sodium sulfate. The column was sequentially eluted with 5 ml of hexane and 10 ml of methylene chloride. The eluent was concentrated to 0.5 ml for analyses. Quantitative analyses were performed by capillary gas chromatography (CGC) with electron capture detector for PCBs (Wade et al., 1988). Some PCB congeners, including 114 and 157, co-elute during CGC and are indistinguishable by electron capture detection. These combined peaks were analyzed using a mass spectrometer detector in the SIM mode.

Statistical Analyses

All statistical analyses were conducted using SAS (SAS Institute Inc., Cary, North Carolina, USA). For each duck species, EROD activity was compared between areas using Student's T-test. For the PCB analysis we compared proportions of observations that were above the detection limits between areas (oiled vs unoiled) using Fisher's Exact test. For each area we had 10 samples and 93 congeners, thus the test compared numbers of positive values (above limit of detection) per 930 possible. We conducted the same analysis using only the 12 congeners known or suspected of inducing P450 1A (congeners 77, 105, 118/108/149, 126, 128, 138, 141, 156/171/202, 158, 167, 169, and 189). Multiple regression analysis was used to simultaneously assess effects of sample area and concentrations of specific PCB congeners on EROD activity.

Results

EROD Activity

Rates of EROD activity in Barrow's goldeneye liver samples averaged higher in birds from oiled Knight Island (94.3 pmol/min/mg protein; $n = 22$) than in those from Montague Island (49.5 pmol/min/mg protein; $n = 19$; $P = 0.0014$; Fig. 2). Hepatic EROD activities of wintering

harlequin ducks also were higher in samples from oiled areas (204.6 pmol/min/mg protein; $n = 19$) than in those from unoiled Montague Island (70.7 pmol/min/mg protein; $n = 18$; $P < 0.001$; Fig. 3).

PCB Analysis

Total PCBs were not measured above detection limits in any harlequin duck plasma sample; detection limits ranged from 0.03 to 0.13 ppm (averaged = 0.07 ppm; oiled areas = 0.07 ppm; unoiled areas = 0.06 ppm). Total PCB and congener concentrations are expressed on a wet weight basis and are not normalized to lipid.

Congener-specific analyses had lower detection limits, ranging from 0.14 to 0.50 ppb. Average congener-specific detection limits were 0.29 ppb for oiled areas, 0.24 ppb for unoiled areas, and 0.26 ppb overall. Of the 93 PCB congeners analyzed, concentrations measured above detection limits occurred in 8.9% of possible instances in birds from oiled areas and 11.9% in birds from unoiled areas. Frequency of values above detection limits was slightly higher at unoiled areas ($P = 0.04$). For congeners suspected of inducing P450 1A in birds, frequencies of observations above detection limits did not differ ($P = 0.82$) between oiled areas (8.3%) and unoiled areas (10.0%).

PCB congener 138 was measured above detection limits in all samples (range = 0.30 to 11.4 ppb), although concentrations did not differ ($P = 0.80$) between oiled (2.15; $n = 10$) and unoiled (1.79; $n = 10$) areas. In a multiple regression analysis, congener 138 concentration was positively related to EROD activity ($F = 53.86$, $P < 0.001$). However, after accounting for variation due to congener 138, birds from oiled areas had considerably higher EROD activity than those from unoiled areas ($F = 19.98$, $P < 0.001$) suggesting that congener 138 concentrations may influence P450 activity, but oiling history explained significant variation after accounting for any effect of congener 138. The relationship between congener 138 concentration and EROD activity was driven by 4 samples (1 from unoiled areas, 3 from oiled) with higher congener 138 values (Fig. 4). Without those samples in the model, there was no relationship between congener 138 and EROD activity, although the term for area was still highly significant ($F = 10.00$, $P = 0.008$).

Discussion

Cytochrome P450 1A activity was significantly higher in harlequin ducks and Barrow's goldeneye from areas of PWS originally impacted with *Exxon Valdez* oil than in birds from unoiled areas. Considerable evidence indicates that PAHs from residual *Exxon Valdez* oil were likely responsible for elevated EROD activities in seaducks and several other vertebrates in oiled areas of PWS (Marty et al., 1997; Woodin et al., 1997; Holland-Bartels, 1998); this suggests that some species of seaducks were still vulnerable to potential deleterious effects of oil exposure as long as 9 years following the oil spill.

Potential Sources of P450 1A-Inducing Compounds

Sources of P450 1A- inducing PAHs in PWS, other than oil from the *Exxon Valdez*, could include natural oil seeps and oil released in Valdez, Alaska, during the 1964 earthquake. However, Short and Babcock (1996) concluded that PAH concentrations in intertidal sediments and mussel (*Mytilus trossulus*) tissues were negligible in PWS immediately prior to the *Exxon Valdez* oil spill. Low concentrations of background hydrocarbons were detected in deep (> 100 m) benthic samples (Short et al., 1999), however, harlequin ducks and Barrow's goldeneyes are not deep foragers. Furthermore, the source of these deep sediment hydrocarbons are coal deposits in eastern PWS, which are not bioavailable (Short et al., 1999) and therefore, cannot induce P450 1A responses from biota. We conclude that background or natural hydrocarbon sources do not explain observed differences in P450 1A induction in seaducks between oiled and unoiled areas of PWS.

Other compounds potentially leading to P450 1A induction are certain PCB congeners. PCBs are ubiquitous throughout the environment, and several congeners are presumed to mediate their toxicity through the aryl-hydrocarbon (Ah) receptor, thereby inducing the CYP 1A gene family (Rattner et al., 1994). The most toxic PCB congeners and therefore, the most potent CYP 1A inducers are three planar congeners, 77, 126 and 169. These congeners were not measured above the limit of detection in any harlequin duck plasma sample, however, all samples contained measurable concentrations of PCB 138 (2, 2', 3, 4, 4', 5' hexachlorobiphenyl). PCB 138 is a di-ortho chlorine substituted analog of the more toxic planar PCB congeners. The two ortho-chlorine substitution decreases the planarity and toxicity of the congener, thereby reducing its potency as a CYP 1A inducer. In fact, the di-ortho analogs are thought to be 0.0001-0.00001 as toxic as the most potent CYP 1A inducer, 2,3, 7,8-TCDD (dioxin) (Safe, 1990).

PCB 138 is one of the most ubiquitous congeners measured in avian species. In Britain tissue analyses from 8 species, including sea birds, raptors and herons, indicated that congeners 138, 153 and 180 were most prevalent (Boumphrey et al., 1993). Threshold concentrations of PCB 138 in duck plasma necessary to induce CYP 1A expression are unknown, however concentrations reported here are low. Concentrations of PCB 138 in black-crowned night heron (*Nycticorax nycticorax*) embryos from a non-industrial reference site (Chincoteague National Wildlife Refuge, VA) had mean values of 7 ppb compared to 77 ppb from a contaminated site (Cat Island, Green Bay, WI) (Rattner et al., 1994). Hepatic EROD activity was 20-fold higher in herons from Cat Island than Chincoteague and positively correlated with total PCB concentrations; however, the contribution of individual congeners to EROD activity was unknown. The relationship between sample tissue type, PCB congener concentrations, and EROD induction has not been researched in birds. However, distribution of congeners in different tissue types appears to be consistent among 16 tissues measured in 3 waterbird species. For each bird, the relative contribution of individual congeners to total PCB concentrations was the same in each organ, although there were differences in total amount of PCBs among tissue type (Boumphrey et al., 1993).

Congener 138 concentration in harlequin duck blood plasma may explain some variation in EROD activity; the four samples with highest congener 138 concentrations also had highest EROD activity. However, this relationship was not sufficient to explain area differences in

EROD activity. Mean concentrations of congener 138 did not differ by area. Moreover, excluding the four samples with the highest congener 138 concentrations eliminated the positive relationship between EROD and congener 138 concentration. However, even this reduced data set showed dramatically different EROD activities between harlequin ducks from oiled and unoiled areas of PWS.

Vulnerability to Continued Oil Exposure

Life history characteristics of harlequin ducks and Barrow's goldeneyes make them particularly susceptible to continued oil exposure and, thus, any subsequent population-level consequences of exposure. These seaduck species occur in intertidal and shallow subtidal habitats in the nearshore environment, which are the same areas that received much of the oil spilled from the *Exxon Valdez* (Galt et al. 1991; Wolfe et al., 1994). In 1992, it was estimated that 15% of the oil spilled from the *Exxon Valdez* (1.65 million gallons) remained in intertidal shorelines and subtidal sediments (Wolfe et al., 1994). Much of this remnant oil was in sheltered bays or beneath beach surfaces (Hayes and Michel, 1999) thus inhibiting further weathering and dispersal. The continuous, but slow degradation of these remaining oil deposits makes continued oil exposure of birds that inhabit these areas plausible.

During winter, harlequin ducks and Barrow's goldeneyes feed almost exclusively on benthic invertebrates (Koehl et al., 1982; Vermeer, 1982; Goudie and Ankney, 1986; Goudie and Ryan, 1991). In the marine environment, bottom sediments and subsequently, benthic invertebrates, are often the final destination for oil constituents (Woodin et al., 1997). Benthic invertebrates do not rapidly metabolize PAHs (Boehm et al., 1996), so ingestion of contaminated prey could continually expose seaducks to low concentrations of oil which could, in turn, induce P450 1A responses. Mussels, a dietary component of both seaducks, in the oil spill zone had negligible concentrations of PAHs prior to the spill; however, accumulation of *Exxon Valdez* oil occurred in mussels throughout the spill-affected area (Short and Babcock, 1996). Similarly, other studies have also documented hydrocarbons in seaduck prey from immediately post-spill through 1995 (Patten et al., 1998; Babcock et al., 1996), suggesting that contaminated prey are a potential source of oil ingestion.

Potential Physiological and Population Consequences of Oil Exposure

Petroleum products are toxic to birds (see reviews by Leighton, 1993 and Leighton et al., 1985). Oil and oil-derived products can damage red blood cells, restrict uptake of nutrients, alter hormone balances, suppress the immune system, inhibit growth, and impair reproduction.

Polycyclic aromatic hydrocarbons are known to induce hepatic EROD activity in herring gulls (*Larus argentatus*) (Lee et al., 1985; Peakall et al., 1989), mallards (*Anas platyrhynchos*) (Gorsline and Holmes, 1981) and starlings (*Sturnus vulgaris*) (Trust et al., 1994). However, it is unclear whether PAH-induced P450 1A activity in birds causes additional toxicological effects (Leighton, 1993). Correlations have been made between early embryonic death and PAH content in crude oil applied to duck eggs (Hoffman, 1979). Additionally, Lee et al. (1986) demonstrated increased mortality with concomitant induction of P450 activity when minute amounts of Prudhoe Bay crude oil were applied to chicken eggs. However, they were uncertain whether

metabolism, and subsequent induction of MFO enzymes were necessary for toxicity. The metabolism of PAHs by the MFO system can produce highly reactive intermediate compounds that interact with other cellular constituents and cause the initiating event leading to mutagenesis or carcinogenesis (Fox, 1993). In laboratory mammals, compounds that bind to the Ah receptor and induce P450 1A responses also cause weight loss, promotion of tumors and immunotoxicity (Fox, 1993).

Oil ingestion and, particularly, external oiling of feathers can have severe metabolic consequences (Jenssen, 1994). Oil disrupts feather structure, reduces insulative properties of feathers, and can result in hypothermia and death. This is the main cause of immediate mortalities of marine birds following oil spills. However, even small amounts of external oil can increase costs of thermoregulation, thus metabolic costs of external oiling could be incurred as long as environmental oil is present. In PWS, oil sheening was observed as late as 1997 from beaches heavily oiled by the *Exxon Valdez* spill (Hayes and Michel, 1999), suggesting that external oiling and subsequent metabolic consequences for birds inhabiting nearshore environments are possible.

Potential physiological consequences of oil exposure could have population-level effects on seaducks. Many lab studies have suggested that oil exposure doesn't have toxic effects on waterfowl (almost always mallards) until high doses are ingested (Stubblefield et al., 1995). Such studies have been used to suggest that harlequin ducks should, similarly, be unaffected by residual *Exxon Valdez* oil spill (Boehm et al., 1996). However, these studies have typically been conducted for relatively short periods (weeks) under benign laboratory conditions. Other studies have documented that oil exposure is a physiological stressor that may not have toxic or demographic consequences in the absence of other stresses; however, with addition of other stressors such as cold temperatures, oiled ducks in the lab suffered considerably higher mortality than unoiled birds (Holmes et al., 1978; 1979). This may be a much more appropriate paradigm for wild seaducks chronically exposed to oil.

Data collected on harlequin ducks following the *Exxon Valdez* oil spill continue to demonstrate population-level effects from oil. Numbers of harlequin ducks surveyed during wing molt declined in oiled portions of Prince William Sound during 1995 to 1997, while populations were stable in unoiled areas (Rosenberg and Petrula, 1998). Winter survival of adult female ducks was lower in oiled areas compared to unoiled areas of PWS (D. Esler, unpubl. data); population model projections incorporating these survival rates matched the population trends observed by Rosenberg and Petrula (1998), suggesting that survival differences were responsible for observed population trends. Goudie and Ankney (1986) suggested that harlequin ducks were on the lower extreme of seaduck body mass necessary for surviving subarctic winters. Under predictable, natural conditions harlequin ducks should have high winter survival. However, harlequin ducks exist close to an energetic threshold, and survival rates may be compromised by even small physiological challenges. We acknowledge that links between oil exposure and population-level effects are speculative, but argue that these links are reasonable based on available information. We conclude that full recovery of some seaduck populations impacted by the *Exxon Valdez* oil spill may be constrained by exposure to residual oil and encourage further research on the mechanisms by which oil exposure may impact wild bird populations.

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TABLE 1. Harlequin duck blood serum samples used for PCB analysis, sorted by area and EROD activity.

Site	EROD Activity[a]	Sample ID	Avg. EROD[b]
Unoiled Area (Montague Island)			
Port Chalmers	4.0	M1	9.6
Stockdale Harbor	7.3	M2	14.55
Port Chalmers	15.2	M1	
Stockdale Harbor	21.8	M2	
Stockdale Harbor	24.0	M3	24.00
Stockdale Harbor	26.2	M4	27.85
Stockdale Harbor	29.5	M4	
Stockdale Harbor	30.3	M5	31.80
Stockdale Harbor	33.3	M5	
Stockdale Harbor	34.6	M6	40.80
Stockdale Harbor	47.0	M6	
Stockdale Harbor	48.9	M7	66.77
Stockdale Harbor	67.0	M7	
Stockdale Harbor	84.4	M7	
Stockdale Harbor	102.6	M8	102.60
Stockdale Harbor	141.5	M9	155.45
Stockdale Harbor	169.4	M9	
Stockdale Harbor	386.4	M10	386.40
Oiled Area			
Crafton Island	92.1	K1	97.65
Crafton Island	103.2	K1	
Main Bay	123.7	K2	123.70

Site	EROD Activity[a]	Sample ID	Avg. EROD[b]
Crafton Island	133.6	K3	139.30
Crafton Island	145.0	K3	
Crafton Island	156.8	K4	164.90
Crafton Island	173.0	K4	197.57
Crafton Island	179.9	K6	181.90
Main Bay	181.9	K5	
Crafton Island	195.2	K6	
Crafton Island	217.6	K6	283.50
Main Bay	263.6	K7	
Main Bay	303.4	K7	
Crafton Island	329.6	K8	353.15
Main Bay	368.5	K9	368.50
Crafton Island	376.7	K8	
Main Bay	n/a[c]	K10	

[a]pmol/min/mg protein
[b] Each line represents an individual bird; lines with common sample numbers were pooled to achieve minimum volume for analysis. Pooling was conducted based on EROD activity and site. Average EROD of pooled samples is presented at the first occurrence of each sample number.
[c]Recaptured bird with implanted radio; liver biopsy not collected.

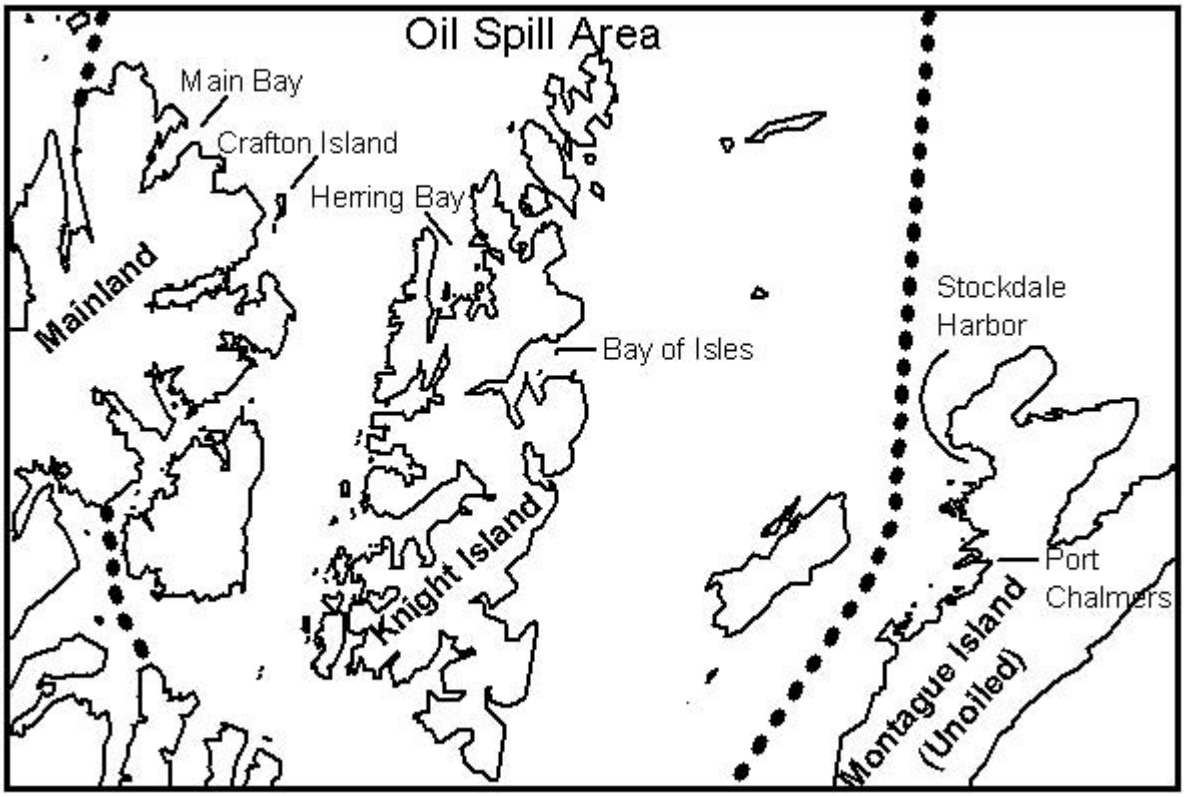

Figure 1. Oiled and unoiled areas of Prince William Sound, Alaska, used as sampling sites to measure hepatic P450 1A induction in harlequin ducks and Barrow's goldeneyes. The area bounded by bold, dotted lines is the area affected by the oil spill.

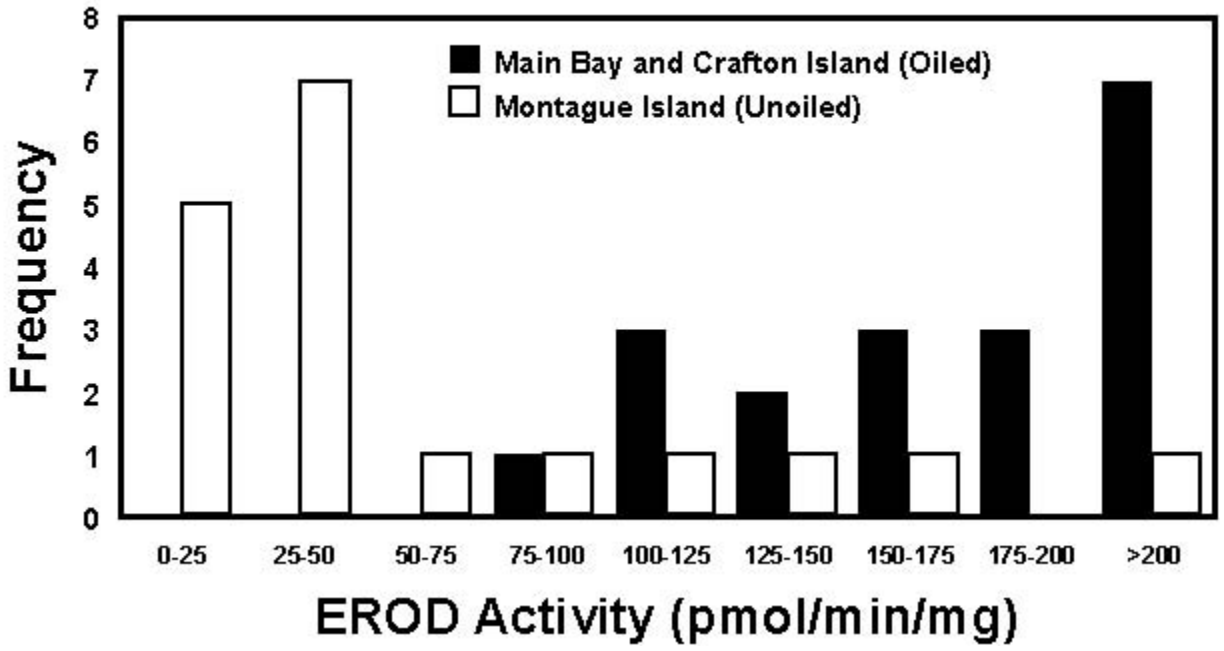

Figure 2. Comparisons of hepatic EROD activity of Barrow's goldeneyes collected from oiled and unoiled areas of Prince William Sound, Alaska.

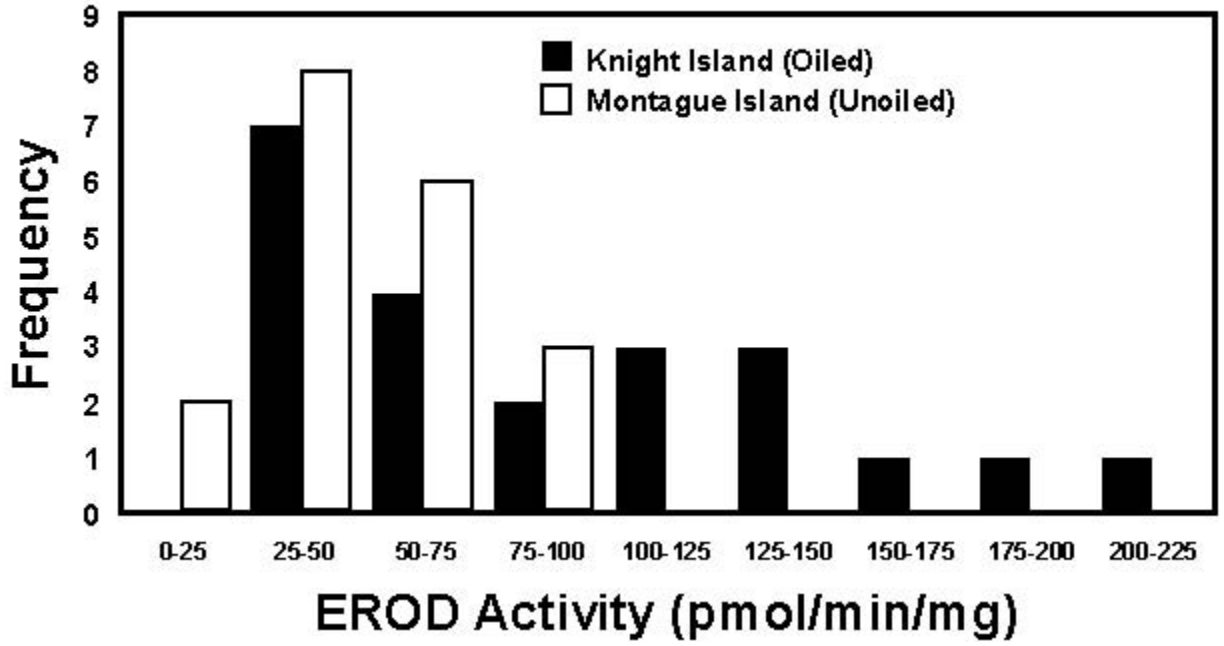

Figure 3. Comparisons of hepatic EROD activity of harlequin ducks captured from oiled and unoiled areas of Prince William Sound, Alaska.

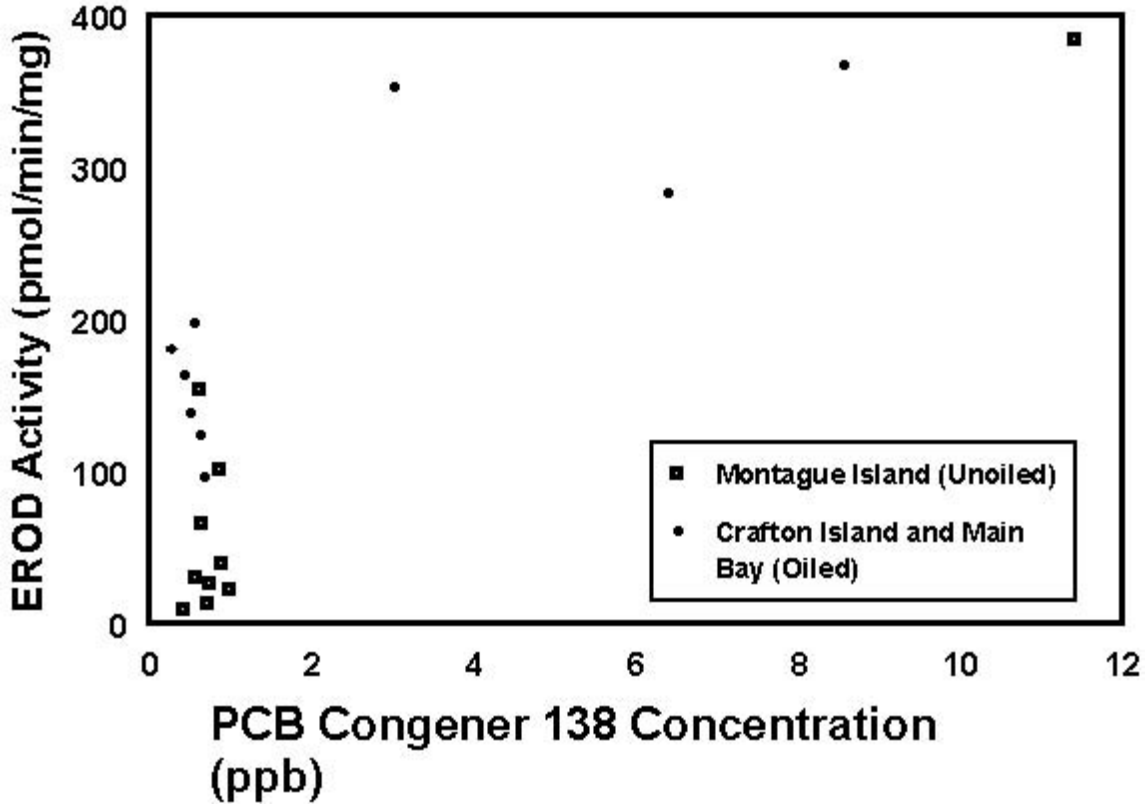

Figure 4. Scatterplot of EROD activity by concentrations of PCB congener 138 in blood plasma of harlequin ducks captured in oiled and unoiled areas of Prince William Sound, Alaska.

River Otter (*Lontra canadensis*) Appendix

(RO)

This River Otter Appendix illustrates the various published works on river otter that were funded in part by the NVP study.

RO-01 Ben-David, M., R. T. Bowyer, L. K. Duffy, D. D. Roby, and D. M. Schell. 1998. Social behavior and ecosystem processes: River otter latrines and nutrient dynamics of terrestrial vegetation. Ecological Society of America. Ecology 79(7):2567–2571.

Abstract: River otters (*Lutra canadensis* Schreber) inhabiting coastal environments scent-mark specific locations along the coast, known as latrine sites. In this study, we used stable isotope techniques to investigate the effects of this scent-marking behavior on terrestrial vegetation at the terrestrial-marine interface. Our analysis of stable isotope ratios of fur and feces indicated that river otters fed mainly on intertidal and subtidal fish. Eight different species of plants, growing in latrine sites of river otters, had significantly higher values of delta^{15}N compared with the same plant species growing on nonlatrine sites. Elevated N concentrations occurred only in grasses and mosses growing in latrine sites. Our results indicate that, through their scent-marking behavior, coastal river otters transfer marine-derived nitrogen into the beach-fringe forest and thus fertilize the terrestrial vegetation in the terrestrial-marine interface.

RO-02 Blajeski, A., L. K. Duffy, and R. T. Bowyer. 1996. Differences in faecal profiles of porphyrins among river otters exposed to the *Exxon Valdez* oil spill. Biomarkers 1:262–266.

Abstract: River otters (*Lutra canadensis*) living in marine environments of Prince William Sound, Alaska, exposed to crude oil from the *Exxon Valdez* spill in March 1989, showed significantly elevated levels of faecal porphyrin over those of otters from non-oiled areas (oiled mean = 48.2 and non-oiled mean = 34.5 nmol g^{-1} dry faeces). Profiles of uro-, hepta-, hexa-, penta-, copro-, and protoporphyrin profiles were qualitatively characterized by high-performance liquid chromatography. These findings suggest that river otters may serve as a suitable indicator species in which porphyrin profiles can be used to monitor the effects of marine and freshwater crude oil exposure. Also, this is the first model showing the effects of an oil spill on porphyrins on a free-ranging mammal using a non-lethal methodology. These effects were detectable 1 year after the spill and following a major effort to clean oil from the shorelines of Prince William Sound.

RO-03 Blundell, G. M., R. T. Bowyer, M. Ben-David, T. A Dean, and S. C. Jewett. 2000. Effects of food resources on spacing behavior of river otters: Does forage abundance control home-range size? Proceedings of the 15th International Symposium on Biotelemetry. (In press)

Abstract: We use three analytical techniques to examine home-range dynamics of river otters in Prince William Sound, Alaska, USA, from February 1997 to January 1998 and discuss problems with analysis of linear home ranges. River otters inhabiting marine environments where fish were abundant had smaller home ranges than animals living in freshwater systems with fewer prey, whereas otters using multiple salmon runs had larger home ranges than otters in other habitats.

RO-04 Blundell, G. M., J. W. Kern, R. T. Bowyer, and L. K. Duffy. 1999. Capturing river otters: A comparison of Hancock and leg-hold traps. Wildlife Society Bulletin 27 (1):184–192.

Introduction: The ability to live-capture study animals is essential to many research and management programs (Schemnitz 1994). An efficient and effective method to live-capture river otters (*Lontra canadensis*) is critical for the success of both theoretical (Ben-David et al. 1996, 1998) and applied studies (Erickson and McCullough 1987, Testa et al, 1994. Bowyer et al. 1995). Both Hancock and leg-hold traps have been used for such purposes. The number of trap nights required to capture a river otter in a Hancock trap ranged from 58 to 123 (Melquist and Hornocker 1983, Shirley et al. 1983, Woolington 1984). Rates of captures for various types of leg-hold traps ranged from 60 to 315 trap nights/otter captured (Shirley et al. 1983, Serfass et al. 1996).

Other potential differences between Hancock and leg-hold traps have been reported. Using Soft-catch[@] leg-hold traps resulted in a high rate of escape (43% of 51 potential captures; Serfass et al. 1996); modifications of the Hancock trap were thought to make it more efficient (Northcott and Slade 1976). However, the cumbersome size of Hancock live traps (95 × 59 × 40 cm; >11 kg) may limit the number of traps that can be transported efficiently or the locations or size of areas where those traps can be set appropriately. Moreover, river otter may learn to avoid capture in Hancock traps (Duffy et al. 1994a).

Few data are available on injuries to river otters from methods used to capture these large mustelids. Shirley et al. (1983) reported 16% of 30 river otters experienced a broken toe from being captured with an unpadded leg-hold trap, but most otters had only minor skin lacerations or suffered no injury. Serfass et al. (1996) compared injuries to otters from Softcatch[@] leg-hold traps with traps lacking padded jaws. Traps with padded jaws caused injury rates of 38% for canine teeth and 38% for appendages (n = 20 otters). Private trappers using unpadded traps caused much greater injuries to otters, but the types of traps and handling techniques varied markedly (Serfass et al. 1996). Hurbert et al. (1996) reviewed efficiency of traps to capture terrestrial carnivores and the injuries caused by those traps. No study has assessed injuries caused to otters by Hancock traps.

We evaluated capture success and injury rate for river otters live-captured in Hancock and unpadded leg-hold traps. We tested for differences in capture efficiency, rate of escape, rate of malfunction, and utility of those types of traps. Additionally, we tested for differences in severity and types of injuries to otters from Hancock and leg-hold traps.

RO-05 Duffy, L. K., M. K. Hecker, G. Blundell, and R. T. Bowyer. 1999. An analysis of the fur of river otters in Prince William Sound, Alaska: Oil-related hydrocarbons 8 years after the *Exxon Valdez* oil spill. Polar Biology 21:56–58.

Abstract: Approximately 8 years after the *Exxon Valdez* oil spill, river otters (*Lutra canadensis*) were trapped from the shoreline in both oiled (Knight Island) and nonoiled (Jackpot Bay) areas of Prince William Sound, Alaska. Captive river otters were wiped with isopropanol-soaked gauze and the gauze extracts were analyzed by gas chromatography with mass spectrometry detection. Differences in pentacosane (C-25) levels in the fur were observed between the oiled and nonoiled sites, while lower molecular weight aliphatics and aromatics were absent. These data are useful when evaluating the role of fur grooming in the long-term exposure of river otters to hydrocarbons and the expression of P450-1A in Knight Island otters.

RO-06 Hecker, M. K., L. K. Duffy, G. M. Blundell, and R. T. Bowyer. 1997. River otters as a sentinel species: Effect and detection of crude oil on the fur of river otters. Pages 100–102 *in* B. Jessup and J. Mazet, editors. Effects of oil on wildlife. Proceedings of the Fifth International Conference on Oil Spills.

Abstract: River otters (*Lutra canadensis*) have been used as a sentinel species in pollution studies throughout North America. A modified wipe test of river otter fur was developed to detect the presence of residual crude oil on the fur of river otters inhabiting Prince William Sound, Alaska. River otter pelts (both tanned and untanned) were used as models and exhibited differences in hair structure and absorption of crude oil. Immunochemical detection, as well as detection by mass spectrometry, after a methanol extraction of crude oil from the fur were compared. Our results showed that an immunoassay provides an inexpensive and reliable test for oil at concentrations greater than 1 ppm. Crude oil on the fur after extraction with methanol could also be detected in the 1 ppm range. Using the immunoassay wipe test, 17 river otters from oiled and nonoiled areas of Prince William Sound sampled in summer 1996 showed no detectable oil >1 ppm. Mass spectrometry can be used to increase the sensitivity of detection.

RO-07 Sauer, T. M., M. Ben-David, and R. T. Bowyer. 1999. A new application of the adaptive-kernel method: Estimating linear home ranges of river otters, *Lutra canadensis*. The Canadian Field-Naturalist 113. 6 pp.

Abstract: Standard techniques for estimating size of home range for semiaquatic mammals usually result in overestimating area because unused tracts of land and water are incorporated into calculations. For river otters (*Lutra canadensis*) that inhabited a narrow strip of habitat along the terrestrial-marine interface, linear length of shoreline previously was used as a measure of home-range size. Although that method produced a conservative estimate, selection of data points using that procedure did not provide any measure of probability or indication of core areas. We used the adaptive-kernel estimator and Geographic Information System for calculating linear length of home ranges for river otters inhabiting a marine environment and assessed the effect of reducing the bandwidth size on those home-range estimates. Using locations collected from four otters in Ester Passage, Prince William Sound, Alaska, USA, during summer 1991, we determined that adaptive kernel with 100% and 95% density contours resulted in a larger estimate than that produced by the previously used method. Decreasing bandwidth did not significantly alter the estimated linear distances of home range. In addition, the use of density contours of 65% delineated core areas; therefore, this technique provided a tool with which researchers can test hypotheses such as seasonal shifts in size and location of home ranges in relation to resource availability and distribution. Our technique may be useful for estimating home ranges of other animals approximating a linear distribution of locations.

Pigeon Guillemot (*Cepphus columba*) Appendix

(PG)

APPENDIX PG-01

MECHANISM OF IMPACT AND POTENTIAL RECOVERY OF PIGEON GUILLEMOTS (*CEPPHUS COLUMBA*) AFTER THE *EXXON VALDEZ* OIL SPILL[1]

A
THESIS

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By

Pamela Edith Seiser

Fairbanks, Alaska

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ABSTRACT

The abundance of pigeon guillemots in oiled areas of Prince William Sound, Alaska, failed to increase after the1989 *Exxon Valdez* oil spill. Population growth may be constrained by the physiological effects of oil exposure, food availability, and nest predation. I conducted a comparative study among unoiled, oiled, and pre-spill data sets, to provide insight on factors limiting population recovery in oiled areas. Blood samples from chicks in oiled and unoiled areas provided little evidence of physiological effects of exposure to oil. Pigeon guillemot diet, productivity, growth rates, and fledging weights in unoiled areas of southwestern Prince William Sound from 1994 to 1998 indicate oiled areas had a lower proportion of high-lipid fish in the chick diet and lower fledging weights, compared to unoiled and pre-spill studies. These results suggest that the lack of recovery in oiled areas is associated with a prey base that results in lower fledging weights, which may reduce juvenile survival.

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ACKNOWLEDGMENTS

My research was a component of a larger study, the Nearshore Vertebrate Predator Project, funded by the *Exxon Valdez* Oil Spill Trustees Council. I have many individuals to thank because my thesis included data from three separate study areas, Jackpot Island, Naked Island and Kachemak Bay, which in turn were supported by three different agencies: the Alaska Cooperative Fish and Wildlife Research Unit, the office of Migratory Bird Management of the U.S. Fish & Wildlife Service and the Alaska Biological Science Center of the U. S. Geological Survey, in Anchorage, Alaska.

First, I would like to thank my committee members: my major advisor Dr. Dave McGuire for his patient instructions in the art of technical writing; Dr. Larry Duffy for his advice on biomarkers; and Dr. Alan Springer for his insights on seabird ecology. I thank the unofficial members of my committee Dr. Dan Roby and Dr. Scott Newman DMV, for contributing their expertise on avian nutrition and physiology. Dr. Greg Golet and Lindsey Hayes collected blood samples from Naked Island and shared their insight on the recovery of pigeon guillemots. I thank *Cepphus* experts George Divoky and Alex Prichard for their helpful discussions. I would also like to acknowledge the contribution of Jackpot Island's 1994 and 1995 data set by Lindsey Hayes and Gail Blundell, respectively.

My study includes data collected over a five-year project by many hard working individuals. At Jackpot Island, they were Adrian Gall, Mike Grene, Phil Joy, Cynthia Restrepo, Kelsey Sullivan, Mike Walgren and Darcie Zeil. Ted Spencer of the Naked Island crew provided assistance and humor, as well as numerous other 'Naked boys and girls': Laura Ballock, Mary Cody, Bryan Duggan, Amy Hahn, Jim Hamon, Melissa Luanglue, Aly McKnight, Angela Palmer, Mark Russell, Scott Schaffer, Bev Short, Oliver Sternicki, Dave Tessler, and Ed Vorisek. At Kachemak Bay, Mike Litzow, April Nielsen, and Sadie Wright generously contributed their time and energy to collect blood samples for my project. Maps were produced by GIS expert and friend Debbie Nigro. Last, but not least, I thank my family, friends, and office mates for the encouragement and supported they provide through my graduate work.

1

CHAPTER ONE

OVERVIEW: THE RESPONSE OF PIGEON GUILLEMOTS TO THE 1989 *EXXON VALDEZ* OIL SPILL

OVERVIEW

In March of 1989, the oil tanker *Exxon Valdez* spilled 42 million L of crude oil into Prince William Sound (PWS). Between 100,000 to 375,000 birds were killed in the spill (Piatt *et al.,* 1990; Ford *et al.,* 1996; Piatt *et al.,* 1996). Negative impacts of the *Exxon Valdez* oil spill (EVOS) on the abundance and subsequent return of PWS avian species back into heavily oiled areas after the spill was still evident in pigeon guillemots, *Cepphus columba*, two years after the spill (Murphy *et al.,* 1997). The profound impact of EVOS on nearshore communities was related not only to the volume of the oil spill, but also to the fate of the spilled oil (Burger, 1993). Pigeon guillemots are tightly linked with the health of nearshore marine habitats (Ewins, 1993; Prichard, 1997). Among seabirds, pigeon guillemots are particularly vulnerable to contaminants in the vicinity of breeding colonies because of their limited foraging range during the chick rearing period (Ewins, 1993). Forty percent of EVOS oil was deposited on PWS shorelines in 1989 (Galt *et al.,* 1991). Despite beach cleaning efforts and natural biodegradation processes, Wolfe *et al*. (1994) estimated that 15% of the EVOS oil remained in intertidal and subtidal areas two years after the spill. The majority of prey base of pigeon guillemots is associated with subtidal and intertidal substrates (Ewins, 1993). While the greater portion of PWS avian communities demonstrated signs of population recovery 2 to 3 years after the spill (Wiens *et al.,* 1996), pigeon guillemot populations in oiled areas, such as Naked Island, have continued to decline below 1990 levels (Hayes and Kuletz, 1997).

From 1979 to 1981, Oakely (1981) and Kuletz (1983) studied the breeding and foraging ecology of Naked Island's pigeon guillemots. The *T/V Exxon Valdez* ran aground in 1989 within 30 km of Naked Island. The degree of oiling along the convoluted shoreline of Naked Island varied from negligible to heavy. When Oakely and Kuletz (1996) returned to Naked Island in 1990, they found 43% fewer pigeon guillemots as compared to their pre-spill censuses (1978 to 1981). Many researchers investigating the impact of EVOS on the PWS ecosystem pointed out that natural changes in the marine environment between pre-spill and post-spill studies may be as significant as the impact of EVOS (Oakely and Kuletz, 1996; Piatt and Anderson, 1996; Wiens and Parker, 1995).

Broad scale changes in climate and oceanographic conditions in the Gulf of Alaska (GOA) between the late 1970's and late 1980's resulted in a shift in the relative abundance and size classes of fish species (Royer, 1993; Piatt and Anderson, 1996; Anderson *et al.,* 1997; Fritz *et al.,* 1993). The Alaska Coastal Current brings GOA waters into PWS resulting in ocean conditions similar to GOA in the southern parts of

PWS (Niebauer *et al.*, 1994). There is evidence that this shift in forage fish species negatively affected GOA seabird survival and productivity. Black-legged kittiwakes, *Rissa tridactyla*, of the outer GOA colonies of Middleton Island, Semidi Islands, and Kodiak Island suffered numerous breeding failures in the 1980's and colony populations dropped in number (Hatch *et al.*, 1992; Springer *et al.*, 1993). As with the outer GOA colonies, kittiwake colonies in southern PWS also experienced low productivity from 1985 to 1989 (Irons *et al.*, 1999; Hatch *et al.*, 1992).

Pigeon guillemot abundance in PWS declined between the 1970's and 1980's in a manner parallel with wide scale declines in the abundance of surface schooling fish species and shrimp (Oakely and Kuletz, 1996; Agler *et al.*, 1999). Similar population responses were observed in other PWS bird and marine mammal species that consumed forage fish (Kuletz *et al.*, 1997; Piatt and Anderson, 1996). However, the declines observed at Naked Island and Knight Island between the eighties and the early nineties were greater along oiled shorelines than along unoiled shorelines (Oakley and Kuletz, 1996; Murphy *et al.*, 1997).

Hayes and Kuletz (1997) reported that the proportion of surface schooling fish in the diet of pigeon guillemot chicks had declined between pre-spill (1979 to 1981) and post-spill studies (1990 to 1996). Surface schooling fish, Pacific herring, *Clupea pallasi*, and Pacific sand lance, *Ammodytes hexapterus*, are important food items for breeding seabirds. Because their summer lipid stores translate to high energy meals for chicks, and their schooling behavior represents a concentrated food source for foraging adults, surface schooling fish represent a potentially high provision rate for chicks (Golet *et al.*, 2000).

Herring and sand lance spawn in the nearshore habitat. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal and spawning habitats (Murphy and Rice, 1999; Rice, 1999). Herring embryos exposed to oil yielded more physically deformed larvae than unoiled embryos (Kocan *et al.*, 1996; Hose *et al.*, 1996). Several studies have reported that EVOS oil in sediments induce xenobiotic responses, induction of cytochrome P450, or liver lesions in intertidal fish species, including high cockcomb, *Anoplarchus purpurescens*, walleye pollock, *Theragra chalcogramma*, and kelp greenling, *Hexagrammos decogrammus* (Woodin *et al.*, 1997; Collier *et al*., 1996; Jewett *et al.*, 1995; Holland-Bartels, 1998). The two potential routes that pigeon guillemots may be exposed to residual oil are through ingestion of contaminated food items, or contact with oil sheens while foraging in oiled areas (King and Sanger, 1979; Piatt *et al.*, 1990; Prichard, 1997).

Recovery of the pigeon guillemot population may be constrained by the physiological effects of oil exposure on chicks and adults, demographic limitations due to pigeon guillemot life history traits, food limitations, or other factors such as nest predation. In my thesis, I assess both the oil and non-oil related factors that may be constraining the post EVOS population growth of pigeon guillemots in PWS. In chapter two, I assess the impacts of residual oil on the clinical health of chicks and adults by comparing the hematological and plasma biochemical profiles of chicks and adults in oiled and unoiled areas. In chapter three, I examine demographic and food limitations. To gauge the current demographic limitations of populations in unoiled areas, I tracked

trends in population, productivity, and fledgling survival, at unoiled Jackpot Island in southwestern PWS, from 1995 to 1998. I compared productivity parameters and the relative quality of the chick diet among unoiled Jackpot Island, pre-spill Naked Island, and post-spill Naked Island studies. To assess quality of the chick diet, I examined the species composition of the chick diet and the frequency of meal deliveries. To evaluate food limitations, I compared the relative quality of the chick diet with chick survival rates, growth rates, and fledging weights. In my final chapter, I address the question 'Is it oil or is it food?' by providing a summary of my findings from chapters two and three. This eco-toxicology approach in assessing the health of post-spill pigeon guillemot populations is unique because few oil spill studies have tested for the lingering sub-lethal effects of residual oil, or gauged population response in accordance with available food resources.

2

CHAPTER TWO

COMPARISON OF PIGEON GUILLEMOT, *CEPPHUS COLUMBA*, BLOOD PARAMETERS FROM OILED AND UNOILED AREAS OF ALASKA, EIGHT YEARS AFTER THE *EXXON VALDEZ* OIL SPILL

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2.1 INTRODUCTION

Population estimates of pigeon guillemots, *Cepphus columba*, in Prince William Sound (PWS), Alaska, have declined from 15,000 individuals in 1972-73 to approximately 3,000 individuals in the mid-1990's (Dwyer *et al.*, 1976; Klosiewski and Laing, 1994; Agler and Kendall, 1997; Sanger and Cody, 1994). A large-scale regime shift in the Gulf of Alaska during the late 1970's (Piatt and Anderson, 1996) likely caused much of this decline, as high-quality forage fish were more widely available in the 1970's than in recent years (Hayes and Kuletz, 1997; Kuletz *et al.*, 1997). Pigeon guillemot populations in PWS were further impacted by the Exxon Valdez oil spill (EVOS; Murphy *et al.*, 1997), which occurred when the supertanker *Exxon Valdez* ran aground on 24 March 1989 and spilled 42 million L of crude oil into PWS. Approximately 40% of this oil was deposited on the shorelines of PWS (Galt *et al.*, 1991). Between 100,000 to 375,000 birds died in the spill, of which 1,500 to 3,000 were pigeon guillemots (Piatt *et al.*, 1990). Seven years after the spill, pigeon guillemots had not recovered to pre-spill numbers (Agler and Kendall, 1997; Oakley and Kuletz, 1996). It is not clear to what extent demography, food availability, or the physiological effects of lingering oil exposure may be constraining recovery of pigeon guillemots in PWS.

Pigeon guillemots are vulnerable to oil spills because they use the nearshore habitat (King and Sanger, 1979; Piatt *et al.*, 1990). They breed in small colonies along rocky coastlines, and roost on intertidal rocks. Guillemots spend much of their time on the sea surface or diving for surface schooling fish, demersal fish, and invertebrates associated with the intertidal and subtidal zones.

The prey of pigeon guillemots are also susceptible to oil contamination. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal habitats (Murphy and Rice, 1999; Rice 1999). For example, Pacific herring, *Clupea pallasii*, embryos exposed to oil yielded more physically deformed larvae than unoiled embryos (Kocan *et al.*, 1996; Hose *et al.*, 1996). Biomarkers of oil ingestion were noted

in PWS fish several years after EVOS. Walleye pollock, *Theragra chalcogramma*, collected from oiled Naked Island in 1990 and 1991, exhibited high levels of fluorescent aromatic compounds in their bile (Collier *et al.*, 1996). Jewett *et al.* (1995) reported that demersal fish in the oiled eelgrass beds of Herring Bay, PWS, demonstrated a high incidence of hemosiderosis lesions in the liver. Kelp greenling, *Hexagrammos decogrammus*, collected in 1996 showed significantly higher expression of P450 activity in oiled Herring Bay versus unoiled Jackpot Bay (Holland-Bartels, 1998). Research in the early 1990's demonstrated that oil exposure had detrimental effects on nearshore predators including river otters, *Lutra canadensis* (Bowyer *et al.*, 1994, 1995; Duffy *et al.*, 1993, 1994), and sea otters, *Enhydra lutris* (Loughlin *et al.*, 1996). Whether residual oil from the EVOS affected pigeon guillemots required further evaluation.

Acute toxic effects of petroleum hydrocarbons are well known (Leighton, 1993), but the lingering effects of chronic oil exposure have not been investigated fully in free ranging piscivorous birds (Fry and Lowenstine, 1985). Leighton (1993) provided an extensive review of avian studies of petroleum oil toxicity. Dosing experiments have shown that the effects of oil ingestion include: (1) lower hatch rate and altered yolk structure (Grau *et al.*, 1977; Szaro *et al.* 1978a); (2) reduced rate of growth (Szaro *et al.*, 1978b; Peakall *et al.*, 1982); (3) slower development and reduced survivorship of chicks (Trivelpiece *et al.*, 1984); (4) liver, kidney and intestine damage in long-term exposure (Khan and Ryan, 1991; Patton and Dieter, 1980; Fry and Lowenstine, 1985); and (5) Heinz-body hemolytic anemia associated with a substantial decrease in packed-cell volume (Leighton *et al.*, 1983).

Because guillemot chicks remain in their natal burrow until they fledge, oil contamination can occur through contact with the oiled feathers of an adult while in the egg or chick stage, or through ingestion of contaminated fish (Leighton, 1993; Peakall *et al.*, 1980). At nine days of incubation, avian embryos are extremely sensitive to oil contacting the egg shell. As little as 5 µl of Prudhoe Bay crude oil has been reported to cause embryo death at this stage (Albers, 1977; Szaro *et al.*, 1978a). Dosing studies of weathered crude oil on congeneric black guillemots, *Cepphus grylle*, suggest that oil ingestion may cause long-term physiological effects which could reduce a young bird's ability to survive at sea (Peakall *et al.*, 1980).

Payne et al. (1986) suggested that detecting simple changes in a biochemical or physiological response in a population may provide information on the presence of toxins. Hematological analyses (differential cell counts) may provide information about the immunological status of birds (Campbell, 1986a). Levels of plasma enzymes provide information on the function of organs, e.g. liver (Campbell, 1986a). Elevated levels of acute-phase protein haptoglobin indicate responses to exogenous toxins, bacterial or viral infections, and physical trauma (Silverman and LeGrys, 1987). Physiological changes occurring during the chick growth period have been suggested by many authors to influence blood parameters (Wolf *et al.*, 1985; Hoffman *et al*., 1985; Kostlecka-Myrcha, 1987; Starck, 1998; Work, 1996; Prichard *et al.*, 1997). To prevent age-dependent variation from biasing assessments, hematological and plasma biochemical profiles should be repeated on chicks at different stages of development.

To make an accurate assessment of clinical tests, reference values of healthy individuals are needed (Hawkey and Samour, 1988), but information on hematological and clinical chemistry on pigeon guillemots or other alcids is limited (Newman *et al*., 1997; Newman and Zinkl, 1998; Prichard *et al.*, 1997; Kosteleck-Myrcha, 1987). We assume therefore that colonies in the unoiled areas represent healthy populations. If oil contamination is limiting recovery of pigeon guillemots in PWS, we expected that blood chemistry and cell counts would differ between oiled and unoiled areas and these differences should be consistent with either toxic responses or lower fitness. In this study, we compare the hematological and plasma biochemical profiles between pigeon guillemot populations in an oiled area of PWS and in unoiled areas of PWS.

2.2 METHODS

During summer 1997, measurements of growth and blood samples from pigeon guillemot chicks were collected in areas oiled by the EVOS and in reference areas that were not oiled (Fig. 2.1). The oiled area we evaluated was Naked Island (60° 40' N, 147° 28' W) in central PWS. The prevailing winds and currents during spring of 1989 deposited oil predominately on the east and northwest shorelines of Naked Island (Galt *et al.*, 1991; Oakley and Kuletz, 1996). The combined colonies of Jackpot Island (60° 19' N, 148° 11' W) and Icy Bay (60° 14' N, 148° 17' W) in southwestern PWS were not oiled and represent the reference areas in this study. For evaluating adults, we also included a third reference area located in Kachemak Bay (59° 35' N, 151° 19' W), which is located in lower Cook Inlet, Alaska.

For each chick, mass and length of wing-chord were measured every five days until the chick fledged. When possible, two blood samples were collected from each chick at approximately 20 and 30 days after hatch. The hatching date of the chick was determined from either direct observation or was estimated by comparing wing-chord length for chicks of unknown age to wing-chord length for chicks of known age. Adults were captured either by noose traps placed on roosting rocks or with a dip net.

One cc of blood was collected from the brachial vein of chicks using a one cc tuberculin syringe with a 25 or 26 gauge needle. Adults were bled from the medial metatarsal vein. Fresh blood was used to make blood smears on glass slides. Two heparinized micro-hematocrit tubes were filled with blood from the puncture site, capped with clay, and stored in coolers. Whole blood was placed in microtainer tubes treated with lithium heparin. These samples were centrifuged within two hours of collection. After centrifuging, plasma was removed with a disposable pipette and divided between two snap-top plastic vials. Vials were frozen in propane freezers. Blood smear slides, micro-hematocrit tubes and one vial of plasma were placed in chilled insulated boxes and shipped to the Avian and Exotic Laboratory of Redondo Beach, California, within 48 hours of collection. The following parameters were measured: red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), hemoglobin (Hp), mean cell hemoglobin content (MCHC), counts of white blood cells (WBC), heterophils,

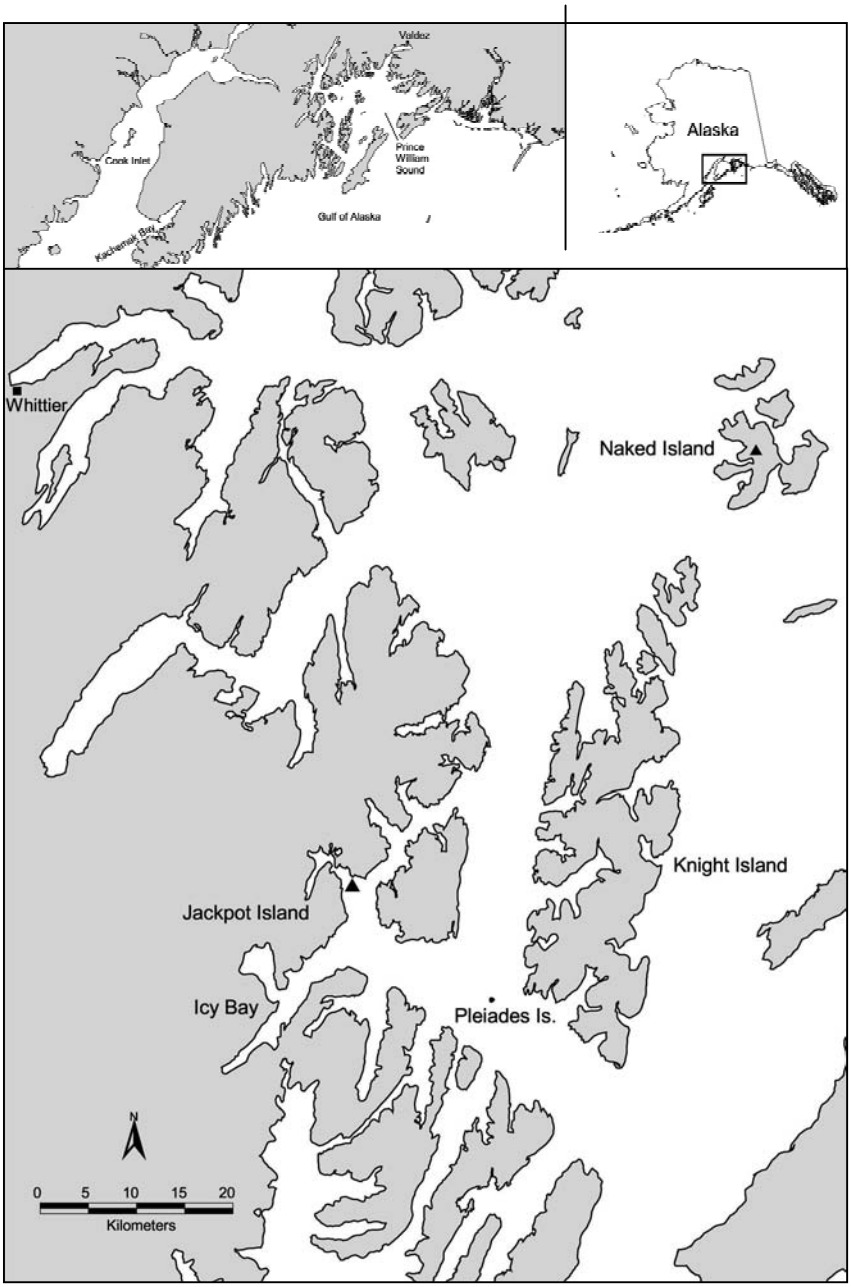

Figure 2.1 Location of the oiled and unoiled reference areas in Prince William Sound and Kachemak Bay, Alaska. The oiled area for this study included several pigeon guillemot colonies on Naked Island in central Prince William Sound. The unoiled reference areas for this study included pigeon guillemot colonies at Jackpot Island and Icy Bay in southwestern Prince William Sound, and pigeon guillemot colonies in Kachemak Bay.

lymphocytes, eosinophils, basophils, activity of creatine phosphokinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transferase (GGT), concentration of calcium, uric acid, plasma protein, total protein, alpha-1 macroglobulin, alpha-2 macroglobulin, beta globulin, gamma globulin, albumin, albumin to gamma globulin ratio, bile acid, phosphorus, and sodium. A second vial of frozen plasma was sent to the University of Alaska Fairbanks for measurement of haptoglobin concentration with electrophoresis kits (Helena Laboratories, Beaumont Texas, USA). Plasma was applied to agarose gels and electrophoresed at 100 volts for one hour. Agarose plates were then fixed with 7.5% trichloroacetic acid and stained with o-dianisidine to detect the Hp-hemoglobin complex. The Hp-hemoglobin complex was quantified by densitometry and results are reported in mg hemoglobin binding capacity per 100 ml of plasma (Duffy *et al*., 1994). Enzyme immunoassay wipes were used to evaluate the presence of polyaromatic hydrocarbon molecules on the plumage of adults. The plumage of adults was wiped with a one-ply section of 5 by 5 cm gauze pad saturated with isopropanol. The gauze pad was then placed in aluminum foil and frozen until analysis. Levels of phenathrene, pentacosane, and hexacosane from the wipes were measured with the EnSysEnviroGard™ Polynuclear Aromatic Hydrocarbon test kit 70608, produced by Millpore Corporation (Bedford, Massachusetts, USA), or detected with gas chromatography-mass spectrometry (Duffy *et al*., 1999).

Data were tested for normality and equal variance with the Kolmogorov-Smirnov test with Littiefors correction and with the Levene median test, respectively. To test the hypothesis that there was no difference between samples collected at 20 days of age and 30 days of age, we used the paired *t*-test or the Friedmans test on ranks, a nonparametric test for a repeated measures design, on the samples collected in the reference area. Blood parameters with significantly different values between sampling ages are considered to be influenced by the development stage of the chicks. A *t*-test or Mann-Whitney test was used, when appropriate, to detect differences in blood parameters between oiled and unoiled areas, and between 30-day post hatch chicks and adults in the reference areas.

2.3 RESULTS

2.3.1 Effects of age: nestlings

We found several age-related differences in the blood samples. For chicks in southwestern PWS, significant differences between the blood samples of chicks 20 and 30 days after hatching included PCV ($P = 0.014$), RBC ($P = 0.002$) and alkaline phosphatase activity ($P = 0.001$). Differences in phosphorus concentrations were marginally non-significant ($P = 0.063$). The mean (± SD) wing-chord lengths of the 20-day and 30-day age groups were 92.8 ± 7.6 cm and 128.7 ± 6.3 cm, respectively. A multiple logistic regression model using variables RBC, PCV, and alkaline phosphatase activity correctly predicted the age group in 18 of 22 blood samples with a concordance of 82% (likelihood ratio test = 7.7, $P = 0.051$). Variables correlated with the nestling wing-chord length of chicks included PCV ($r = 0.59$, $P = 0.001$, $n = 26$), RBC ($r = 0.58$,

P = 0.001, n = 24), alkaline phosphatase (r = 0.57, P = 0.003, n = 24), phosphorus (r = -0.39, P = 0.059, n = 24) and Hp (r = 0.56, P = 0.004, n = 24).

2.3.2 Effects of age: adults versus nestlings

The blood profiles of the adult birds from reference areas of Jackpot Island, Icy Bay and Kachemak Bay were distinct from the blood profile of the chicks from the reference area of Jackpot and Icy Bay. The age-related differences among chicks, which included PCV, RBC, alkaline phosphatase, and phosphorus, extended to our comparison between adults versus chicks. By the time a chick fledges, which occurs between 33 and 54 days of age, its weight is comparable to that of an adult, but its wing growth is not complete (Ewins, 1992; Ewins, 1993). For adults from southwestern PWS, the mean (± SD) for wing-chord length and body weight were 184 ± 4 cm and 508 ± 50 g, respectively. The wing-chord length at 20 days and 30 days after hatching was 49% and 70%, respectively, of wing-chord length in adults. The body mass at 20 days and 30 days after hatching was 66% and 86%, respectively, of the adult body mass. Because we had only samples from four adults in southwestern PWS, we incorporated adults from Kachemak Bay (n=3) into our sample of adults from unoiled areas. In the unoiled areas, adults had higher PCV (P = 0.001), RBC (P = 0.003), Hp (0.004), AST (P = 0.010), and albumin concentrations (P = 0.011), and lower alkaline phosphatase (P < 0.001) and lower phosphorus concentrations (P < 0.001) than 30-day old chicks in southwestern PWS. Adults also tended to have lower WBC (P=0.072), calcium concentration (P = 0.063), and bile acid concentration (P = 0.094) than chicks.

2.3.3 Oiled vs. Unoiled Populations: nestlings

In the 20-day age group, chicks sampled from the oiled population at Naked Island had lower calcium (P = 0.002), plasma protein (P = 0.008), and alkaline phosphatase activity (P = 0.025), and a higher lymphocyte count (P = 0.006) than chicks in the unoiled area of southwestern PWS (Table 2.1). In the 30-day age group, Naked Island chicks had significantly lower calcium (P = 0.043) and MCV (P = 0.015) than chicks from southwestern PWS (Table 2.2).

2.3.4 Oiled vs. Unoiled Populations: adults

Our sample size of adults was small. The number of adult blood samples from Naked Island, southwestern PWS, and Kachemak Bay were 10, 4 and 3, respectively. Adults at Naked Island were captured between 29 July and 3 August. Three of the adults in the reference areas were captured in June and two in August. Adults captured in the oiled area had significantly higher AST activity (P = 0.017), lower RBC (P = 0.006), Hp (P = 0.004) and GGT (P = 0.015) than adults in the reference areas (Table 2.3). The AST activity for the adults in the oiled area was nearly double the levels for the adults in the

reference areas. The plumage wipes from adults at Naked Island (n = 10) indicated low levels of phenathrene, pentacosane and hexacosane (mean ± SD: 0.004 ppm ± 0.002, 0.178 ppm ± 0.059, and 0.202 ppm ± 0.047, respectively).

Table 2.1 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks sampled at 20 days of age. Samples were collected in 1997 from oiled Naked Island colonies and unoiled Jackpot Island & Icy Bay colonies, in Prince William Sound, Alaska.

	Oiled Area Naked Island			Unoiled Area Jackpot Island & Icy Bay		
	mean	SD	n	mean	SD	n
Red Blood Cells (cu mm^{-3})	2.6	0.4	14	2.58	0.4	17
Packed Cell Volume (%)	44	4	15	43	6	18
Mean cell volume (cu mm^{-3})	159	16	14	160	13	17
Hemoglobin (g dl^{-1})	12.7	1.6	14	11.6	1.6	16
MCHC (g dl^{-1})	30.7	5.4	14	28	4.3	16
White Blood Cells (10^3 mm^{-3})	13	5	14	16	6	18
Heterophil *	49	12	14	61	10	17
Lymphocytes *	49	12	14	37	10	17
Eosinophil	0.6	1.3	14	0.7	1.3	16
Basophil	1.4	1.3	14	1.1	1.4	18
Calcium (mg dl^{-1})*	8.9	1.9	14	11.0	1.2	18
CK (u l^{-1})	530	233	14	776	541	17
LDH (u l^{-1})	937	234	14	897	471	18
AST (u l^{-1})	277	106	14	221	119	16
Uric Acid (mg dl^{-1})	18.3	8.7	14	20.0	11.2	16
Plasma Protein (g dl^{-1})*	3.1	0.5	14	3.8	0.6	18
Total Protein (g dl^{-1})	4.5	0.6	14	4.8	0.8	18
Alpha-1 (g dl^{-1})	0.39	0.11	14	0.44	0.18	18
Alpha-2 (g dl^{-1})	0.70	0.31	14	0.75	0.32	18
Beta (g dl^{-1})	0.88	0.21	14	0.91	0.35	18
Gamma Globulin (g dl^{-1})	0.70	0.16	14	0.75	0.15	18
Albumin (g dl^{-1})	1.86	0.33	14	1.94	0.51	18
Albumin/Gamma Globulin (g dl^{-1})	0.72	0.17	14	0.68	0.15	18
Bile Acid Assay (umol l^{-1})	38.8	35.6	14	61.9	105	14
Alkaline phosphatase (u l^{-1})*	372	151	14	279	82	17
GGT (u l^{-1})	25.2	12.5	14	20.6	14.8	13
Phosphorus (mg dl^{-1})	9.6	4.8	13	6.2	1.7	17
Sodium (mmol l^{-1})	129	17	11	141.0	5	13
Haptoglobin (Hp binding dl^{-1})	109	40	15	124	51	16

*Means significantly different ($P < 0.050$) between chicks sampled at Naked Island and Jackpot-Icy Bay.

Table 2.2 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks at 30 days of age. Samples were collected in 1997 from oiled Naked Island colonies and unoiled Jackpot Island & Icy Bay colonies, in Prince William Sound, Alaska.

	Oiled Area Naked Island			Unoiled Area Jackpot Island & Icy Bay		
	mean	SD	n	mean	SD	n
Red Blood Cells (mm^{-3})	3.16	0.40	24	2.95	0.42	13
Packed Cell Volume (%)	48	4	25	47	6	15
Mean cell volume (cu mm^{-3})*	148	13	24	160	10	13
Hemoglobin (g dl^{-1})	13.8	1.6	22	13	1.8	14
MCHC (g dl^{-1})	29	5	22	27	4	14
White Blood Cells (10^3 mm^{-3})	13	6	24	12	5	17
Heterophil	62	11	24	56	12	17
Lymphocytes	36	11	24	42	12	17
Eosinophil	0.3	0.5	24	0.2	0.4	17
Basophil	1.1	1.0	24	1.3	1.8	17
Calcium (mg dl^{-1})*	9.0	1.8	19	10.3	1.3	15
CK (u l^{-1})	613	528	20	554	221	15
LDH (u l^{-1})	863	482	21	863	325	15
AST (u l^{-1})	313	169	19	304	233	15
Uric Acid (mg dl^{-1})	12.3	11.1	21	16.7	8.7	14
Plasma Protein (g dl^{-1})	3.5	0.8	22	4.0	1.5	17
Total Protein (g dl^{-1})	5.0	1.7	22	4.6	0.9	15
Alpha-1 (g dl^{-1})	0.50	0.37	22	0.40	0.19	15
Alpha-2 (g dl^{-1})	0.68	0.40	22	0.72	0.39	15
Beta (g dl^{-1})	0.98	0.35	22	0.90	0.49	15
Gamma Globulin (g dl^{-1})	0.75	0.38	22	0.73	0.19	15
Albumin (g dl^{-1})	0.75	0.17	22	0.70	0.21	15
Albumin/Gamma Globulin (g dl^{-1})	2.14	0.75	22	1.84	0.50	15
Bile Acid Assay (umol l^{-1})	38	45	15	106	158	14
Alkaline phosphatase (u l^{-1})	502	367	18	443	152	15
GGT (u l^{-1})	16	15	14	16	11	13
Phosphorus (mg dl^{-1})	7.4	4.5	21	5.6	1.9	15
Sodium (mmol l^{-1})	133	16	16	142	13	13
Haptoglobin (Hp binding dl^{-1})	99	38	20	122	44	14

*Means significantly different ($P < 0.050$) between chicks sampled at Naked Island and Jackpot-Icy Bay.

Table 2.3 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of adult pigeon guillemots sampled in 1997 from oiled Naked Island, Prince William Sound and unoiled areas of Jackpot Island & Icy Bay, Prince William Sound and Kachemak Bay, Lower Cook Inlet in Alaska.

	Oiled Area Naked Island			Unoiled Area Jackpot Island, Icy Bay and Kachemak Bay		
	mean	SD	n	mean	SD	n
Red Blood Cells (cu mm^{-3})*	3.01	0.35	10	3.76	0.59	6
Packed Cell Volume (%)	53	5	10	58	6	7
Mean cell volume (cu mm^{-3})	168	9	10	163	10	6
Hemoglobin (g dl^{-1})	18.3	3.3	10			
MCHC (g dl^{-1})	34.3	7.12	10	33.2	11.4	4
White Blood Cells (10^3 mm^{-3})	8	2	10	8	1	7
Heterophil	58	13	10	64	13	7
Lymphocytes	37.9	8.8	10	33.4	12.1	7
Eosinophil	0	0	10	0	1	7
Basophil	4	5	10	3	2	7
Calcium (mg dl^{-1})	8.6	1.6	9	9.1	1.2	7
CK (u l^{-1})	244	168	9	375	339	7
LDH (u l^{-1})	892	296	10	915	143	7
AST (u l^{-1})*	979	816	10	461	199	7
Uric Acid (mg dl^{-1})	14.85	5.83	10	14.6	6.5	7
Plasma Protein (g dl^{-1})	4.7	2.3	10	3.9	0.7	6
Total Protein (g dl^{-1})	5.5	0.98	10	5.6	1.7	7
Alpha-1 (g dl^{-1})	0.45	0.24	10	0.43	0.29	7
Alpha-2 (g dl^{-1})	0.67	0.42	10	0.90	0.45	7
Beta (g dl^{-1})	0.90	0.58	10	0.71	0.30	7
Gamma Globulin (g dl^{-1})	0.69	0.17	10	1.02	0.97	7
Albumin (g dl^{-1})	2.75	0.71	10	2.63	0.71	7
Albumin/Gamma Globulin (g dl^{-1})	1.03	0.30	10	0.94	0.27	7
Bile Acid Assay (umol l^{-1})	40.3	74.5	7	2.05	2.6	7
Alkaline phosphatase (u l^{-1})	93	70	8	137	102	6
GGT (u l^{-1}) *	3	5	9	10.8	8.2	7
Phosphorus (mg dl^{-1})	2.2	1.8	8	1.7	0.8	7
Sodium (mmol l^{-1})	138.6	17.1	7	143.8	9.3	4
Haptoglobin (Hp binding dl^{-1})	122	28	8	93	50	7

*Means significantly different ($P < 0.050$) between adults sampled at oiled areas and unoiled areas.

2.4 DISCUSSION

The clinical hematology and biochemistry of seabirds is not as well known as for waterfowl, poultry or pet species (Newman and Zinkl, 1998). Blood parameters vary among species according to life history patterns, diet, and activity level. Pigeon guillemots differ from more commonly studied birds in that they have rapidly growing semi-precocial chicks, their diet is composed of marine fish, and they are adapted to diving to depths greater than 20 m (Ewins, 1993). Interpreting our results is also made difficult because of the paucity of biochemical studies on this species. The few reference values for this species are from studies with sample sizes of less than ten individuals (Newman and Zinkl, 1998; Newman *et al.*, 1997; Prichard *et al.*, 1997; Haggblom *et al.*, 1988; Bradley and Trefall, 1974). Our study extends the biochemical information for chicks of this species by providing reference values for different stages of development that are based on larger sample sizes.

2.4.1 Effects of Development

Physiological changes occurring during post hatch development of chicks affect many hematological and biochemical parameters (Starck, 1998; Vinuela *et al.*, 1991; Kostlecka-Myrcha, 1987). Age-related variation in blood parameters is an important consideration when collecting samples from pigeon guillemot colonies, because the range in chick ages may be as great 42 days (Drent, 1965). This is caused by asynchronous nesting or the laying of replacement clutches (Ewins, 1993; Drent, 1965). It has been well documented in many avian species that adults have higher PCV, RBC, and Hp than immature birds (Work, 1996; Wolf *et al.*, 1985; Kostlecka-Myrcha, 1987; Fairbrother *et al.*, 1990), but there is little documentation of the changes in these parameters within the nestling period for free-living species (Kostelecka-Myrcha, 1987). Anemia has been associated with oil contamination (Hartung and Hunt, 1966; Szaro *et al.*, 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Leighton *et al.*, 1983). Clinical signs of anemia are low PCV, RBC, MCV or MCHC. Therefore it was critical for us to identify these age-specific differences in red blood cell parameters before evaluating the health of immature birds. During the nestling period, there are dramatic changes in the profile of the red blood cells as embryonic forms, natal forms, and adult forms replace one another (Schenk *et al.*, 1978). Kostelecka-Myrcha (1987) documented PCV increases and MCV decreases during the nestling period of the little auk, *Plautus alle*, as smaller sized adult red blood cells replaced the red blood cells after hatching. The greatest increases in RBC occurred during the first 10 days after hatch (Kostelecka-Myrcha, 1987; Hoffman *et al.*, 1985). Post-hatch development of erythropoietic tissue is closely related to growth of body mass. As the chick approaches adult size or asymptotic body mass, bones are ossifying in preparation for flight and erythropoietic tissue decreases to adult levels (Starck, 1998). Pigeon guillemot chicks reach asymptotic growth between 30 and 40 days of age (Ewins, 1993). Kostelecka-Myrcha (1987) noted a non-significant

increase in Hp level during the latter half of the nestling period. Our study and the study of Haggblom *et al.* (1988) confirm that similar age-related changes in Hp occur in pigeon guillemots chicks. We expect subtle changes in red blood cells and Hp to continue after chicks fledge.

Elevated alkaline phosphatase (AP) activity in birds is associated with increased osteoblastic activity such as skeletal growth and repair, egg production, or nutritional deficiencies (Lumeij, 1994). Therefore the normal range of AP activity in rapidly growing chicks is higher than in adults (Wolf *et al*., 1985; Hoffman *et al*., 1985; Vinuela *et al.*, 1991; Work, 1996). We found AP activity nearly doubled between the samples for chicks 20 days and 30 days after hatching. The activity of AP reported by Newman and Zinkl (1998) for fledglings were similar to the AP activity for 20-day old chicks in our study. In red kites, *Milvus milvus*, Vinuela *et al.* (1991) reported that AP activity peaked at 38 days after hatch, when the growth of long bones were near completion. Pigeon guillemot chicks also had higher phosphorus and marginally higher calcium levels than adults. Vinuela *et al.* (1991) noted that increases in calcium and phosphorus levels correlated with increases in AP activity during the nestling period of red kites. In brown pelicans, *Pelecanus occidentalis*, Wolf *et al.* (1985) found that AP activity and phosphorus concentration were highest during the first 10 months of development and remained moderately elevated through the first two years of life. Pigeon guillemots are smaller than pelicans, but their skeletal growth continues after fledging for at least two months (Ewins, 1992). These patterns suggest that AP activity, phosphorus and calcium concentrations of guillemot chicks will peak prior to fledging then gradually drop to adult range within the first six months of life.

Elevated WBC is a symptom of infection. Interpretation of elevated WBC in juvenile birds is difficult because their normal range is variable and higher than adults (Fudge, 1996). For terns, shearwaters, and petrels, Work (1996) reported that older chicks tend to have higher WBC than adults. Puerta *et al.* (1990) reported similar results for common cranes. We could not detect differences in WBC between 20-day and 30-day old chicks, but these chicks had higher WBC than adults.

Similar to our results, Prichard *et al.* (1997) and Work (1996) reported that chicks had lower AST activity than adults. Newman and Zinkl (1998) found that young pigeon guillemots between five and ten weeks old have AST activity greater than or equal to the activity in adults. Elevated AST activity is associated with hepatocellular damage, septicemia and muscle injury. Bollinger *et al.* (1989) studied the effect of different capture methods on waterfowl AST activity and reported that AST activity becomes elevated with physical exertion. We suggest that chicks have lower AST activity than adults because they are sedentary and their muscles are less developed. Compared to adults, chicks offer little resistance to capture and are less likely to experience muscular exertion and injury.

Age-related differences in Hp concentration have been documented in mammals. Stellar sea lion, *Eumetopias jubatus*, pups that are less than 15 days old have significantly lower haptoglobin (Hp) levels than adults (Zenteno-Savin *et al.*, 1997). In humans, neonates do not have detectable levels of Hp until two months of age (Henry, 1991). Prichard and co-workers (1997) reported that pigeon guillemot chicks had significantly

lower Hp levels than adults. Adults in our study had lower mean Hp levels than reported by Prichard (1997), which may explain why we did not find similar age-related differences. Prichard (1997) noted that Hp was correlated with the rate at which adults deliver meals to the nest. In our study Hp was significantly correlated with the rate of weight gain immediately prior to the drawing of blood from chicks. This relationship supports Prichard's speculation that Hp is sensitive to the nutrition of chicks. We also documented a positive correlation between Hp and RBC, which suggests that Hp levels may be linked to the development of erythropoietic tissue during chick development.

2.4.2 Comparison between populations in oiled and unoiled areas

Various oil-dosing studies have been conducted on birds, but the symptoms of toxicity of oil ingestion have varied with species, age, the chemical composition of the oil, the dosing levels, and the presence of additional stress factors (Hartung, 1995; Leighton, 1993). Ingestion of sublethal levels of crude oil may constitute a nonspecific stressor for birds and render them more vulnerable to stress factors such as persistent cold temperatures and bacterial diseases (Holmes *et al.*, 1979). To evaluate the presence of injury at the oiled colonies in this study, we measured blood parameters that were indicators of physiological health of organ systems that involve the liver function, kidney function, the haematopoietic system, immune function, and electrolyte balance.

The avian liver responds to oil ingestion with hypotrophic activity (Szaro *et al.*, 1978b; Patton and Dieter, 1980; Stubblefield *et al*., 1995) and induction of hepatic cytochrome P-450 (Peakall *et al*., 1989; Lee *et al*., 1985). Enlargement of the liver may be a compensatory response to metabolize the high burden of toxic material introduced in experimental diets (Patton and Dieter, 1980; Stubblefield *et. al*, 1995) or an inflammation response to cell injury. Hepatocellular damage and necrosis are associated with elevation in the activity of plasma liver enzymes (Lewandowski *et al*., 1986). In Leighton's (1993) review of oil toxicity research, he found that the evidence of injury to the liver was inconsistent among studies, which may be associated with enzyme responses that are specific to species (Franson *et. al*, 1985). Our indicators of liver injury were elevated bile acid, AST and LDH activity in the plasma. In pigeons, *Columba livia*, elevated levels of bile acid (Lumeij, 1988) and AST are the most sensitive indicator of experimentally induced liver injury (Lumeij, 1988; Campbell, 1986b). Ingestion stimulates the release of bile acid. Fasted peregrine falcons, *Falco peregrinus*, experienced a three-fold increase in plasma bile acid concentration after ingestion of meat (Lumeij and Remple, 1992). During our study adults fed their nestlings at a rate of 0.4 to 1.0 fish h^{-1}. We did not control the food intake of chicks and this would explain some of variation in bile acid concentrations between individuals. Post-prandial increases in bile acid concentration represent 1-fold to 2-fold increases, while hepatobilary disease results in 5-fold to 10-fold increases relative to the reference range (Lumeij, 1991). Elevated levels of bile acid concentration (exceeding 200 micro mol l^{-1}) indicate persistent loss of hepatic function (Fudge, 1996). The bile acid concentrations of chicks at Naked Island were in the ranges reported for pigeons and peregrine falcons (Lumeij, 1988; Lumeij and Remple, 1992).

While AST and LDH are considered non-specific because they occur in many tissues, Campbell (1986b) found that AST and LDH were sensitive indicators of liver disease in carnivorous birds including red tail hawks, *Buteo jamicensis*, and great horned owls, *Bubo virginianus*. Elevated BA, AST or LDH concentrations were uncommon among chicks in both the oiled and unoiled areas, and we did not observe a significant difference in mean activity of BA, AST or LDH between chicks of Naked Island and southwestern PWS. Other researchers working with weathered Prudhoe Bay crude oil found no effect of oil dosing on liver enzyme responses of alcid chicks (Leighton, 1993; Prichard, 1997) and mallards, *Anas platyrhynchos* (Rattner, 1981; Stubblefield *et al.*, 1995). The blood variables associated with liver function and hepatocellular damage do not indicate deleterious effects on livers of chicks at Naked Island.

Renal tubular necrosis was documented in Cassin's auklets, *Prychoramphus aleuticus*, after oil was applied to their breast feathers (Fry and Lowenstine, 1985). Increases in uric acid in the plasma may indicate adverse effects on renal function (Allen, 1988; Fudge, 1996). In veterinary practices uric acid levels greater than 20 mg dl^{-1} are abnormal (Allen, 1988; Fudge, 1996). Newman and coworkers (1997) noted that uric acid levels in adult piscivorous marine birds are typically higher than in other avian species. They suggest that high protein diets combined with the osmoregulation demands of living in a marine environment causes higher concentrations of serum uric acid. In our study, both chicks and adults had uric acid levels that were below 20 mg dl^{-1}, which is within the reference range previously reported for adult pigeon guillemots (Newman and Zinkl, 1998; Newman *et al.*, 1997). Therefore, the uric acid levels of chicks in the oiled area of our study do not appear to indicate the presence of impaired renal function or damage.

Anemia was documented in several species of birds following exposure to oil (Hartung and Hunt, 1966; Szaro *et al*., 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Fry and Addiego, 1987; Leighton *et al*., 1983). Reduced PCV and Heinz-body hemolytic anemia was documented in young herring gulls, *Larus argentatus*, and Atlantic puffins, *Fratercula arctica*, after experimental ingestion of crude oil (Leighton *et al.*, 1983). Yet, ingestion of high doses of Prudhoe Bay crude oil did not result in anemia in both adult rhinoceros auklets, *Cerrorhinca monocerata*, (Newman, personal communication) and mallards (Stubblefield *et al*., 1995). Hemolytic anemia was documented in adult white-winged scoters, *Melanitta fusca*, rescued from an oil spill, but blood samples were taken several days after the birds were captured (Yamato *et al*., 1996). The decrease in physical activity, the stress of handling, and the change in diet associated with captivity may influence erythropoiesis in adult alcids (Newman, personal communication). Anemia is the result of reduced erythropoiesis, accelerated erythrocyte destruction (hemolytic anemia), or blood loss. Clinical signs of anemia are low PCV, RBC, MCHC or MCV. There is little variation in PCV among species, and values below 32% are considered diagnostic of anemia (Hawkey and Samour, 1988). In our study, the values for PCV, MCHC and hemoglobin were within the ranges that are normal for immature birds, which indicates that there was probably no anemia for chicks in the oiled area of our study. The MCV values for 30-day old chicks at Naked Island were significantly less than the MCV for chicks in southwestern PWS and in Kachemak Bay,

Alaska (Seiser, unpublished data). It is not clear why MCV values are lower in the oiled area.

Immunosuppression has been noted in various oil dosing studies (Leighton, 1993). Reduced lymphocytes and reduced resistance to bacterial pathogens have been recorded in mallards (Holmes *et al*., 1979; Rocke *et al*., 1984). In adult rhinoceros auklets, ingestion of crude oil elicited no inflammatory response in WBC or differential cell counts, but young alcids may respond differently (Newman, personal communication). Leighton (1986) reported morphological changes to the lymphoid glands of young Atlantic puffins and herring gulls. In our study, WBC and differential cell counts (lymphocytes, heterophils, eosinophils and basophils) were our indicators of the state of the immune system. The ratio of lymphocytes to heterophils for the 20-day old chicks at Naked Island was significantly different from the ratio for chicks in southwestern PWS, but this pattern did not persist for the 30-day old chicks. We found that Naked Island did not have significantly lower values of WBC or differential cell counts than the unoiled area in southwestern PWS, which suggests that the immune system was not stressed or impaired in a way that would influence cell production.

Hypertrophy of salt glands has been documented in marine birds dosed with crude oil (Peakall *et al.,* 1980, 1982, 1983; Miller *et al*., 1978). Osmoregulatory impairment can be accompanied by increases in plasma sodium levels. Peakall et al. (1980) noted a transient rise in plasma sodium levels in black guillemot chicks dosed with 0.1 ml and 0.2 ml of Prudhoe Bay crude oil. Similar results have been found in herring gulls (Miller *et al*., 1978) and mallards (Eastin and Rattner, 1982). In contrast, Prichard (1997) found sodium levels of pigeon guillemot chicks did not respond to dosing with 0.2 ml of weathered Prudhoe Bay crude oil. The sodium levels for chicks in the unoiled area of our study were similar to levels for the control chicks in the study by Prichard *et al.* (1997). Because the sodium levels for the chicks at Naked Island were not significantly different from the levels for chicks in southwestern PWS, we conclude that there is no evidence for hypertrophy of salt glands.

The results reported here also extend the data base for Hp levels in pigeon guillemots. Haptoglobin is an acute phase protein that has been widely used in human and other mammal medical practices as an indicator of inflammatory diseases, infectious diseases, trauma or stress. Gevaert and co-workers (1991) demonstrated that Hp concentrations increased after the pigeons were infected with salmonellosis. Although Hp has been employed to assess potential stressors in compromised wildlife populations (Duffy *et al*., 1993; Duffy *et al*., 1994; Zenteno-Savin *et al*., 1997; Prichard *et. al.,* 1997), it has not been widely used for assessing health in free-ranging birds. The recovery of river otters from the initial impact of the EVOS was documented with the use of Hp (Duffy *et al*., 1993: Duffy *et al*., 1994). In comparisons between declining and stable populations of pinnipeds, significantly higher Hp concentrations were associated with the declining populations of harbor seals, *Phoca vitulina*, and sea lions (Zenteno-Savin *et al*., 1997). Prichard et al. (1997) examined the use of Hp as a potential biomarker of oil ingestion in pigeon guillemot chicks, but found that variation in growth rates and feeding rates among chicks from different colonies confounded their interpretation of Hp response to the ingestion of weathered crude oil. In our study, there was no evidence of

poor health identified by our suite of health indicators, which is consistent with the similar Hp levels we observed in chicks from oiled and unoiled areas.

Because nearly all the chicks that were sampled for blood in our study ultimately fledged, we conclude that our handling and blood sampling did not affect survival. This observation also supports our diagnosis of clinically healthy chicks. In contrast, the overall fledging success (fledglings per hatchling) for Naked Island and Jackpot Island was 46% and 68%, respectively. In Kachemak Bay, Prichard (1997) also noted that the majority of nestling mortality occurred in the first 12 days after hatch. Predators or food shortages are the most common sources of mortality of young chicks (Hayes and Kuletz 1997, Nelson, 1987). Mink, a major predator of nestlings in PWS, was not present on Jackpot Island in 1997, but was at Naked Island. The shoreline of Naked Island suffered both oil contamination and physical disturbance from efforts to clean beaches after the spill. Both events tend to have negative effects on the prey base of pigeon guillemots. Therefore, we limit our conclusions on the health of chicks to the latter half of the nesting period. Currently, hematological and biochemical variables of the pigeon guillemots we studied provide little evidence of oil-related injury for chicks that hatched in 1997, eight years after the Exxon Valdez oil spill. In contrast to chicks, the pilot study we conducted on adult health suggests that the issue of oil-related injury in pigeon guillemot adults cannot be dismissed without further study.

Pigeon guillemot adults have greater opportunities for exposure to oil than nestlings. Adults feed on invertebrates including crabs, shrimps, and bivalves (Oakley 1981; Kuletz, 1983; Sanger, 1987), but rarely provision their chicks with invertebrates (Oakley, 1981; Ewins, 1993). In the winter, invertebrate consumption may increase because of seasonal changes in distribution of prey fish. Pacific sand lance, *Ammodytes hexapterus*, are inaccessible because they are burrowed in the sediment, and young cod move to deeper waters (Oakley, 1981; Sanger, 1987). Bioaccumulation of polynuclear aromatic hydrocarbons (PAH) is greater in invertebrates than fish. Invertebrates cannot metabolize PAH as efficiently as fish, because of differences in the activity of mixed function oxgenenase enzymes and metabolic rate between invertebrates and fish (Gibson, 1977). Therefore, adults potentially have a greater dietary source of PAH's than nestlings (Bolger *et al.*, 1996; Baumard *et al.*, 1998).

It is important to recognize that our sample of adults is small and was obtained opportunistically. The majority of the samples from the unoiled areas were obtained in June, while the samples from the oiled area were collected in late July and early August. Also, we do not know the sex of the birds we sampled. Sex and reproductive condition have been documented to affect plasma biochemistry (Wolf *et al.*, 1985; Fairbrother *et al.*, 1990; Gee *et al.*, 1981). Because interpretation of differences between blood parameters for adults from the oiled and unoiled areas in our study is complicated by sampling issues, the interpretation we present is preliminary and should be viewed with some caution.

In comparison to adults in the unoiled area of our study, GGT activity was significantly lower for adults in the oiled area. GGT activity is commonly measured in mammal clinical practices to detect cholestatic diseases of the liver or the consumption of drugs and other toxic substances that induce the microsomal enzyme system (Henry,

1991). For example, fungi infested feed produces elevated plasma GGT activity in domestic chickens (Espada *et al*., 1994). GGT activity is not a sensitive indicator of avian hepatocellular injury (Campbell, 1986b). Egg laying also appears to elevate serum GGT activity. In domestic mallard hens, Fairbrother *et al.* (1990) observed that serum GGT activity was 10-fold higher during the egg-laying period compared to the incubation period. Newman and Zinkl (1998) measured the mean serum GGT activity for several seabird species, and reported a mean GGT activity of 16.5 IU l^{-1} with a range of 0 to 60 IU l^{-1} for five pigeon guillemot adults captured during the egg laying period. These values were slightly higher than the values we observed for adults in the unoiled areas of our study, which were also sampled early in the breeding season. For adults at Naked Island, which were sampled late in the breeding season, the GGT activity was within the range previously reported for adult rhinoceros auklets, *Cerorhinca monocerata*, common murre, *Uria aalge,* incubating western gull, *Larus occidentalis*, and non-breeding white pelicans, *Pelecanus onocrotalus* (Newman and Zinkl, 1998; Puerta *et al.*, 1991). It is not clear if the lower GGT activity we observed for adults in the oiled area represents a normal seasonal trend in GGT activity for adult pigeon guillemots.

The AST activity of adults in the oiled area was significantly higher and nearly double the AST activity of adults in the unoiled areas of our study and double the AST activity of adult pigeon guillemots observed in other studies (Newman *et al*., 1997; Newman and Zinkl, 1998). Elevated AST activity is associated with both hepatocellular damage and muscle injury (Bollinger *et al*., 1989). Muscle injury associated with capture causes elevated CK or LDH activity in waterfowl species (Bollinger *et al.,* 1989; Franson *et al.,* 1985; Fudge, 1996). We did not observe significant differences in CK or LDH between adults in oiled and unoiled areas of our study. Because similar capture methods were used in the oiled and unoiled areas of our study, we suggest that the elevated AST concentrations in the adults from the oiled area are more consistent with hepatocellular injury than muscle injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Because adults have greater opportunities for exposure to residual oil than nestlings, we recommend additional studies to fully evaluate the health of adults residing in oiled areas.

3

CHAPTER THREE

POPULATION TRENDS AND REPRODUCTIVE BIOLOGY OF PIGEON GUILLEMOTS IN UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA, BETWEEN 1994 & 1998: INSIGHTS ON THE RECOVERY OF PIGEON GUILLEMOTS IN OILED AREAS

3.1 INTRODUCTION

Pigeon guillemots were impacted by the *Exxon Valdez* oil spill (EVOS). Piatt *et al.* (1990) estimated between 1,500 to 3,000 pigeon guillemots were killed in 1989. When the pigeon guillemot population of oiled Naked Island began to decline in 1993, concern arose that it was a symptom of sub-lethal effects of chronic exposure to residual oil. However, several factors may cause the population to decline and limit growth, such as physiological effects of oil on chicks and adults, food shortages, and nest predators. At Naked Island, Hayes and Kuletz (1997) correlated population declines between pre- and post-spill periods with declines in the abundance of sand lance, an important prey species of pigeon guillemots.

The decline in sand lance abundance may be attributed to either localized oiling or broad scale changes in climatic-oceanographic conditions. In the Gulf of Alaska, many piscivorous seabird and marine mammal populations dependent on high-quality forage species declined during the 1970's and 1980's (Agler *et al.*, 1999; Piatt and Anderson, 1996; Springer *et al.*, 1993). Declines in high-quality forage species, such as herring, sand lance and capelin were associated with climatic and oceanographic changes in the Gulf of Alaska in the late 1970's (Piatt and Anderson, 1996). To provide insight concerning the role of oil and food in constraining recruitment at oiled areas in PWS, I conducted a five-year study to document population trends, breeding success, and chick diet at an unoiled area in southwestern PWS.

3.2 STUDY AREA

Jackpot Island (60° 19' N, 148° 11' W) is located near the mouth of Jackpot Bay in southwestern PWS, Alaska (Fig. 3.1). The oiled area study site, Naked Island (60° 40' N, 147° 28' W) is in central PWS, approximately 55 km NE of Jackpot Island. Descriptions of Naked Island are found in Oakely and Kuletz (1996), Golet *et al.* (2000) and Galt *et al.* (1991).

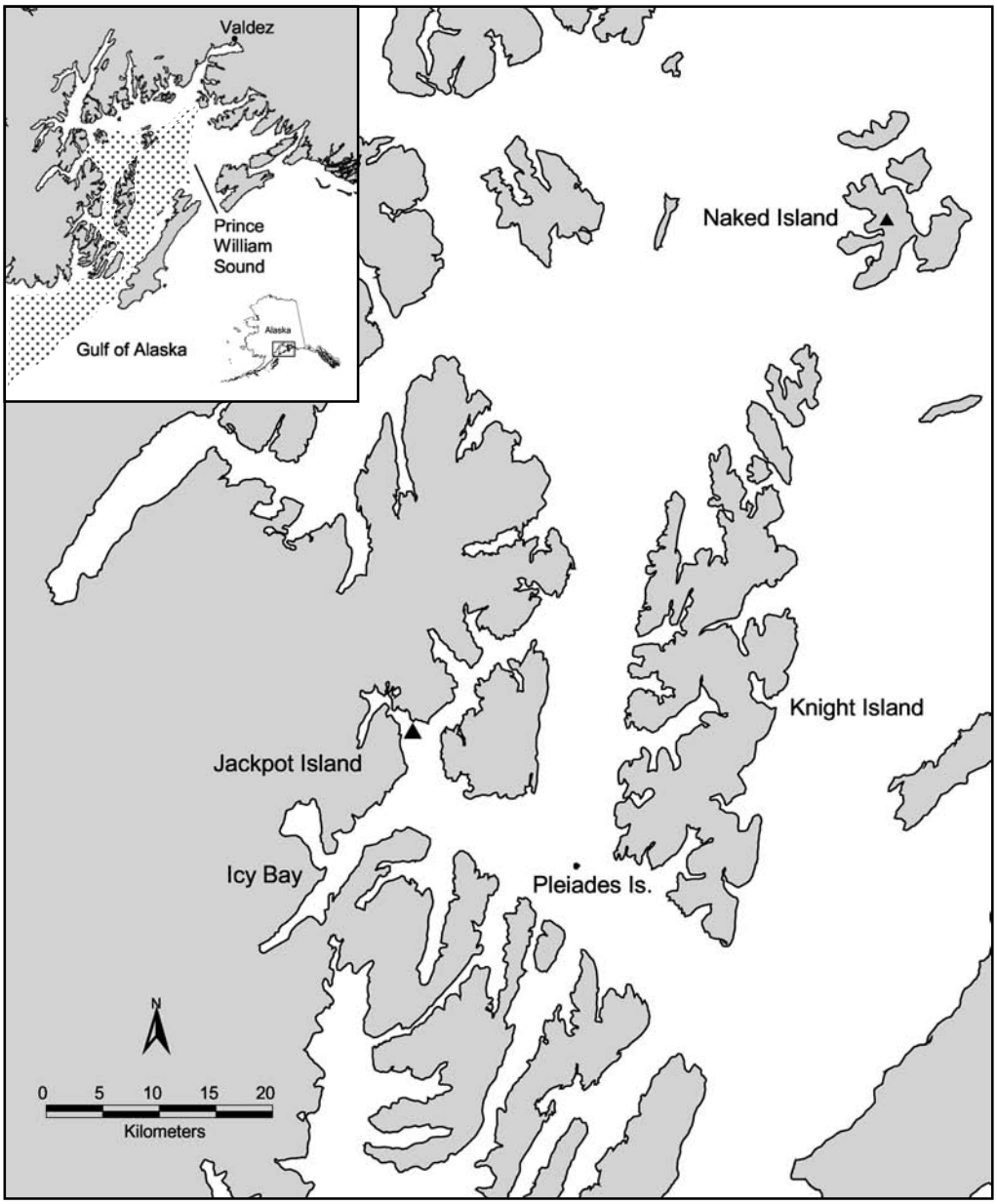

Figure 3.1 Location of the Jackpot Island and Naked Island study areas in Prince William Sound, Alaska. The inserted map of Prince William Sound shows the area oiled by the 1989 *Exxon Valdez* oil spill.

In 1993, Sanger and Cody (1994) noted that the density of breeding pigeon guillemots at Jackpot Island (103 birds km^{-1}) was the highest in PWS. Jackpot Island is separated from the mainland by 0.9 km of water. Deep waters (> 120 m) surround Jackpot Island, but to the north, Dangerous Passage and its associated bays offer shallower water (< 40 m) and to the south, guillemots forage at the submarine moraine at the mouth of Icy Bay. The large land mass of Chenega Island shielded Jackpot Island and its adjacent guillemot foraging areas from direct oiling in the aftermath of the EVOS (Galt *et al.*, 1991).

Jackpot Island (1.6 ha) is vegetated with Sitka spruce, *Picea sitchensis*, and western hemlock, *Tsugu heterophylla*. Pigeon guillemots on Jackpot Island predominantly nest in earthen burrows that are located under tree roots that jut out from the edges of cliffs, approximately 3 to 7 m above the mean high tide line. Other burrow nesters on the island include horned puffins, *Fratercula corniculata,* and common mergansers, *Mergus merganser*. Jackpot Bay is an important nursery area for Pacific herring, *Clupea pallasii* (Stokesbury *et al.*, 1997). Prey species common to the area include Pacific sand lance, *Ammodytes hexapterus,* crescent gunnel, *Pholis laeta,* northern ronquil, *Ronquilis jordani,* arctic shanny, *Stichaeus puctuatus,* Pacific cod, *Gadus macrocephalus,* Pacific tomcod, *Microgadus proximus,* walleye pollock, *Theragra chalocogramma,* and several species of salmon (Salmonidae) and sculpins (Cottidae).

3.3 METHODS

In this chapter, I compare productivity parameters and diets among three PWS pigeon guillemot studies: post-spill unoiled Jackpot Island, post-spill oiled Naked Island and pre-spill Naked Island. I collected the Jackpot Island (1994-1998) data using methods described below. Similar methods were used at the Naked Island study site. The pre-spill Naked Island (1979-1981) data were obtained from Oakely and Kuletz (1996) and post-spill oiled Naked Island data (1994-1997) from Hayes and Kuletz (1997) and Golet *et al.* (2000).

From 1994 to 1998, I documented the number of the birds attending the Jackpot Island colony, the phenology of the nesting season, the survival of eggs and chicks, the composition of the chick diet, the delivery rate of food to chicks, and the growth rate and fledging weight of chicks. In early June, during the morning high tide cycle, I conducted boat-based counts of the number of pigeon guillemots attending the Jackpot Island colony following census methods commonly used for guillemots (Ewins 1985; Drent, 1965; Kuletz, 1983). In 1997 and 1998, I also censused colonies at the Pleiades Islands, Gage Island, Flemming Point, Point Countess, West Arm of Whale Bay, and two locations in Icy Bay (denoted Icy Bay 2040 and Icy Bay 2035). The locations of all seven colonies are listed in the Beringian Seabird Colony Catalog (USFWS, 1999).

My estimates of productivity were restricted to nests found during the egg stage. Hatching dates of chicks were determined from direct observations or were estimated by comparing the wing length for chicks of unknown age to the wing length recorded for chicks of known age (Thoresen and Booth, 1958; Oakely, 1981). I calculated laying date

by subtracting 32 days (Drent, 1965) from my estimated hatching dates. For the majority of fledglings, my estimated fledge date was within 2 days of the actual date that the fledglings evacuated the burrow.

I began inspecting nest sites in late June, coinciding with hatch dates reported by Oakely and Kuletz (1996). Because incubating guillemots are sensitive to human disturbance (Drent *et al.*, 1964; Vermeer *et al.*, 1993; Cairns, 1980), I restricted the number of visits during the incubation period. Active nests were visited every third day in 1995 and every fifth day in the other years. To determine fledging weight and date, I increased my visitation rate to every other day after chicks reached the age of 30 days or when wing length became greater than 120 mm. All previously used nest sites were checked for re-occupation. During the incubation period nests are cryptic, but during the chick rearing period we could easily detect nests because of fecal stains at the burrow entrance, vocalization of the chick, or delivery of fish by adults. There were very few nest sites on Jackpot Island for which I could not physically or visually assess the presence of chicks.

On each visit to a nest, I measured the body mass of each chick to the nearest 1 g using a hand-held spring scale and measured the maximum flattened wing-length to the nearest 1 mm. I found the linear phase of growth for PWS chicks was 8 to 20 days post hatch (~ 40 to 90 mm wind length), similar to Emms and Verbeek (1991) and Koelink (1972). I conducted regression analyses between mass and age during the linear growth phase and used the slope of the relationship for my estimate of growth rates (g day^{-1}). To examine the effects of brood size and sibling competition on growth rate and fledging weight, I classified chicks as singleton chicks (chicks in one-chick broods), alpha chicks (first hatching chicks of two-chick broods) or beta chicks (second hatching chicks of two-chick broods).

I conducted provisioning watches to determine composition of the chick diet and the rate that adults provision their chicks with food (delivery rate). My observation platform was a boat anchored approximately 30 m offshore. During these 16-hour provisioning watches I recorded the time that the adult brought a prey item to the nest and identified the prey item to the lowest possible taxon. My hourly delivery rates were based on the 16-hour observation periods (0600 h to 2200 h). Annual mean delivery rates were computed from the mean delivery rate of individual nests.

Prey items were also classified into 4 groups: surface schooling fish (herring, sand lance, smelt, or salmon), gadids (Pacific cod, Pacific tomcod, or walleye pollock), non-schooling fish (such as gunnels, ronquil, and sculpins) or other species (uncommon species not included in the 3 previous groups, such as flatfish, lingcod, *Ophinod elongatus,* and greenling, *Hexogrammos* spp.). The composition of the chick diet was based on the total sum of identified chick meals recorded during all provisioning watches.

All the chicks I handled were marked for future identification with a unique combination of two colored bands on the right leg and a single colored band on the left leg. The color of the left leg band represented the hatch year of the chick. Pigeon guillemots first breed at the age of 3 or 4 years (Drent, 1965; Nelson, 1991). In 1998, when I expected to observe the 1994 and 1995 cohorts of fledglings return to the colony to breed, I dedicated five days to observing banded birds. Observations of banded birds

were also noted during provisioning watches. I estimated the percentage of the 1997 and 1998 fledglings surviving to their third year based on the number of banded fledglings observed at Jackpot Island during the 1997 and 1998 breeding season.

I tested data for normality and equal variance with the Kolmogorov-Smirnov goodness of fit test and the Levene median test, respectively. I tested for significant differences among years using analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test, as appropriate. When these tests resulted in significant differences, I identified significant differences among groups by conducting multiple pair-wise comparisons with the Bonferroni t-test or the non-parametric Dunn's method. I assumed statistical significance if $P < 0.05$. Means are reported with standard deviation.

3.4 RESULTS

3.4.1 Population trends and nesting effort

The number of pigeon guillemots attending the Jackpot Island colony increased 36% from 74 to 101 birds over the five-year study period (Fig. 3.2). For seven colonies in southwestern PWS, comparison between my 1998 census and the 1993 census conducted by Sanger and Cody (1994) indicates population increases at six colonies and no change at one colony (Table 3.1). Based on the report of 78 birds at Jackpot Island in 1993 by Sanger and Cody (1994), the annual changes in the population at Jackpot Island between 1993 and 1998 were –5%, 7%, 9%, 0% and 17%, respectively.

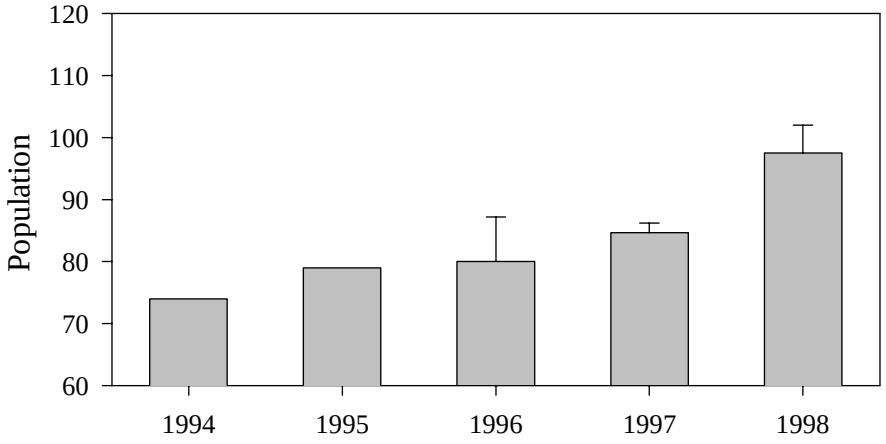

Figure 3.2 The number of pigeon guillemots attending the Jackpot Island colony in Prince William Sound, Alaska, from 1994 to 1998. Multiple census were conducted in 1996, 1997 and 1998.

There was little variation in number of nesting attempts among the five years (Fig.3.3); nesting attempts varied between 36 in 1996 and 40 in 1995. Nesting effort, which is defined as active nests per number of pairs in the June census, was higher during the first two years (97% in 1994, 100% in 1995) than nesting effort observed in the last

three years (81% in 1996, 88% in 1997, 72% in 1998). Over the five-year study period, we found 184 clutches dispersed over 72 different nest sites. New nest sites were discovered each year, but more commonly, nests from the previous year were re-occupied. Between 1995 and 1998, the percentage of current active nest sites occupied in the previous year ranged from 55% to 72%. The three nests where I found adults killed by mink were not occupied the following year. Of the 36 nest sites discovered in 1994, eleven were not occupied the following year, six were occupied for five consecutive years, five for four consecutive years, nine sites for three consecutive years, and five nest sites for two consecutive years.

Table 3.1 June census counts for eight pigeon guillemot colonies in southwestern Prince William Sound for 1993, 1997 and 1998.

Year	Jackpot Island	Pleiades Islands	Gage Island	Flemming Point	Point Countess	Whale Bay West arm	Icy Bay 2040	Icy Bay 2035
1993[a]	78	48	16	8	6	8	6	6
1997	86	48			7	8	7	6
1998	100	76	22	15	9	11	9	6

[a] Source: Sanger and Cody (1994)

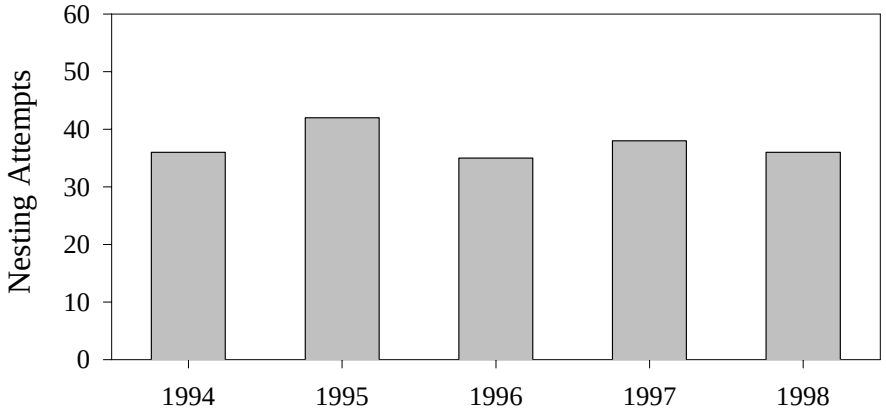

Figure 3.3 The number of active pigeon guillemot nests found on Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998.

3.4.2 Productivity

Estimates of median laying and hatch dates varied 15 days over the course of this study, and the 1995 and 1996 dates were earlier than the median dates for the other 3 years (Table 3.2). The 1995 median fledging date was significantly earlier than the

median fledging date in each of the other three years (Table 3.2; Kruskal-Wallis test, H = 19.7, P = < 0.001, df = 3).

Table 3.2 Estimates of median laying, hatching and fledging dates for the pigeon guillemot colony on Jackpot Island, Alaska, from 1994 to 1998.

Year	Median Laying Date			Median Hatch Date			Median Fledging Date		
	mean	SD	nests	mean	SD	nests	mean	SD	nests
1994	1 June	6	18	3 July	6	18	9 August	5	24
1995	24 May	4	14	26 June	4	14	4 August	5	12
1996	26 May	6	15	25 June	6	15			
1997	2 June	7	12	2 July	7	12	10 August	6	15
1998	6 June	6	9	8 July	6	9	12 August	3	9

Among the five years, mean clutch size, hatching success, fledging success and productivity were 1.82 ± 0.09 eggs $nest^{-1}$, 0.55 ± 0.18 chicks egg^{-1}, 0.48 ± 0.30 fledglings $chick^{-1}$, and 0.27 ± 0.22 fledglings egg^{-1}, respectively (Table 3.3). The 1994 breeding season was the most productive of the five years, with the highest number of hatchlings per nest, fledglings per nest, fledglings per egg laid, and total fledglings produced (Fig. 3.4 and Fig. 3.5a).

Table 3.3 Mean clutch size, hatching success and nestling survival for pigeon guillemot nests found during the egg stage on Jackpot Island, Alaska, from 1994 to 1998.

Year	Nests	Clutch Size (eggs/nest)	Hatching Success (chicks/egg)	Nestling Survival (fledglings/chick)	Productivity (fledglings/egg)
1994	24	1.92	0.80	0.76	0.61
1995	29	1.90	0.56	0.45	0.25
1996	21	1.73	0.61	0.00	0.00
1997	31	1.74	0.44	0.68	0.31
1998	28	1.79	0.33	0.53	0.18
5-year mean		1.82 ± 0.09	0.55 ± 0.18	0.48 ± 0.30	0.27 ± 0.22

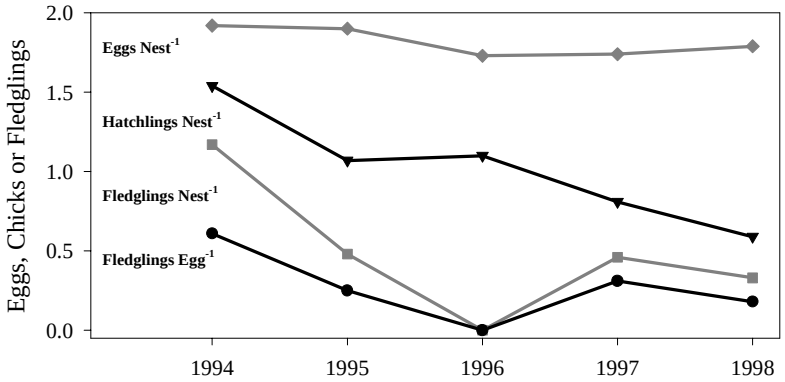

Figure 3.4 The mean clutch size, hatchlings per nest, fledglings per nest and productivity (fledglings egg^{-1}) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. Means are based on nests that were found during the egg stage.

Between 1994 and 1998, the percentage of nests with at least one fledgling was 75%, 41%, 0%, 26%, and 22%, respectively. Thus, Jackpot Island supported twice as many successful breeding pairs during the first two years than the last two years. The 1995 breeding season was less productive than the 1994 breeding season, the 1996 breeding season was a failure because of predation, and few fledglings were produced during 1997 and 1998 because of low hatching success. Hatching success was lower in the last two years because of nest abandonment during the incubation stage (Fig. 3.5b). With the exception of 1996, losses to predators were low on Jackpot Island (Fig. 3.5c). Predation losses in 1995 were attributed to a pair of northwestern crows nesting on the island, and the catastrophic losses in 1996 were caused by the presence of mink on the island. In 1997 and 1998, there was little evidence of nest predation as abandoned eggs and dead chicks remained in the burrows the entire breeding season.

3.4.3 Chick Diet

Surface schooling fish, which include herring and sand lance, formed at least one third of the diet of Jackpot Island chicks in four out of the five years (Fig. 3.6). Herring was the dominant species of schooling fish in the diet, and composed 42%, 29%, 20%, 0.4% and 41% of the number of fish delivered between 1994 and 1998, respectively.

Over the five year period, sand lance ranged from 0.5% to 13% of the diet. Non-schooling demersal fish, which include gunnels, pricklebacks, and sculpins, were as common as surface schooling fish in chick diet. In 1997 there was a major shift in the composition of the chick diet, in which schooling fish were rare and adults provided chicks with higher numbers of non-schooling demersal fish (Fig. 3.6). During 1994 gadids formed nearly one quarter of the chick diet, but in the following years gadids were less frequent. Other prey fish species, which include flatfish and greenlings, were uncommon and comprised no more than 3% of the chick diet in any given year.

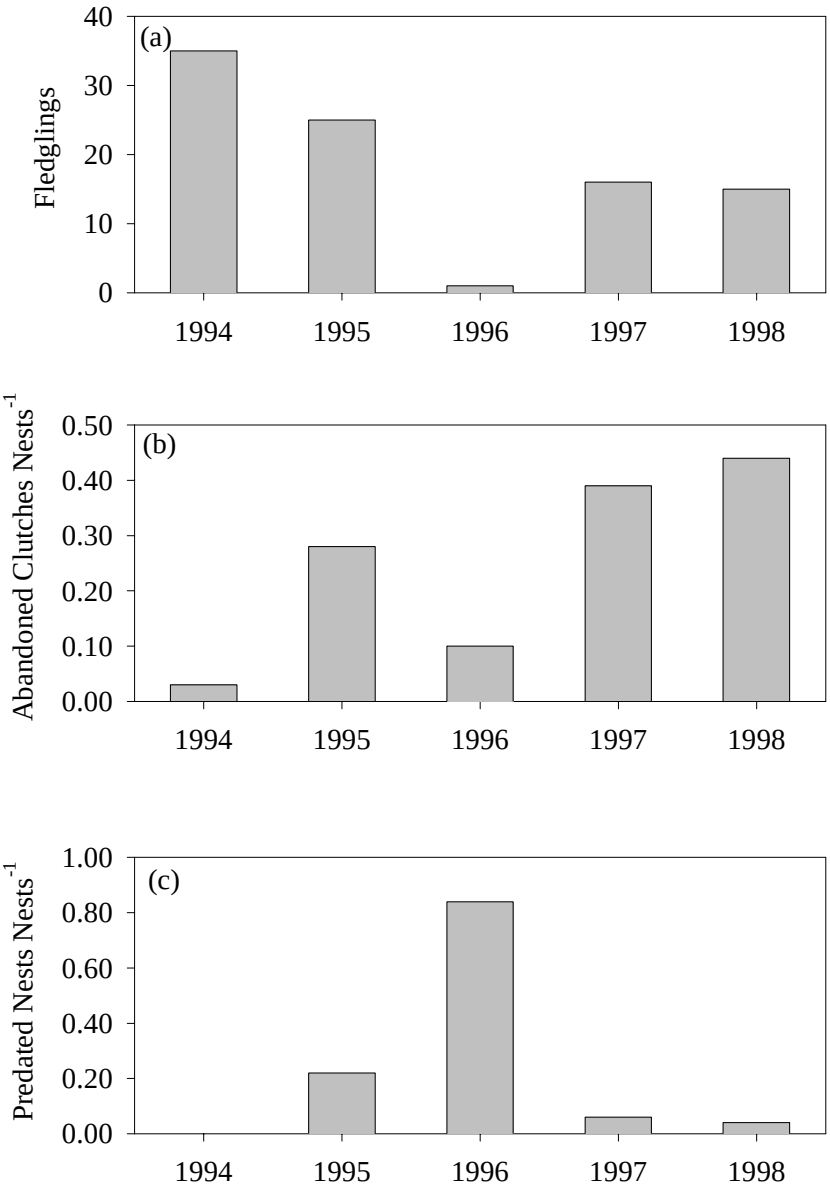

Figure 3.5 Total fledglings (a), abandonment rate (b), and predation rate (c) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. The total number of pigeon guillemot fledglings included all nesting attempts; the percentage of pigeon guillemot nests abandoned during the incubation stage is based on all nesting attempts; and the percentage of pigeon guillemot nests experiencing predation is based on nests found during the egg stage.

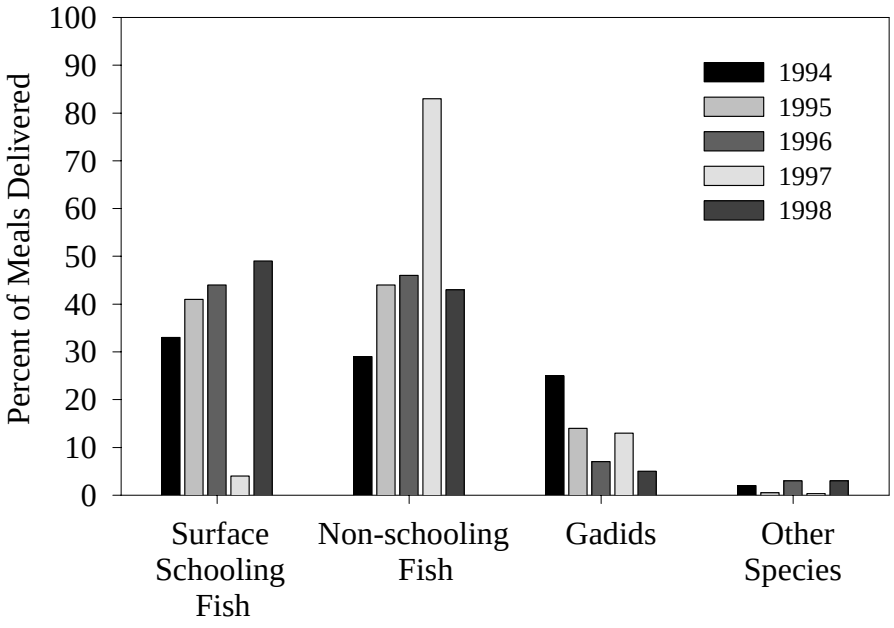

Figure 3.6 Composition of the pigeon guillemot chick diet at Jackpot Island, Alaska, from 1994 to 1998. Surface schooling fish include Pacific herring (*Clupea pallasi*) and Pacific sand lance (*Ammodytes hexapterus)*. Non-schooling fish include pricklebacks (Stichaeidae), gunnels (Pholidae), ronquils (Bathymasteridae) and sculpins (Cottidae). Gadidae include Pacific cod (*Gadus macrocephalus*), Pacific tomcod (*Microgadus proximus*) and walleye pollock (*Theragra chalocogramma)*. Other species represent food items not included in the three previous groups, such as flatfish (Bothidae and Pleuronectidae) and greenling (Hexagrammidae).

3.4.4 Delivery Rates

The sample of delivery rates in 1994 included only one observation period, and I have no data on delivery rates for chicks older than 8 days in 1996 because of mink predation during that year. Therefore, I eliminated both the 1994 and 1996 data from the following analyses of delivery rate variability among years and between brood size. Although delivery rates per chick were not significantly different among the 1995, 1997, and 1998 breeding seasons (Two-way ANOVA, year effect $F = 2.379$, $P = 0.114$, $df = 2$), delivery rates tended to be higher in 1995 (Fig. 3.7). Delivery rates per chick were significantly higher for nests with one chick than nests with two chicks (Fig. 3.7; Two-way ANOVA, brood size effect $F = 15.707$, $P < 0.001$, $df = 1$). The interaction between year and brood size was not significant ($P = 0.559$). Similar to analysis for delivery rates per chick, delivery rate per nest for 1995 (0.86 ± 0.28 fish nest^{-1} hr^{-1}, $n = 23$ nests), 1997 (0.79 ± 0.25 fish nest^{-1} hr^{-1}, $n = 16$ nests), and 1998 (0.75 ± 0.20 fish nest^{-1} hr^{-1}, $n = 7$ nests) was not significantly different among years (One-way ANOVA, $F = 1.270$, $P = 0.296$, $df = 3, 46$).

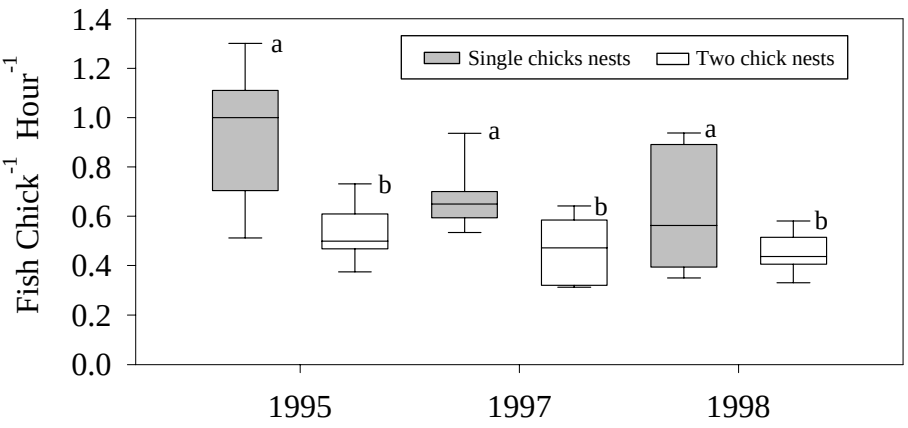

Figure 3.7 Comparison of the mean delivery rates per chick for the pigeon guillemot colony at Jackpot Island, Alaska, among the 1995, 1997 and 1998 breeding seasons and between nests with one and two chicks. Lines in the box plots indicate the median, and the 5th, 25th, 75th and 95th percentiles. Letters identify significantly different groups ($P < 0.05$).

3.4.5 Growth Rates and Fledging weights

The growth rate of chicks was not significantly different among years (Fig. 3.8a; (One-way ANOVA; $F = 0.619$, $P = 0.651$, $df = 4, 58$). Growth rates between nest mates were significantly different (Paired t-test, $t=3.12$, $P=0.008$, $df = 12$): single chicks grew significantly faster than the alpha and beta chicks of 13 pairs of siblings (One-way ANOVA; $F = 3.087$, $P = 0.53$, $df = 2, 60$). Beta chicks grew at slower rates (14.7 ± 3.5 g d^{-1}, $n = 13$) than their alpha siblings (16.4 ± 2.8 g d^{-1}, $n = 13$) or singleton chicks (17.6 ± 2.7 g d^{-1}, $n = 21$). Fledging weight in 1994 (500 ± 37 g) tended to be high in comparison with 1995 (467 ± 46 g), 1997 (463 ± 41 g), and 1998 (482 ± 42 g) (Fig. 3.8b; One-way ANOVA, $F = 2.021$, $P = 0.121$, $df = 3, 56$). Fledging wing-length in 1994 (141 ± 6 mm) tended to be smaller than 1995 (145 ± 7 mm), 1997 (144 ± 7 mm), and 1998 (145 ± 3 mm) (One-way ANOVA, $F = 2.336$, $P = 0.083$, $df = 3, 57$). Although fledging weight was significantly correlated with growth rate ($r = 0.336$, $P= 0.032$, $n = 41$), growth rate explains only 11% of the variation in fledging weight. Compared to their nest mate, beta chicks spent more days in the nest and tended to have slightly lower fledging weights (Paired t-test, $t=1.974$, $P = 0.072$, $df = 12$) and wing lengths (Paired t-test, $t = 1.818$, $P = 0.092$, $df = 13$).

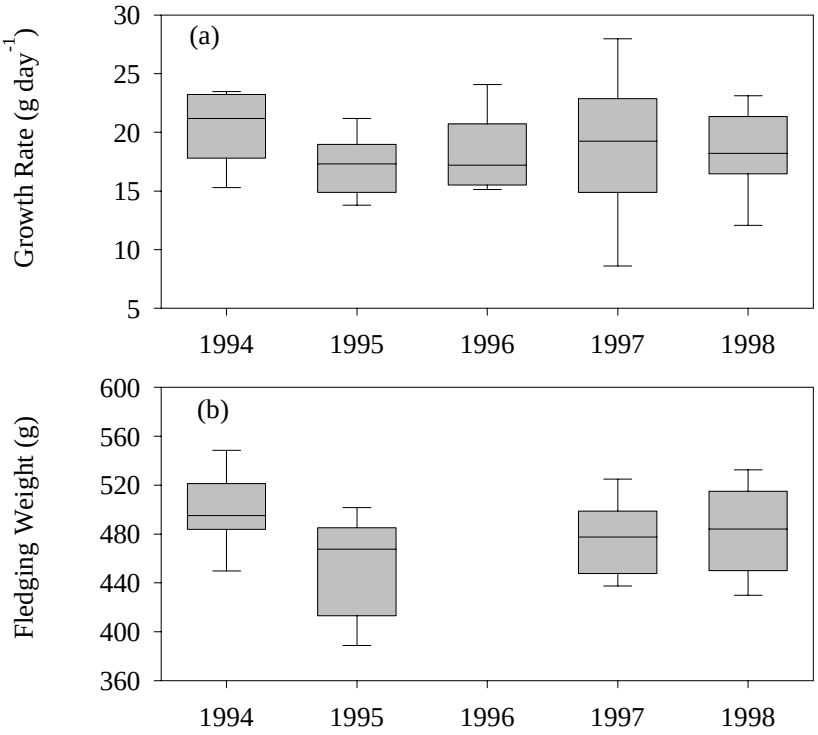

Figure 3.8 The linear growth rate (a) and fledging weight (b) of pigeon guillemot chicks on Jackpot Island, Alaska, from 1994 to 1998. Lines in the box plots indicate the median, and the 5th, 25th, 75th and 95th percentiles.

3.4.6 Fledgling Survival

I banded 28, 22, 0, 16 and 15 fledglings, in 1994, 1995, 1996, 1997 and 1998, respectively. In 1997, I located eight of the birds banded in 1994. Six of the birds were observed at Jackpot Island, one bird was observed at the Pleiades Islands and one bird was observed at a colony in Icy Bay. The following year, the searched area was limited to Jackpot Island. In 1998, I recorded two additional birds from the 1994 hatch year and five birds from the 1995 hatch year. My observations indicate that at least 36% of fledglings banded in 1994 survived to their third year. My conservative estimates of the proportion of fledglings returning to their natal colony in their third year is 21% for the 1994 cohort and 23% for the 1995 cohort. I documented four banded birds breeding at 3 years of age.

3.5 DISCUSSION

Recent increasing population trends at colonies in southwestern PWS suggest that favorable environmental conditions exist in Prince William Sound for the expansion of pigeon guillemot populations. However, at the end of this 5-year study, the abundance of pigeon guillemots at oiled Naked Island remained below their 1994 levels (G. Golet personal communication). These contrasting population trends suggest one or more demographic parameters, such as productivity, fledgling survival or adult survival, varies between the oiled and unoiled areas. Population growth at Naked Island may be limited by physiological effects of oil exposure, quality of diet, or other factors such as predators.

I could find no evidence that recovery was constrained by the physiological effects of oil exposure on chicks, although this hypothesis still needs to be rigorously evaluated for adults (Seiser *et al.,* 2000). The abundance of high-quality fish during the breeding season is a factor of particular interest because Hayes and Kuletz (1997) observed pre-spill and post-spill differences in the proportion of high-quality fish delivered to chicks at Naked Island. Sand lance and herring are surface schooling fish and are noted for their rich lipid stores and relative high energy density (kJ g^{-1}) (Anthony *et al.,* In Press). Temporal or regional differences in diet may represent limitation to population development if associated with lower productivity or survival rates (Carins, 1987). Declines in the relative abundance of these surface schooling fish in the chick diet has been associated with lower breeding success for Atlantic puffin, *Fratercula arctica,* (Lid, 1981; Anker-Nilseen, 1987) and arctic terns, *Sterna paradisaea* (Montevecchi, 1993).

I evaluated the hypothesis that availability of high-quality food is playing a role in constraining population growth in oiled areas by comparing diet and survival of chicks from unoiled Jackpot Island and pre-spill Naked Island to that of oiled Naked Island. In my discussion, I restricted the post-spill Naked Island data set to the five-year period coinciding with my Jackpot Island study. My pre- and post-spill comparison of Naked Island differs from Hayes and Kuletz (1997), because I do not include the two breeding seasons immediately following the grounding of Exxon Valdez oil tanker. Oakley and Kuletz (1996) examined the acute effects of EVOS on pigeon guillemot breeding success and diet, whereas I focus on a period when pigeon guillemot breeding success and diet may be influenced by chronic effects of EVOS to the nearshore community.

I ranked the relative quality of diet of chicks based on the proportion of high-quality fish in the diet and the rate that chicks received fish. I then examined nestling survival rate, growth rates and fledgling weight of the various colonies to determine if the observed difference in diet of chicks may have affected demographic parameters of productivity and fledgling survival rates.

3.5.1 Food Constraints

Diet of Chicks

Herring and sand lance accumulate lipid stores during summer months to sustain themselves during winter fasting periods (Blaxter and Holiday, 1963). Because of these substantial lipid stores, herring and sand lance tend to have higher energy density (kJ g^{-1}) than the other food items in the chick diet (Van Pelt *et al.,* 1997; Hislop *et al.,* 1991; Paul *et al.,* 1998; Anthony *et al.,* In press). I compare the proportion of surface schooling fish, demersal fish, gadids and other fish delivered to chicks at Jackpot Island to the proportions delivered to pre- and post-spill chicks at Naked Island to evaluate whether differences in diet composition have the potential to constrain recovery of pigeon guillemots in oiled areas of PWS.

Based on data in the literature, I have ranked the whole-body energy content among four categories of fish from high to low, as follows: surface schooling fish, demersal fish, gadids, and other fish. Paul and Paul (1998) reported significant regional, seasonal and annual variation in whole body energy content of PWS forage fish. Other researchers are currently addressing these diet issues for PWS pigeon guillemots (D. Roby, personal communication). In my evaluation of the quality of chick diet, I assume whole body energy of fish is constant over time and region. The validity of this assumption will be addressed in other studies.

The composition of the diet varied among the unoiled Jackpot Island colony reported here and oiled and pre-spill colonies at Naked Island (Chi-square; $\chi^2 = 31.8$, $P < 0.001$, $df = 8$). Compared to the diet of pre-spill chicks at Naked Island, I documented a lower abundance of sand lance and greater abundance of gadids in the diet of Jackpot Island chicks, which was similar to Hayes and Kuletz (1997) report on the diet of post-spill chicks at Naked Island. However, I found the abundance of herring in the diet of Jackpot Island chicks was significantly higher than the abundance of herring in the diet of both pre-spill and post-spill chicks at Naked Island (One-way ANOVA: $F = 5.568$, $P = 0.024$, $df = 2, 10$). In contrast to the Naked Island studies, the majority of surface schooling fish delivered to Jackpot Island chicks were herring rather than sand lance. Surface schooling fish represented 33 ± 16% of the Jackpot Island chick diet, which is intermediate between the proportion at Naked Island during the pre-spill period (48 ± 11%) and post-spill period (21 ± 7%) (One-way ANOVA: $F = 4.704$, $P = 0.036$, $df = 2, 10$). During the warm-water year of 1997, herring sharply declined in the diet of Jackpot Island chicks, while the Naked Island chicks experienced a modest gain in the abundance of surface schooling fish in their diet. With the exception of 1997, chicks at Jackpot Island had a greater proportion of schooling fish in their diet (40 ± 5%) than the chicks at post-spill Naked Island (T-test, $t = 6.277$, $P < 0.001$, $df = 6$).

During the warm-water year of 1997, adults delivered more non-schooling demersal fish to chicks at Jackpot Island compared to other years. Among the other years, the proportion of non-schooling demersal fish delivered to chicks at Jackpot Island (39 ± 8%) was similar to the proportion delivered to Naked Island chicks during the pre-

spill years (38 ± 5%), but less than during the post-spill years (58 ± 9%, n = 3). Gadids occurred in similar frequency in the diet of chicks at Jackpot Island (15 ± 7%, n = 5) and post-spill Naked Island (16 ± 13%, n = 4). In both areas, the abundance of gadids declined after 1994. However, the post-spill abundance of gadids in the chick diet remained greater than pre-spill diets at Naked Island (4 ± 4%). Other fish species comprised a minor proportion of diets of post-spill chicks: Jackpot Island (2 ± 2%) and Naked Island (5 ± 2%). The proportion of other fish species (10 ± 9%) was slightly higher for pre-spill chicks at Naked Island.

The lower abundance of high-quality fish in chick diets in oiled areas of PWS has the potential to constrain growth rates and survival of juvenile pigeon guillemots if delivery rates do not compensate for the lower energy content of chick meals. During periods of food shortages, brood reduction will offset the effect of low delivery rates. Single chicks received a significantly higher number of meals than individuals in two chick nests at Jackpot Island as well as in other studies (Prichard, 1997). I did not test the effect of brood size on delivery rates because information on brood size was not available for Naked Island delivery observations. The mean delivery rates I observed at Jackpot Island (0.86 ± 0.18 fish $nest^{-1}$ hr^{-1}, n = 4) and pre-spill Naked Island (0.90 ± 0.20 fish $nest^{-1}$ hr^{-1}) were not significantly higher than the post-spill Naked Island (0.74 ± 0.13 fish $nest^{-1}$ hr^{-1}; One-way ANOVA; F = 1.256, P = 0.330, df = 2, 9).

The observation of lower abundance of high-quality fish at post-spill Naked Island without a significant change in rate of fish delivered to chicks to compensate for the lower quality suggests lower energy content in the diet of Naked Island chicks during the post-spill years. The lower quality of the chick diet found at Naked Island can not be interpreted as a population limitation unless it is associated with lower productivity or fledgling survival (Cairns, 1988).

Productivity

Food limitations at the egg laying, incubation and chick rearing periods occur at different temporal scales or at different levels of prey supply (Carins, 1988). Therefore I examined the three components of productivity individually for evidence of food limitation (Table 3.4). The mean clutch size at Jackpot Island over the five-year study period (1.82 ± 0.09 eggs $nest^{-1}$) is only slightly higher than the mean clutch sizes observed at Naked Island during the pre-spill period (1.69 ± 0.14 eggs $nest^{-1}$) and during the post-spill period (1.72 ± 0.07 eggs $nest^{-1}$). Because there are no significant differences in the clutch size between these studies (One-way ANOVA; F = 1.766, P = 0.216, df = 2, 11), egg production is not impeding post-spill population growth.

During this study, I documented high abandonment rates at the Jackpot Island colony in two out of five years. Kuletz (1983) reported unusually low hatching rates at Naked Island in one out of three years. However, during the post-spill years Naked Island colonies experienced little interannual variation in hatching rates. Therefore, mean hatching success at Jackpot Island (0.55 ± 0.18 chicks egg^{-1}) was lower than the mean

Table 3.4 Comparison of clutch size, hatching success, fledgling success and productivity among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies. Means are based on pigeon guillemot nests found during the egg stage.

Period (years)	Study Area	Clutch Size (eggs/nest)	Hatching Success (chicks/egg)	Fledgling success (fledglings/chick)	Productivity (fledglings/egg)
Post-spill 1994-1998	Jackpot Island (unoiled)	1.82 ± 0.09	0.55 ± 0.18	0.48 ± 0.30 n = 5 0.61 ± 0.14 n = 4	0.27 ± 0.22 n = 5 0.35 ± 0.21 n = 4
Post-spill 1994-1998	Naked Island[a] (oiled)	1.72 ± 0.07	0.62 0.13	0.42 ± 0.17 n = 5	0.35 ± 0.15
Pre-spill 1979-1981	Naked Island[b]	1.69 ± 0.14	0.78 ± 0.07	0.77 ± 0.19[d]	0.47 ± 0.15
ANOVA	All	P = 0.216	P = 0.056	P = 0.041	P = 0.530

[a]Source: Golet *et al.* 2000 for 1994-1997 data and G. Golet contributed the 1989 data.
[b]Source: Oakley and Kuletz 1996, Golet *et al.* 2000
[c]Four year mean excludes 1996 breeding season at Jackpot due to high mink predation.
[d]Bonferroni t-test pair-wise comparisons ($P < 0.05$)

hatching success observed at Naked Island during both the pre-spill years (0.62 ± 0.13 chicks egg^{-1}) and the post-spill years (0.78 ± 0.07 chicks egg^{-1}; One-way ANOVA; $F = 3.790$, $P = 0.056$, $df = 2, 11$). Low hatching rates for guillemots has been associated with food shortages, presence of mammalian predators or frequent disturbance by humans (Ainley *et al.*, 1990; Drent *et al.*, 1964; Drent 1965; Hodder and Graybill, 1983; Emms and Morgan, 1987).

At Naked Island, Kuletz (1983) captured adults in their burrows during the 1980 nesting season and reported the high abandonment rate for birds she disturbed. At Jackpot Island, I avoided capturing incubating adults. The pattern of high nest abandonment in the last two years of the 5-year study suggests that factors besides our presence on the island prompted the birds to abandon their nests. There is indirect evidence that availability of prey during the incubation period may have declined in the last two years of this study. During the 1997 nesting season I observed substantial abandonment of nests coupled with a scarcity of herring among the fish delivered to chicks. The scarcity of juvenile herring in 1997 was associated with higher than average sea surface temperatures in the Gulf of Alaska that lasted from May 1997 to March 1998 (calculated from records of the National Data Buoy Center, NOAA). However, birds nesting at the Naked Island colonies did not experience similar food limitations. Sand lance in the Naked Island areas responded to warm waters by forming surface schools earlier in 1997 than observed in the two previous years (Brown, 1997).

In contrast, the abandonment I observed in 1998 occurred when herring were abundant in the chick diet. However, precipitation was twice as high in June of 1998 compared to the four other years of the study, and the second highest recorded in 16 years at Main Bay weather station (WRCC, 1999). High rainfall in June of 1998 may have represented poor foraging conditions for the birds at Jackpot Island. Kuletz (1983) documented that the rate adults provisioned chicks declined during periods of poor weather.

Thus, the abandonment I observed in both 1997 and 1998 may have been caused by an overall scarcity of food or poor foraging conditions. This interpretation is consistent with observations at the Farallon Islands by Ainley *et al.* (1990), who noted that low hatching success of pigeon guillemots was associated with warm-water years and low abundance of primary prey species, rockfish, *Sebates* spp., and that high hatching success was associated with cold water years that resulted in exceptional food availability.

Evaluating the role of food in the lower post-spill nestling survival at Naked Island is confounded by reports of increased nest predation (Oakley and Kuletz 1996). Mink are a major nest predator in PWS. The failure of the 1996 breeding season at Jackpot Island was caused by mink predation. The colonies at Naked Island suffered losses to mink predation on an annual basis and poor nesting success in 1998 was attributed to mink predation. Of the three pair-wise comparisons of nestling survival, only the comparison between pre-spill Naked Island and post-spill Naked Island was significantly different (One-way ANOVA; $F = 4.471$, $P = 0.041$, $df = 2, 10$; Bonferroi t-test, $t = 2.978$, $P = 0.042$).

Productivity was similar between Jackpot Island (0.35 ± 0.21 fledgling egg^{-1}, n = 4 years) and post-spill Naked Island (0.35 ± 0.15 fledgling egg^{-1}) because the lower hatch rates at Jackpot Island were balanced by lower survival of nestlings at Naked Island. The productivity of pre- spill Naked Island birds (0.47 ± 0.15 fledgling egg^{-1}) was not significantly greater than that of post-spill Naked Island birds (One-way ANOVA; F = 0.678, P = 0.530, df = 2, 10). Because of the small range observed between pre-spill and post-spill productivity, I suggest that other demographic factors, such as juvenile survival to breeding age or adult survival rates, are responsible for the post-spill populations trends at Naked Island.

Growth Rates, and Fledging Weight

In comparison to the measurement of fledglings per egg, growth rates and fledgling weights (Table 3.5) may be better measurements of overall reproductive performance because of their influence on post-fledgling survival (Greenwood *et al.,* 1993). From 1994 to 1998, the mean linear growth rate of chicks at Jackpot Island (18.6 ± 1.1 g^{-1} day-^{1}) was not significantly different from the pre-spill growth rate at Naked Island (20.4 ± 2.3 g^{-1} day^{-1}), or the post-spill growth rate at Naked Island (18.2 ± 2.6 g^{-1} day^{-1}) (One-way ANOVA; F = 1.123, P =0.363, df = 2, 10). In contrast, the mean fledging weight of chicks at Jackpot Island (482 ± 18 g chick^{-1}) was similar to the pre-spill fledging weight at Naked Island (480 ± 40 g;) and significantly greater than the post- spill fledging weight at Naked Island (446 ± 14 g) (One-way ANOVA; F = 9.788, P < 0.001, df = 2, 10). Post spill chicks at Naked Island fledged with similar wing length but at lighter weights than Jackpot Island and pre-spill chicks.

Table 3.5 Comparison of growth rates and fledging weights among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies.

Period (years)	Study Area	Growth Rate (grams /day)	Fledging Weight (grams)
Post-spill 1994-1998	Jackpot Island (unoiled)	18.6 ± 1.1	482 ± 18
Post-spill 1994-1998	Naked Island[a] (oiled)	18.2 ± 2.6	446 ± 14[c]
Pre-spill 1979-1981	Naked Island[b]	20.4 ± 2.3	480 ± 40
ANOVA	All	P = 0.363	P = 0.001

[a]Source: Golet *et al.* (2000) for 1994-1997 data and G. Golet contributed the 1989 data. [b]Source: Oakley and Kuletz (1996) and Golet *et al.* (2000). [c]Bonferroni t-test pair-wise comparisons (P < 0.05)

Guillemot chicks that are fed predominantly sand lance (>50%) have higher growth rates (Prichard, 1997, Golet *et al.*, 2000) and peak fledging weights (Golet *et al.*, 2000) than chicks that are fed predominantly gadids or non-schooling demersal fish. Similar results have been noted for rhinoceros auklets, *Cerorhinca monocerata,* (Bertram and Kaiser, 1993; Wilson and Manuswal, 1986) and Atlantic puffins, *Fratercula arctica,* (Harris and Hislop, 1978). Romano *et al.* (1999) reported that tufted puffins, *Fratercula cirrhata,* raised on schooling fish diets had greater fat reserves than birds raised on walleye pollock diets. In field studies at the Farallon Islands, Shultz and Sydeman (1997) reported that low fledging weights were associated with years of low food abundance. During the post-spill period, the combination of similar linear growth rates at Jackpot Island and Naked Island with the greater fledging weight at Jackpot Island suggests that food constraints were primarily realized late in the chick-rearing period and most likely associated with development of fat reserves.

Food limitations for fledglings are different than those of nestlings because fledglings do not receive food from their parents and fledglings are less experienced at capturing prey than adults. Fledgling survival may be increased through greater energy reserves and advanced development (Thompson and Flux, 1988). Fledging weight has been positively related to the survival of juveniles in many species (Manx shearwaters, *Puffinus puffinus,* Perrins *et al.*, 1973; South African gannet, *Sula capensis,* Jarvis, 1974; blue tit, *Parus caeruleus*, Nur, 1984; black-legged kittiwake, *Rissa tridactyla,* Coulsen and Porter, 1985; blackbird, *Turdus merula,* Magrath 1991; but see Harris and Rothery, 1985 on Atlantic puffins, *Fratercula arctica*.) For the 1994 and 1995 year-classes of guillemots, I found no significant difference in fledging weights between birds I observed in later years and birds I assumed dead. However, these fledglings were produced during a summer of high food abundance as indicated by delivery rates and abundance of schooling fish in the chick diet. Harris and Rothery (1984) suggested fledging weight is not critical to survival of puffins when post-fledging food resources are abundant. Although fledging weight has been suggested as an index of fledgling survival, this has not yet demonstrated for pigeon guillemots.

3.5.2 Demographic Limitations to Recovery

During my study, I observed a 36% increase in the number of adults attending the Jackpot Island colony, but annual population growth was not consistent among years. The 1998 increases in populations observed for several other southwestern and central PWS colonies suggest that conditions favorable for breeding success and fledgling survival existed throughout PWS in the mid-1990's. This pattern of colony growth may represent recruitment of a strong year-class after several years of lower recruitment.

Demographic factors that could contribute to lack of recovery at Naked Island compared to Jackpot Island, include lower production, higher net emigration, higher post-fledging mortality, or a combination of these factors. Because the production at Naked Island and Jackpot Island are similar, production does not appear to be the factor responsible for the lack of recovery at Naked Island, which is in agreement with the observations of Hayes and Kuletz (1997). With respect to emigration, we have one

documented case of a chick banded at Naked Island that subsequently nested at Jackpot Island in 1997 and 1998. Although this observation suggests the potential for emigration, we do not know the relative difference in emigration rates of pigeon guillemots between Jackpot Island and Naked Island. The higher fledging weights I observed at Jackpot Island in comparison to Naked Island suggest that recovery at Naked Island may be constrained through reduced fledging survival. It is also possible that recovery at Naked Island may be constrained through reduced adult survival.

Factors that contribute to the mortality of adult seabirds include predators, entanglement in fishing nets, food shortages, the long-term effects of oil exposure and disease. I observed mortality of nesting pigeon guillemot caused by mink predation, similar to that reported by others working in guillemot colonies (Petersen, 1981; Folkestad, 1982; Barrett and Vader, 1984,), however we do not know if adult predation is higher at Naked Island than colonies in unoiled areas. Similarly, we have no reason to believe that there are differences between Naked Island and unoiled populations in adult mortality caused by gillnet fisheries; gillnets are known to be a significant source of mortality for seabirds (DeGange *et al.,* 1993; Carter and Sealy, 1982; Takekawa *et al.,* 1990). The solitary foraging habits and moderate diving depth of guillemots may make them less susceptible to gillnet losses unless the nets are in the vicinity of colonies (Evans and Nettleship, 1985). Gillnets are used in PWS herring and salmon fisheries. During this study the herring fisheries was closed for three years. Wynne (1990, 1991) reported no mortality of pigeon guillemots associated with the PWS Copper River salmon gillnet fisheries.

Naked Island could also be experiencing higher adult mortality because of the long-term effects of oil exposure. In chapter two I presented preliminary evidence that adults from oiled areas have elevated aspartate aminotransferase concentrations, which is consistent with hepatocellular injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Additional studies to fully evaluate the health of adults residing in oiled areas would help to evaluate the issue of whether adult mortality caused by the long-term effects of oil exposure plays a role in the lack of recovery at Naked Island.

Several studies have found declines in seabird populations associated with declining abundance of herring or sand lance (Atlantic puffins: Lid, 1981; Harris and Wanless, 1991; black-legged kittiwakes: Heubeck and Mellor, 1994). The higher fledging weights I observed at Jackpot Island compared to Naked Island, suggest that food limitation may be expressed in the later part of the breeding season. If late-season food shortages affect body condition of adults, then these shortages may affect survival. I recommend that adult survival and late-season body condition of adults be monitored for breeding birds at Naked Island and Jackpot Island to determine whether late-season food shortages have the potential to cause higher adult mortality.

For an injured population to return to their initial levels after a major mortality event, such as an oil spill, environmental conditions must favor breeding success, survival of juveniles to breeding age and survival of breeding adults. My analysis indicates that lack of recovery of pigeon guillemot populations in oiled areas of PWS is likely associated with lower quality prey, which results in lower fledging weight, and

which may constrain recovery through reduced fledgling survival. Food shortages and the long-term effects of oil exposure may also constrain recovery if they result in lower adult survival.

CONCLUSIONS

1. From 1994 to 1998, I observed a positive trend in the Jackpot Island pigeon guillemot population. For four consecutive years, the number of birds at Jackpot Island met or exceeded the previous year's counts.

2. Jackpot Island experienced high rates of nest abandonment in 1997 and 1998. High sea-surface temperatures in June of 1997 and high rainfall in June of 1998 may have caused poor foraging conditions during the incubation period. These factors may have contributed to the high abandonment rates observed during those two years.

3. Productivity losses to mink predation occurred only in one out of five breeding seasons at Jackpot Island. The presence of mink on the island in 1996 resulted in higher morality rates for both adults and nestlings. The relative isolation of Jackpot Island from mink predation may explain the difference in nesting density between Jackpot Island and the shoreline of the mainland.

4. The proportion of high-lipid fish in the diet of Jackpot Island chicks was higher than the post-spill Naked Island, but lower than pre-spill Naked Island. The abundance of herring was significantly higher in the diet of Jackpot Island chicks compared to pre- and post-spill Naked Island chicks. Delivery rates were not significantly different among the three studies. Therefore, the quality of chick diet was higher at Jackpot Island than post-spill Naked Island, but not pre-spill Naked Island.

5. Mean fledgling success, productivity rates and growth rates at Jackpot Island and post-spill Naked Island were similar. However, fledging weights were significantly higher at Jackpot Island. This observation suggests that food limitations at Naked Island was experienced in the later stages of the nesting period. Lower fledging weights at Naked Island may lead to a lower post-fledgling survival rate.

6. Population trends at unoiled Jackpot Island and oiled Naked Island did not exhibit similar temporal patterns. Because mean productivity levels did not differ between the two areas, the disparity in fledging weight may partially account for these varying population trends. It is unknown if food limitation at Naked Island extends to breeding adults. I recommend comparative studies on adult survival rates and late summer body condition.

4

CHAPTER FOUR

STATUS OF RECOVERY OF PRINCE WILLIAM SOUND'S PIGEON GUILLEMOT POPULATION.

Pigeon guillemots and their foraging areas were impacted by the 1989 *Exxon Valdez* oil Spill (EVOS) (Piatt *et al.*, 1990; Spies *et al.*, 1996). In the years immediately following EVOS, no significant increase in pigeon guillemot abundance were reported at several spatial scales within Prince William Sound (Oakely and Kuletz, 1993; Murphy *et al.*, 1995; Agler and Kendall, 1996). The pigeon guillemot population had not recovered according to the conventional measurement of recovery, the numeric replacement of individuals directly killed by oiling. This definition assumes that current environmental conditions support recruitment. Oakley and Kuletz (1996) pointed out that survey estimates prior to the spill (1972, 1984-85) indicated that the abundance of pigeon guillemots in PWS were declining. Present oceanic conditions may not support population growth. Therefore, comparing population trends in oiled areas to adjacent unoiled areas might be a more suitable indicator of recovery than comparing post-spill populations to pre-spill abundance levels. My observations at unoiled Jackpot Island and several other colonies in southwestern PWS indicate that it was possible for pigeon guillemot populations to substantially expand in the post-spill period, between 1994 and 1998. However, during the same period, the abundance of pigeon guillemots at oiled Naked Island dropped below the population level measured in 1994. Several factors may constrain population growth in oiled areas: the physiological effects of oil exposure on guillemots, food limitations, predation, and other factors, such as disease. I assessed the role of oil exposure and food limitations in the recovery of pigeon guillemot populations affected by the EVOS.

Before this study, information on the breeding success and diet of pigeon guillemots in PWS was only available for Naked Island colonies in central PWS. In 1979, ten years prior to the spill, Naked Island supported 1,200 pigeon guillemots (Oakely and Kuletz, 1996). Since the spill, the annual counts of pigeon guillemots along Naked Island shorelines have oscillated between 400 to 700 birds (Hayes and Kuletz, 1997). The pre-spill (1979 to1981) and post-spill studies (1989 to 1991, 1994 to 1998) of Naked Island pigeon guillemots represent 12 years of data on breeding success and diet. Because of the time laps between studies and oiling of Naked Island, researchers did not have the information necessary to determine whether the lack of population growth in the nineties would have occurred in the absence of EVOS or not (Oakley and Kuletz, 1996; Hayes and Kuletz, 1997; Golet *et al.*, 2000).

To provide insight on mechanisms behind the population trend in oiled areas, I collected information on the health, food habits and population dynamics of pigeon guillemots at Jackpot Island. This unoiled reference site is located in southwestern PWS. To make comparisons between an oiled area and a reference site, the reference site must

meet three criteria: (1) the foraging habitats of the two areas must be similar except for oiling; (2) the movement of birds between the reference site and the oiled site must be limited, and (3) the reference site must have an adequate number of accessible nests for logistical and statistical purposes.

Jackpot Island was selected as a reference site because it offered a large concentration of nests located at a fair distance (55 km) from oiled Naked Island. Selecting reference sites with similar oceanographic conditions to oiled study sites is difficult because oil was not randomly distributed in PWS, nor was the degree of oiling consistent along shorelines. Wind and current patterns responsible for the distribution of oiling along PWS shorelines may also be correlated with other less obvious habitat variables (Laur and Haldorson, 1996). I found that birds in the two areas feed on similar prey species, but other measurements of foraging habitat varied. The shoreline density of nests in the greater Jackpot area was low, compared to Naked Island's. Breeding densities may be related to the quality of the forage area, availability of nesting sites, or predator densities. The lower shoreline density of pigeon guillemots in unoiled areas is an unavoidable weakness in my study. Despite these limitations for a direct comparison to Naked Island, the diet of Jackpot Island birds typifies the oil exposure levels and foraging habits of breeding birds in the unoiled area of Prince William Sound.

To evaluate the health of birds in 1997, I compared the hematological and plasma biochemical profiles among populations of pigeon guillemots in oiled and unoiled areas. If the effect of chronic exposure to residual oil is significant enough to limit the recovery of pigeon guillemots in PWS, then I expected the blood parameters to differ between populations in oiled and unoiled areas in a pattern that would be consistent with toxic responses. I examined chicks and adults separately, because adults have greater opportunities for exposure to residual oil than nestlings residing in burrows. With the 30-day old chick, I found calcium and mean cell volume were significantly different between populations in oiled and unoiled areas. However, these blood biomarkers provided little evidence of continuing oil injury to chicks. Preliminary data from adults indicated elevated aspartate aminotransferase activity (AST) for adults in the oiled area, which is consistent with hepatocellular injury. These findings indicate that exposure to residual oil elicited a physiological response in pigeon guillemots. The consumption of invertebrates by adults (Oakely, 1981; Ewins, 1993) may contribute to difference in biomarker responses between adults and chicks. Bioaccumulation of polynuclear aromatic hydrocarbons is greater in invertebrates than fish (Gibson, 1977). The energetic costs of this physiological response and its influence on adult survival and productivity are unknown. I recommend studies that fully evaluate health and survival rates of adults residing in oiled areas.

Alcid productivity is affected by availability and abundance of food in the vicinity of their nests (Evans and Nettleship, 1985). To evaluate food resources and limitations during the breeding season, I examined the diet, survival, growth performance and fledging weight of chicks. Hayes and Kuletz (1997) suggest that the availability of high-lipid fish was limiting the growth of the population at Naked Island. They reported a decline in the abundance of high-lipid fish in the diet of chicks between the pre-spill (1979-81) and post-spill (1989-90, 1994-96) studies. In PWS, distribution of pigeon

guillemot colonies in non-glaciated waters, overlap with the summer distribution of juvenile herring and sand lance documented by Brown (1997, 1998). Thus populations in both oiled and unoiled areas of PWS would be influenced with changes in abundance of high-lipid fish. Little is know about the abundance of fish in areas with tidewater glaciers, because silt and ice prevent aerial and sonar detection of surface schooling fish. I expanded on the pre-and post-spill comparisons of Hayes and Kuletz (1997) to include post-spill data for an unoiled area. I also truncated the post-spill Naked Island data set to the 5-year period corresponding to the data from the unoiled area.

I evaluated the quality of chick diet based on the proportion of the meal that were high-lipid fish and the frequency at which meals were delivered to the chicks. I found that the proportion of high-lipid fish in the diet of Jackpot Island chicks was higher than post-spill Naked Island chicks, but not pre-spill Naked Island chicks. Yet, delivery rates were not significantly different among the three studies. Similar to the observations by Hayes and Kuletz (1997), I concluded that the quality of post-spill diet was lower than the pre-spill diet. However, the quality of the post-spill diet was higher for chicks at Jackpot Island than for the chicks at Naked Island. The abundance of herring at Jackpot contributed to post-spill regional differences in diet quality. Although food limitation was not expressed in the linear growth rates of chicks, the difference in diet quality between Jackpot Island and Naked Island was expressed later in the chick rearing period and translated into lower fledging weights for Naked Island chicks. Fledging weight has been suggested as an index of fledgling survival (Perrins *et al.,* 1993; Jarvis 1974). Consequently, the lower fledging weights at Naked Island suggest that recovery at Naked Island may be constrained through reduced juvenile survival.

Populations increase only by recruitment of natal juveniles and immigration. I noted that mean productivity rates over the five-year period were not significantly different between Jackpot and Naked Island, but lower fledging weights at Naked Island may lead to regional differences in recruitment rates. Martin (1987) suggested that productivity be defined by both the number of fledglings that survive to breed and by the negative effects of the breeding effort on the parent. Tinbergen *et al*. (1984) reported that the cost of reduced survival for adults was expressed in years when winter food abundance was low. Guillemot species experience seasonal shifts in their diet from a fish-dominated diet in the summer to a mixed diet of fish and invertebrates in the winter (Vermeer *et al.,* 1987). I recommend that winter food limitations be examined for pigeon guillemots in oiled and unoiled areas.

Cairnes and Elliot (1987) theorize that the rate of population recovery from a large mortality event such as an oil spill depends on the size and location of neighboring colonies. The spatial distribution of breeding pigeon guillemots in PWS does not present a high immigration potential for Naked Island. According to the colony surveys of Sanger and Cody (1994), the density of pigeon guillemots in the area surrounding Naked Island and its associated islands in central PWS, is much lower than the Naked Island complex. The observation by Murphy *et al.* (1997) that the abundance of pigeon guillemots occupying oiled shorelines did not increase the first two years after the spill supports the notion that immigration potential in PWS is low. The failure of the Naked Island population to maintain levels above the 1990 census level (723 birds) suggests that

recruitment as well as immigration are insufficient to compensate for adult morality and emigration losses.

To maintain a stable population, breeding pairs must produce at least two fledglings over the course of their lifetime. Based on present production (0.6 fledgling per nest) and fledgling survival (36%) rates at Jackpot Island, a breeding pair would have nest 9 years to insure two of their fledglings survived to breeding age, an annual survival rate of 90%. However, lower adult survival rates lower reported in the literature for pigeon guillemot (80%; Nelson, 1981) and black guillemot (85%; Asbirk 1979; 89%; Frederiksen, 1998). Lower abundance of high-lipid fish in the oiled area and the long-term effects of oil exposure are potential mechanisms to create regional differences in adult survival rates. Again, I recommend future research on adult survival rates.

Herring was the major contributing factor to the high-lipid diet at Jackpot Island. The herring population in PWS peaked in 1989 and crashed in 1993, just prior to this study. In 1989 herring composed 25% of the diet of Naked Island chicks (Oakely and Kuletz, 1996). After 1993, only 5% fish in the Naked Island chick diet was herring (G. Golet personal communication). In contrast, a third of fish delivered to chicks at Jackpot Island were herring. The local abundance of juvenile herring is attributed to the size of local spawning biomass as well as favorable spring wind and current patterns for retention of planktonic larva (Stokesbury *et al.,* 1997). Few herring spawn in Southwest PWS and the source of Jackpot Bay's juvenile herring population has not been traced (Stokesbury *et al.,* 1997). Naked Island has both an intermittent spawning population (Funk, 1995) and a favorable location for receiving larva from other spawning areas (B. Norcross, personal communication). Differences in the abundance of herring may be attributed to physical differences in nearshore habitats, natural cycles in herring populations, or to the lingering effects of EVOS.

The difference in abundance of herring and sand lance at Jackpot Island and Naked Island was also noted in the diet of marble murrelets (Kuletz and Kendall, 1999), and in Brown's (1997, 1998) aerial surveys of surface schooling fish. Little is known about historic population trends in the abundance of sand lance because lack of commercial interest in the species. The abundance of high-lipid fish in the diet of oiled Naked Island chicks continued to remain low from 1990 to 1998. Declines in high-lipid fish abundance between the pre-spill and post-spill studies may be related to declines in primary productivity (Bertram *et. al.,* 1991), diseases and oiling (Hose *et al*., 1996, Carls *et al.,* In press).

If the effect of EVOS on the pigeon guillemot populations was limited to the 1989 mortality event, then I would expect by 1994, that the population dynamics in oiled and unoiled areas would follow similar trends. In fact population trends differed between oiled Naked Island and unoiled Jackpot Island, which suggests regional differences in one or more demographic parameters. Productivity was similar between the two areas but indirect measurements suggest potential for lower juvenile and adult survival rates for populations residing in oiled areas. Blood biomarkers provided evidence that residual oil in the nearshore environment is a potential health risk to adult birds. But, I lack information to determine at what scale oil toxicity currently inhibits adult survival or productivity. I presented evidence that food limitations at Naked Island may lead to

lower fledgling survival. The recovery of pigeon guillemots from EVOS depends on the status of food resources in the nearshore habitat. The low abundance of high-lipid fish during the breeding season was one mechanism limiting population expansion in oiled areas. Current environmental conditions in the oiled areas of PWS do not appear to support growth and recovery of pigeon guillemot populations.

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Biosketches of
Nearshore Vertebrate Predators Team
(B)

PERSONNEL

Dr. Brenda Ballachey is a Research Physiologist at the U.S. Geological Survey (USGS), Alaska Biological Science Center. She was Project Leader for sea otter National Resource Damage Assessment studies from 1990 through 1996 and has been involved in all aspects of post-spill research on sea otters. She has authored or coauthored more than 25 peer-reviewed publications and is currently a co-principal investigator for the Nearshore Vertebrate Predator (NVP) project, examining effects of residual oil on health and recovery of sea otters and other NVP study species.

Mr. Jim Bodkin, Research Wildlife Biologist, is the Team Leader for studies of coastal marine research at the USGS Alaska Biological Science Center in Anchorage. He has 22 peer-reviewed scientific publications and directs an active sea otter research program. He has studied and published articles on sea otter population biology, natural history, and community ecology since 1988. Jim has been a principal investigator in *Exxon Valdez* oil spill related research since March 1989.

Dr. R. Terry Bowyer, Professor of Wildlife Ecology, is the Deputy Director of the Institute of Arctic Biology at the University of Alaska Fairbanks. Dr. Bowyer has an extensive publication record (more than 80 scientific articles). He has conducted extensive research on river otters and impacts of *Exxon Valdez* oil spill on this species.

Dr. Thomas A. Dean is the President of the ecological consulting firm Coastal Resources Associates, Inc., in Vista, California. He has over 20 years of experience in the study of nearshore ecosystems and has authored more than 20 publications, including several papers dealing with sea urchin and kelp interactions. He has extensive experience in long-term monitoring studies with marine plants and invertebrates. He has had a major role in both the shallow subtidal and intertidal *Exxon Valdez* oil spill investigations since 1989.

Dr. Lawrence Duffy, Professor of Chemistry and Biochemistry at the University of Alaska Fairbanks, has been working in the area of toxicology for 17 years and is a member of the International Society of Toxicology. He has studied various bacterial and mammalian toxins. Since the *Exxon Valdez* oil spill, he has published several papers related to developing biomarkers. He is currently on the editorial board of the Science of the Total Environment. At the University, he teaches “Environmental Biochemistry and Biotechnology” and is a member of the Environmental Chemistry Program and Mammal Group.

Dr. Daniel Esler, Research Wildlife Biologist with the USGS, Alaska Biological Science Center, is now a University Research Associate with the Centre for Wildlife Ecology, Simon Fraser University. He has conducted waterbird research in arctic and subarctic regions of Alaska and Russia for the past 11 years. Since 1995, he has served as Project Leader for harlequin duck studies as part of the *Exxon Valdez* Oil Spill Trustee Council-sponsored NVP project. He has authored more than 20 peer-reviewed journal publications and numerous reports and presentations addressing research and issues in waterbird conservation.

Dr. Leslie Holland-Bartels is the former head of the Marine and Freshwater Ecology Research Program for the Alaska Biological Science Center and current Director of the USGS Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. In Alaska, she directed the research of 17 senior scientists in the areas of seabirds, marine mammals, anadromous fisheries, and associate habitat and population issues. She has 24 years experience in aquatic ecology and more than 30 publications in national scientific journals on subjects including contaminants, ecology of invertebrates, fisheries, water quality, and aquatic ecology.

Dr. Stephen C. Jewett currently serves as a Research Professor and the Scientific Diving Officer at the School of Fisheries and Ocean Science, University of Alaska Fairbanks (UAF), since 1975. While at UAF, he has been involved in numerous benthic and intertidal investigations throughout Alaska that emphasize assessment and/or monitoring. He has authored more than 30 publications in scientific journals and books. In addition to his role in the NVP project, he was co-principal investigator on the *Exxon Valdez* oil spill shallow subtidal investigations (1989–95) in Prince William Sound and is currently examining cytochrome P450 in nearshore fishes in the Sound.

Dr. Lyman McDonald is a Senior Biometrician and the President of Western EcoSystems Technology, Cheyenne, Wyoming. He has 30 years of comprehensive experience in the application of statistical methods to design, conduct, and analyze environmental and laboratory studies. He has designed and managed both large and small environmental impact assessment and monitoring programs.

Dr. A. David McGuire is an Assistant Professor of Biology and Wildlife and an Assistant Leader of the Alaska Cooperative Fish and Wildlife Research Unit at the University of Alaska Fairbanks. His research interests include operation of ecological processes at large spatial scales, ecological modeling, and global change biology.

Dr. Charles E. O'Clair, Fishery Research Biologist, is now retired from the National Marine Fisheries Service, Auke Bay Laboratory, in Juneau, Alaska. He has more than 16 peer-reviewed scientific publications. His research experience includes 9 years of damage assessment and restoration process research related to the *Exxon Valdez* oil spill. Other research experience includes 12 years of field and laboratory work on the effects of oil pollution and logging practices on marine benthic invertebrates and research on the ecology and behavior of Dungeness, King, and Tanner crabs.

Dr. Alan Rebar is the Dean of the School of Veterinary Medicine and the Professor of Veterinary Clinical Pathology at Purdue University. He is internationally recognized as an expert in the field of clinical pathology and toxicology. He has been involved in *Exxon Valdez* oil spill studies of sea and river otters since 1991.

Dr. Paul W. Snyder is an Assistant Professor of Pathology and Immunotoxicology and the Director of the Clinical Immunology Laboratory of the Department of Veterinary Pathobiology,

Purdue University. He is also a Diplomat of the American College of Veterinary Pathologists. His research interests are in the area of mechanism-based studies on the pathology and immunology of xenobiotics on biological systems.

Dr. Glenn R. VanBlaricom is an Assistant Unit Leader (Wildlife), Washington Cooperative Fish and Wildlife Research Unit, and an Associate Professor of Fisheries in the School of Aquatic and Fishery Sciences, University of Washington. He has conducted research on coastal ecosystems since 1970 and has been involved in research on sea otters and their ecosystems for 22 years. He has more than 30 peer-reviewed scientific publications.

Cooperators:

Mr. Timothy D. Bowman is a Wildlife Biologist for the U.S. Fish and Wildlife Service, Migratory Bird Management Project. He was principal investigator for the *Exxon Valdez* oil spill damage assessment study on bald eagles and has conducted aerial and ground surveys of waterfowl and seabirds throughout Alaska. He has nine publications in national peer-reviewed journals.

Dr. Gregory H. Golet is a Wildlife Biologist for the U.S. Fish and Wildlife Service. He has studied seabirds in Prince William Sound since 1989 and has published in national peer-reviewed journals.

Dr. John Stegeman is a Research Scientist at Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. He is internationally recognized as an expert in the area of Cytochrome P450 biomarkers of hydrocarbon exposure.

Exxon Valdez Oil Spill
Restoration Project Final Report

Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

Restoration Project 99025
Final Report

Volume 2
Appendices

L. E. Holland-Bartels, Editor

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December 2002

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Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Predators Following the 1989 *Exxon Valdez* Oil Spill

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Study History: This project began with the acceptance of the 5-year study plan by the Trustee Council in March 1995. The FY 95 funds were provided to develop sampling protocols, test methodologies, and to initiate those portions of the overall study that could begin in late summer 1995. The first full field season for this study was initiated in FY 96, followed by a similar field effort in FY 97, and focused reduced effort in FY 98. Program reviews by the Chief Scientist and Trustees of work reported in this document occurred in February 1996 and 1997 and January 1998. The final report has undergone external scientific peer review conducted through the Chief Scientist's office as well as journal review as noted in the individual chapters.

Abstract: The 1989 spill of some 42 million L of crude oil into Prince William Sound, Alaska, represents not only the largest tanker spill in United States history, but the world's largest spill in northern waters. Acute effects have been studied extensively. However, efforts to quantify the spill's long-term chronic effects and develop defensible restoration measures have been plagued by varying levels of scientific uncertainty. That such uncertainty exists is not unexpected. The spill occurred in Prince William Sound's highly variable physical setting typified by its complex oceanography and fjord-like geomorphology. Additionally, uncertainty was driven by the scarcity of precise pre-spill population estimates and spotty life-history information for most species. The research reported herein in, structured in eight primary papers and 27 supporting papers (appendices), documents the state of recovery and assessments of continuing constraints to population recovery for four vertebrate predators (sea otter *Enhydra lutris*, harlequin duck *Histrionicus histrionicus*, river otter *Lontra canadensis*, and pigeon guillemot *Cepphus columba*) whose recovery status remained uncertain some 5 years after the *Exxon Valdez* oil spill. These species are used in a collective weight of evidence approach to better understand the process of coastal community recovery. Each species is examined for the strength of information it brings in health, population, and trophic metrics to support or reject the hypothesis of continuing oil effects in the nearshore system versus the alternatives that food constraints or demographic bottlenecks limit these focal species. While data for individual species contain various levels of uncertainty, scientific confidence is developed in the following picture when examined across species, metric, and hypothesis: Within the nearshore coastal environment, sporadic releases of residual oil are occurring, and benthic species, primarily invertebrates, are being exposed in a temporally and spatially patchy manner sufficient to transport oil up through the food chain. Thus, for the two invertebrate-feeders, sea otter and harlequin duck, evidence exists over several lines of investigation to suggest that local-scale populations continue to be constrained not by food availability or natural demographic processes, but by increased levels of mortality coincident with continued exposure to residual oil. Conversely, weight of evidence suggests that

only limited direct oil-related effects are being transferred through the fish trophic pathway. Sufficient evidence suggests recovery is occurring in river otter populations, while the lack of recovery in pigeon guillemot may be attributed to food limitations (both natural and indirectly related to the spill) and/or slow demographic response to initial acute mortalities. Individual lines of investigation often contained uncertainty, but the collective weight of evidence presented in this multipaper volume indicates lack of full recovery of the nearshore ecosystem from the *Exxon Valdez* oil spill nearly a decade following the event. Integrated, multispecies approaches can allow sufficient weight of evidence to develop despite inherent system variability or data limitations and, thus, facilitate both better societal understanding of such pollution events and development of appropriate restoration responses.

Key Words: Alaska, Barrow's goldeneye, biomarkers, body mass, *Cepphus columba*, clams, condition indices, cytochrome P450, demography, diet, ecosystem, emigration, *Enhydra lutris*, *Exxon Valdez* oil spill, food limitation, habitat selection, harlequin ducks, health, hematology, *Histrionicus histrionicus*, home range, hydrocarbons, immigration, intertidal, *Lontra canadensis*, masked greenlings, mortality, mussels, nearshore, pigeon guillemots, plasma biochemistry, pollution, population recovery, predator-prey interaction, prey, prey availability, prey consumption rate, prey demography, Prince William Sound, reproduction, river otters, sea otters, sea urchins, serum chemistry, sex-ratio, subtidal, surveys, survival, trophic.

Project Data: Final Restoration Report 99025, a collaborative and multiagency effort, used an integrated approach to assess recovery status of the nearshore ecosystem of Prince William Sound following the *Exxon Valdez* oil spill of 1989. As a result of this design, scientists from some 15 research organizations located in over 10 states participated and were required to openly share research results to all participants in near real-time. This distributed-organization and research-sharing requirement necessitated the development of a detailed data management plan and a process by which data could be shared and remotely accessed. Such a design was developed and documented in Holland-Bartels (1996)[1]. For the period of active study, all study data were served for project scientists by the U.S. Geological Survey's Alaska Biological Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503 (Table 1). At project completion, all study data were returned to principal investigators to be managed and archived per policy of their respective agencies. Access to these data is made by arrangement with senior authors (or agency) of the report chapters.

Citation: Holland-Bartels, L.E., editor. 2002. Mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 *Exxon Valdez* oil spill, volume 2 - appendices. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska.

[1]Holland-Bartels, L. 1996. Mechanisms of impact and potential recovery of nearshore vertebrate predators: Restoration Project 95025 Annual Report. Report to the *Exxon Valdez* Oil Spill Trustee Council, Anchorage, Alaska, USA.

Table 1. Data management summary for Nearshore Vertebrate Predator Study at study completion, 1998.

				Files present?			Compliance?	
NVP component	Files (#)	Total size (mb)	Data on file	History	Metadata	SOP	History	Metadata
Focal Species								
Sea otters	501	165.0	all:1995–98	some	some	yes	some	yes
Harlequin ducks	108	3.18	all:1995–98	yes	yes	yes	yes	yes
River otter	15	3.41	all:1996–98	yes	yes	yes	yes	yes
Pigeon guillemot	24	4.07	1996–97 (no 98)	yes	yes	yes	yes	yes
Prey Data								
Duck food	23	0.55	all:1995, 1997	yes	yes	yes	yes	yes
Intertidal clams	71	3.28	all:1995–97	yes	yes	yes	yes	yes
Mussels	137	6.99	1996 (no 97, 98)	yes	yes	yes	yes	yes
Subtidal clams	21	1.53	all:1995–97	yes	no	yes	yes	-
Subtidal fishes	51	1.25	all:1995–97	yes	yes	yes	yes	yes
Sea urchins	94	2.48	all:1996–97	yes	yes	yes	yes	yes
Other Files								
Invertebrate predators	22	1.88	all:1995–96	yes	one	yes	yes	yes
Side-scan sonar	72	4.40	all:1995	yes	yes	yes	yes	yes

Mechanisms of Impact and Potential Recovery of Nearshore Vertebrate Preadotrs Following the 1989 *Exxon Valdez* Oil Spill

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Synthesis Appendix

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APPENDIX SYN-01

Integrating Ecosystem Studies: a Bayesian Comparison of Hypotheses[1]

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[1]Published: 1998. Pages 495–507 *in* F. Funk, J. N. Ianelli, T. J. Quinn II, and P. J. Sullivan, editors. Proceedings of the International Symposium on Fishery Stock Assessment Models for the 21st Century. Alaska Sea Grant College Program AK-SG-98-01.

*****Abstract:

Ecosystem studies are difficult to interpret because of the complexity and number of pathways that may affect a phenomenon of interest. It is not possible to study all aspects of a problem, thus subjective judgement is required to weigh what has been observed in the context of components that were not studied but may have been important. This subjective judgement is usually a poorly documented and ad hoc addendum to a statistical analysis of the data. We present a Bayesian methodology for documenting, quantifying, and incorporating these necessary subjective elements into an ecosystem study. The end product of this methodology is the probability of each of the competing hypotheses. As an example, this method is applied to an ecosystem study designed to discriminate among competing hypotheses for a low abundance of sea otters at a previously oiled site in Prince William Sound, Alaska.

*Introduction:

Ecosystem approaches are increasingly advocated as a way of improving the science and management of natural systems (Lackey *in press*). For instance, studies of the effects of anthropogenic stressors on a species can be misleading if they ignore possible indirect effects acting through predator or prey populations (Higashi and Patten 1989). Further, natural changes in these other components of the ecosystem may cause changes in the focal population, masking or exaggerating the effects of the stressor (Piatt and Anderson 1996). Many studies of the impacts of human actions on a particular species now include research on other components of the ecosystem thought to be important to the focal species.

Nonetheless, there are practical limitations to an ecosystem approach. Because of cost and logistical constraints, not all ecosystem components can be studied and therefore some indirect impacts may be missed. Experimentation or replication may not be possible, and it may thus be difficult to unambiguously assign causes to any observed differences in populations between impacted and non-impacted sites, or before versus after an impact at a single site. It is also highly likely that among the suite of studies, some will give results that are to some degree contradictory.

For these reasons, interpreting the results of an ecosystem study requires some degree of expert judgement. Synthesizing the results of numerous studies of parts of a complex problem is difficult, and it may thus be difficult for investigators to reach conclusions in a rational fashion. Further, different scientists faced with the same evidence may arrive at different conclusions. As the subjective interpretation of results tends to be an ad hoc and poorly documented process, the sources of disagreement may be difficult to uncover and resolve. This paper presents a structured method for documenting and quantifying the expert interpretation of the results of an ecosystem study.

*Proposed Methodology:

The methodology presented here is designed for testing ecosystem-level hypotheses. It integrates studies of diverse components of the ecosystem, summarizing the results as the relative evidence for each hypothesis from each study and the overall evidence for each hypothesis from the ensemble of studies. Its Bayesian features consist of incorporating and quantifying the subjective step of interpreting results, and calculating a probability that each hypothesis is true.

The method consists of the following steps:

1. Generate hypotheses
2. Summarize the experiments and their results
3. Create a table of the expected results under each hypothesis if each experiment were ideal
4. Calculate the probability of the observed result under each hypothesis using statistical considerations
5. Adjust probabilities by considering potential violations of statistical assumptions
6. Adjust probabilities to account for differences between the hypotheses tested and the hypotheses of interest
7. Summarize the evidence for each hypothesis, accounting for dependencies among experiments

Steps 3-6 deal with eliciting statements of probability from experts. Such elicitations can be problematic if experts are unfamiliar with translating their experiments into numerical probabilities (Morgan and Henrion 1990, ch. 7). Our sequence of steps is designed to overcome such problems by sequentially considering several sources of uncertainty, progressing from the most to least familiar. At each of the seven steps, in particular those where subjective judgement is required, the rationale leading to the decision should be thoroughly documented.

**Step 1. Generate hypotheses. The first step is to have the experts identify the hypotheses that are the competing explanations for the phenomenon under investigation. It is important that the hypotheses be both exhaustive and mutually exclusive. If not, the confidence assigned to some hypotheses will be overstated, as the evidence for them will in some respects be counted twice.

Often, there will be reason to believe that several of the hypothesized phenomena might act simultaneously. There are two principal ways of constructing mutually exclusive hypotheses if this is a possibility. The first is to consider a “multiple causes” hypothesis. The second is to redefine the hypotheses to allow minor effects of other factors. For instance, the two hypotheses “effect is produced by factor A” and “effect is produced by factor B” can be made mutually exclusive by redefinition as “effect is principally produced by factor A” and “effect is principally produced by factor B”.

**Step 2. Summarize the available data. In this step, the studies and their results are summarized. For clarity, it is often more useful to use a short verbal description of the results. For instance, a study of differences in prey abundance between control and treatment might be summarized as “much greater abundance found at the control site”.

**Step 3. Consider ideal studies. The third step in this process is to lay out a table with the different hypotheses as the top row and the different experiments as the left-most column (Table 1). Then, have the experts fill out this table as if each study were an ideal experiment; i.e. there were no possibility of either false positive or false negative results.

Table 1. Hypothetical results of a set of ideal experiments.

	Hyp. 1	Hyp. 2	Hyp. 3	Hyp. 4
Study A	positive	negative	negative	positive
Study B	negative	negative	positive	negative
Study C	positive	positive	positive	negative

In the hypothetical example above, Study A would distinguish between Hypotheses 1 or 4 and Hypotheses 2 or 3. In combination, the three studies would be able to determine which hypothesis was true.

**Step 4. Statistical considerations. While ideally the three studies would determine which hypothesis was true with 100% accuracy, in the real world misleading results may be obtained. One of the ways this may happen is through random sampling error. Often, almost any result is possible under any of the hypotheses. Nonetheless, the observed result will be more probable under some hypotheses than others.

The objective of this step is to calculate these relative probabilities, otherwise known as the likelihoods of each of the hypotheses (Gelman et al. 1995 ch. 1). Often, with continuously-distributed variables, the likelihood is a probability density rather than a probability per se. Likelihoods (Table 2) are usually obtained from standard statistical distributions such as the normal or binomial. The exact distribution used depends upon the assumptions made about the experimental data, such as whether each point is independent and identically distributed, whether the sampling variance is constant, etc.

Table 2. Table of likelihoods. P(Result of A|Hyp. 1) means the probability of getting the observed result of Study A if Hypothesis 1 were true.

	Hypothesis 1	Hypothesis 2
Study A	P(Result of A|Hyp. 1)	P(Result of A|Hyp. 2)
Study B	P(Result of B|Hyp. 1)	P(Result of B|Hyp. 2)

This is the first of a series of steps in which experts are asked to assign probabilities to the competing hypotheses. Some experts are unfamiliar with quantitative probability statements and scientists in particular are often uncomfortable making assertions about the relative merits of competing hypotheses without conclusive evidence. This step is important in that it introduces experts to assigning probabilities to the hypotheses, yet does so in a rigorous way using familiar statistical calculations.

**Step 5. Account for possible biases in the test or experimental results. The assumptions of statistical tests are rarely exactly met. Samples may not be completely independent, important sources of error may not be included in the statistical model (e.g., ignoring error in the measurement of the independent variable), and measurements may have some unknown biases. Historically, statistical confidence tends to overstate the certainty of scientific results (Henrion and Fischoff 1986).

In constructing the table of likelihoods of results, this overconfidence needs to be accounted for. Generally, the effect of such errors is to make the probabilities of the result under each hypothesis more similar. Based on their knowledge of the experiment, experts should determine which assumptions of the test are likely to be violated, and to what degree. These judgements are to some extent subjective, but once made the statistical literature or computer simulations can provide guidance on their likely effects. In consultation with a statistician, the experts should adjust the table of probabilities to account for such violations.

**Step 6. Account for differences between the statistical hypothesis being tested and the biological hypothesis that is actually of interest. Often, an experiment to test a hypothesis tests it only indirectly. The results may thus be ambiguous if the indirect indicator could occur in several ways, some of which are not related to the hypothesis.

For example, if the hypothesis were that some population was affected by an environmental contaminant, an investigator might test the environment for the presence of the contaminant and test individuals for signs of poor health. A positive result in either case would not necessarily implicate the contaminant; the contaminant might be present yet not be causing health effects, or poor health might be due to causes other than the contaminant.

As in step 5, the effect of a difference between the hypothesis tested and the hypothesis of interest is to even further equalize the probabilities of the observed results under each hypothesis. The appropriate amount of adjustment of the table entries depends on the probability of other (possibly unknown) alternative explanations for the test results.

Such assessments are unavoidably subjective and require the judgement of experts. Hopefully, by this point in the process the experts are comfortable with assessing the relative probability of the data under each hypothesis and how violations of assumptions may result in misleading experimental results. It is crucial that they consider alternative explanations for their data yet not be paralyzed by such possibilities. They should be willing to examine data that seems to strongly favor one hypothesis and consider whether there are other, possibly unstudied ecosystem pathways that could produce similar results and state how probable they feel such pathways are.

**Step 7. Summarize the evidence. In this step, the table of probabilities is summarized to derive the overall weight of evidence for each hypothesis provided by the ensemble of studies. If the studies are independent, then elementary statistical theory says the joint likelihood of each hypothesis is simply the multiplication of its probability under each study (Eq. 1). The overall

likelihood of each hypothesis is then simply the product of its column of probabilities (here R1, R2, and R3 signify the results of experiments 1, 2 and 3, respectively).

Likelihood of hypothesis = P(R1|hyp.) x P(R2|hyp.) x P(R3|hyp.) [1]

The different hypotheses can then be compared in terms of their relative likelihoods. This comparison is easier if the likelihoods are re-scaled so that the sum of all of the likelihoods is 1. From a Bayesian perspective, each re-scaled likelihood could then be interpreted as the probability that a hypothesis was true.

***Complication A. Dependencies among results. There are two ways that experimental results might not be independent. First, the data from two experiments may have been taken from the same random sample. Second, two experiments may measure the same ecological phenomenon two different ways. In either case, it is not appropriate to treat the results as providing independent evidence bearing on the alternative hypotheses; i.e., simply multiplying the probabilities of the two experiments together will overweight the evidence.

There are several possible methods to account for dependencies among experimental results. If experiments are highly interdependent, they should be lumped and a single probability of each hypothesis calculated for the ensemble results. If experiments are only partially dependent, the correlation of results must be accounted for. If the correlation can be calculated, probability theory provides methods for calculating a joint probability. If not, a value must be obtained from experts, although experts have been found to perform poorly at providing a numerical value for correlation coefficients (Morgan and Henrion 1990 ch. 7).

A more intuitive method for dealing with partially correlated results is to ask investigators to provide an estimate of the "effective" number of experiments. For instance, investigators may feel that dependence between two experiments is such that they jointly provide only as much evidence as 1.5 independent experiments. Then, the appropriate adjustment would be to raise each of the probabilities to the 0.75 power (e.g., Eq. 2). In general, if N experiments are correlated so that the effective number is E, probabilities for hypotheses for each experiment should be adjusted by raising them to the E/N power.

Likelihood of hypothesis = $P(R1|hyp.)^{0.75}$ x $P(R2|hyp.)^{0.75}$ [2]

***Complication B. Prior probabilities. Bayesian statistics involves multiplying the likelihoods by a set of prior weights (the prior probabilities) for the hypotheses before re-scaling to calculate the posterior probabilities. In the Bayesian approach, these prior probabilities reflect the weight accorded each hypothesis before the experiments were conducted. Assuming the probability of each hypothesis to be proportional to the joint likelihoods treats each hypothesis as being equally likely a priori, thus letting the data determine the relative probability of each hypothesis. While this is intuitively appealing, it may not be appropriate.

For instance, if the analysis were being used in a legal proceeding, it might be appropriate to give the benefit of the doubt to the defendant by assigning small prior weights to hypotheses implicating the defendant. Similarly, in investigating current scientific theory a high prior weight might be assigned to the currently accepted paradigm, so that a novel competing theory would not get much credence unless the evidence for it was overwhelming. An alternative to using prior weights is to calculate probabilities only from likelihoods, but require a very high probability that a hypothesis is true before acting on it. Whatever the prior weights, if data strongly support one hypothesis over the others the final probabilities will reflect this.

Standard Bayesian practice is to compare the evidence for competing hypotheses using Bayes factors (Kass and Raftery 1995). The Bayes factor is simply the ratio of the posterior probabilities of two competing hypotheses divided by the ratio of the prior probabilities assigned before the experiments were conducted. When the prior probabilities of the hypotheses are equal, this is simply the ratio of the posterior probabilities.

*An example -- sea otters after the Exxon Valdez oil spill:

On March 4, 1989, the supertanker Exxon Valdez spilled nearly 42 million liters of crude oil in Prince William Sound, Alaska (Spies et al. 1996). This spill is hereafter referred to with the acronym EVOS. Sea otter populations in oiled areas suffered high mortality (Loughlin et al. 1996). Other components of the ecosystem were likewise severely affected. Five years after the spill, residual oil was present in sediments and mussel beds in some areas of the spill (Spies et al. 1996). Even today, residual oil is found in some areas.

The Nearshore Vertebrate Predator (NVP) project (Holland-Bartels et al. 1996), a multi-university and agency investigation funded by the EVOS Trustee Council, is aimed at determining whether top predators in Prince William Sound are still suffering the effects of the oil spill. The question is difficult to answer unambiguously because of the complicated nature of the ecosystem and the lack of data from the period before EVOS. The NVP project studies predator populations from several points of view, and also looks at other components of the ecosystem on which these predators depend. If a population is still being affected by EVOS, the study is designed to ascertain whether the effects are due to the continuing toxic effects of oil, a slow rate of recovery from past mortality, or an indirect effect on some critical ecosystem component.

With limited resources and such an intensive approach, few populations can be studied. Sea otter abundance at Knight Island, which was oiled in 1989, is lower than at Montague Island, which was not. The NVP sea otter study has focused on these two populations, trying to find the reason for these differences in abundance. The principal hypotheses are:

1. **Direct toxicity of residual oil.** Residual oil is present and reducing the fecundity and/or survival of otters at the oiled site.
2. **Reduced forage due to oil effects.** The initial impact of oil or residual oil is reducing prey available to sea otters.
3. **Slow recovery due to demographic limitations.** Aside from the initial otter mortality from EVOS, residual oil is absent or does not affect otters or their food. However,

limitations on the maximum growth rate of the population have prevented the population from reaching capacity yet.

4. **Natural differences in capacity.** The oiled site has poorer or less abundant otter habitat.

A variety of studies have been undertaken to determine which hypothesis is the most likely. These include:

1. **Demographic comparisons**. Population abundance, age structure, and reproductive rates were compared between islands.
2. **Individual health.** Otters were captured at both locations. Individuals were weighed and measured, and blood samples taken. In particular, blood cells and serum chemistry were examined for signals of poor health, and a specific signal of exposure to oil (the enzyme P450) was tested for.
3. **Prey abundance and foraging success.** The abundance and size distribution of major prey items of sea otters were compared among islands. In addition, foraging sea otters were observed to determine relative rates of success in obtaining prey items.

Statistical hypothesis tests were performed for many of the studies but are not reported here. We chose not to calculate likelihoods based solely on statistical distributions --step 4 of our methodology -- because the limitations imposed by the design of the study tended to emphasize the considerations dealt with in steps 5 and 6. There are multiple predictions from each of the hypotheses, not all of which are distinct. Any particular study result may eliminate some hypotheses but leave several others. More likely, any particular study result would be ambiguous, as there is a small likelihood of almost any result from each hypothesis. In particular, the detection of a phenomenon does not necessarily imply that this was the cause of the difference in abundance between the two islands. For instance, oil could be present but yet not greatly affect survival. Likewise, prey abundance could differ between one site and another but be unrelated to the difference in otter abundance.

Thus, the interpretation of the results of the studies required some judgement. Our chief tool was to ask ourselves, “What is the probability we would get the result we observed from Study ___ if Hypothesis ___ was true?” We attempted to quantify our impression of the strength of each piece of evidence by filling out the table of probabilities, sequentially considering what the result would mean in an ideal world, what the statistical tests implied, how the assumptions of the tests might be violated, and what mechanisms might cause the results to be misleading.

We felt our ability to discriminate among probability levels was fairly coarse. Accordingly, we initially filled in the table of probabilities verbally, using the categories “high”, “moderate – high”, “moderate”, “low – moderate”, and “low”, which we later replaced with 0.9, 0.7, 0.5, 0.3, and 0.1, respectively (Table 3).

Table 3. First attempt at integrating studies. Top row gives hypotheses, and left column gives experiments with the results in parentheses. "M" refers to Montague Island (control), and "K" to Knight Island (oiled). The main body of the table gives the probability of obtaining each experimental result under each hypothesis. The bottom two rows summarize the result as the product of the probabilities for each hypothesis (i.e. the joint likelihood) and the probability products re-scaled to sum to 100%.

EXPERIMENT & (RESULT)	"A" DEMOGRAPHIC LIMITATION	"B" FOOD LIMITATION	"C" OIL PERSISTENCE	"D" RECOVERY HAS OCCURRED
OTTER DENSITY (K << M)	0.9	0.9	0.9	0.3
REPRO. RATES (EQUAL)	0.9	0.5	0.7	0.9
BLOOD CHEMISTRY (EQUAL)	0.9	0.7	0.3	0.9
P450 (EQUAL)	0.7	0.7	0.1	0.9
PREY ABUNDANCE (M < K)	0.9	0.1	0.1	0.1
FORAGING SUCCESS (M < K)	0.9	0.1	0.7	0.1
Joint Likelihood	**0.4133**	**0.0022**	**0.0013**	**0.0022**
Probability of Hypotheses	**98.6%**	**0.53%**	**0.32%**	**0.52%**

The result of our first analysis was to assign more than a 98% probability to the hypothesis that the population differences were due to a demographic limitation in the rate of recovery of the Knight Island population from spill mortality. All other hypotheses combined had less than a 1.5% probability of being true. We were unhappy with this result, as this high degree of confidence did not reflect our personal higher degree of uncertainty. We felt that the evidence for this hypothesis was not that strong.

In examining the reasons for this initial result, we identified three principal sources of error. First, we overstated the power of the studies to discriminate among hypotheses. For instance, we assigned a 0.90 probability of seeing greater prey abundance at the oiled site if demography was limiting recovery, but only a probability of 0.10 under any of the other hypotheses. We did not adequately address step 6 of our methodology; for instance, there would

be a fairly good chance of seeing higher prey abundance at the oiled site under several alternative hypotheses.

Second, the range of hypotheses we considered was too narrow. In retrospect, we felt there was a strong possibility that all of the hypotheses might be incorrect, and some other factor might be responsible for differences between areas. This resulted in an unrealistically high probability for the hypothesis most consistent with the data.

Third, we did not adequately account for dependencies among experimental results (step 7, complication A). While we lumped most blood chemistry measures into one result, we kept the assay for the enzyme P450 (a more direct measure of exposure to oil) as a separate experiment. Since this assay could indicate the same phenomenon, and was measured on the same sample of animals, we felt the two results were effectively equivalent to only 1.5 experiments. Similarly, measures of prey size, prey abundance, and foraging success to some extent measured the same phenomenon. In retrospect, we decided to consider them as equivalent to 2 experiments.

We therefore revised the tabled probabilities, taking what we hoped was a more realistic look at the power of the studies and adding another alternative hypothesis to those we had listed. While we were able to think of several specific alternatives, we felt the true explanation for population differences might be something we hadn't considered. Therefore, we added only one hypothesis; an "unknown causes" category. Meanwhile, the completion of analyses of blood chemistry and the enzyme P450 suggested that residual oil might be present at the oiled site, and new information became available about the size distribution of prey species (Table 4).

Table 4. Second attempt at integrating studies. Top row gives hypotheses, and left column gives experiments with the results in parentheses. "M" refers to Montague Island (control), and "K" to Knight Island (oiled). The main body of the table gives the probability of obtaining each experimental result under each hypothesis. The bottom two rows summarize the result as the product of the probabilities for each hypothesis (i.e. the joint likelihood) and the probability products re-scaled to sum to 100%.

EXPERIMENT & (RESULT)	"A" DEMOGR. LIMIT.	"B" FOOD LIMIT.	"C" OIL PERSIST.	"D" RECOVERED	"E" UNKNOWN CAUSES
OTTER DENSITY (K << M)	0.9	0.9	0.9	0.3	0.9
REPRO RATES (EQUAL)	0.9	0.5	0.7	0.9	0.9
BLOOD CBC'S & CHEMISTRY (WEAK INDICATION OF LIVER DAMAGE AT K)	0.5	0.5	0.7	0.3	0.5

P450 (M < K)	0.3	0.3	0.9	0.3	0.3
PREY ABUNDANCE (M < K)	0.9	0.1	0.5	0.3	0.5
PREY SIZE (M < K)	0.9	0.1	0.7	0.3	0.7
FORAGING SUCCESS (M < K)	0.9	0.1	0.7	0.3	0.7
Joint Likelihood	0.1581	0.0011	0.1744	0.0040	0.0764
Probability of Hypotheses	38.2%	0.3%	42.1%	1.0%	18.5%

The revised table again supports the hypothesis that the populations differ because the population in the oiled area has not had the time to recover fully from the losses due to the oil spill. However, it shows even greater support for the hypothesis that residual oil is still affecting the population. The hypothesis that some unknown factor accounts for the difference between populations is also quite probable.

Two hypotheses were eliminated from consideration, principally because of the forage abundance studies. Forage was more abundant and foraging success higher at the oiled site. These results were not at all consistent with the food limitation hypothesis, and were also unlikely if the population at the oiled site had recovered to its carrying capacity. However, it should be noted that the "unknown causes" hypothesis, which has a fairly high probability of being true, is not necessarily related to the spill. Thus it would be inappropriate to say the probability that the population is no longer suffering effects of the spill is only 0.01.

We will refine and expand this analysis as more data become available and more experts are consulted. These results are not our final interpretation, and should be viewed as a preliminary analysis. We provided this example solely to illustrate the use of the methodology.

*Discussion:

The Bayesian aspects of the proposed methodology are (1) use of subjective expert judgement in interpreting indirect tests of hypotheses, and (2) integration of experimental results and expert judgement into an overall probability for each hypothesis using Bayesian probability calculations. A large literature exists on using Bayesian methods to compare hypotheses (Kass and Raftery 1995).

Bayesian methods have been criticized from a variety of standpoints (e.g., Dennis 1996). The principal criticism is that Bayesian methods inject subjectivity into scientific analyses that should be objective. However, in extrapolating from the results of diverse studies on small aspects of a larger question, subjectivity in the form of expert judgement is unavoidable. We propose a methodology that formalizes the intuitive process experts use in interpreting the results of

ecosystem studies. This approach clearly distinguishes subjective interpretation from experimental results, and clearly shows the reasoning used.

Our methodology provides a tool for investigators to organize their thinking. The ecosystem and the results of the numerous studies may be too complex to be readily grasped in their entirety. By allowing investigators to approach the synthesis of the studies one element at a time, our method increases the tractability of the process.

The methodology also facilitates openness and discussion, since subjective components of the synthesis of the studies are documented and quantified. It clearly shows why a particular conclusion was reached, and what evidence investigators felt was ambiguous or particularly strong. Areas of disagreement among investigators are also easily identified.

Our methodology is based on principles derived from other methods widely used for eliciting probabilities from experts (summarized in Morgan and Henrion 1990, Ch. 7). Examples of such methods include the Stanford/SRI protocol (Spetlzer and Stael von Holstein 1975, Merkhofer 1987) and the Wallsten/EPA protocol (Wallsten and Whitfield 1986). We've tailored our methodology to the specific goal of summarizing the relative support for alternative hypotheses from an interrelated but necessarily incomplete set of studies.

Most methods for probability elicitation pay great attention to getting experts comfortable with the idea of translating their knowledge and judgement into probability statements, and to overcoming a tendency of experts to give probabilities that overstate the level of certainty (Tversky and Kahneman 1982, Morgan and Henrion 1990, Ch. 7). Our solution to these difficulties is to take experts through a specific sequence of probability elicitation steps. These start with specifying deterministic outcomes, then progress through familiar specifications of probability (likelihood calculations) to less familiar probability specifications (the effects of violation of statistical assumptions and of not directly testing the hypothesis of interest). This sequence gradually introduces the process of making probability statements. It also sequentially introduces more and more forms of uncertainty, continually forcing the expert to reflect on whether the degree of confidence he's previously expressed is appropriate.

Our example illustrates both the utility and limitations of the methodology. The summary table lists the hypotheses and the experimental results. Probabilities within the table explicitly document the experts' interpretation of the consistency of the results of each experiment with each hypothesis. The summary probabilities excluded two hypotheses but retained three others, one of which appears to be only half as probable as the other two.

However, the 18.5% probability assigned to the "Unknown Causes" hypothesis makes interpretation of the other probabilities somewhat ambiguous. Much of the probability assigned to this hypothesis may indicate that recovery has occurred, and the differences we found are caused by some unknown factor(s) unrelated to the spill. It is also possible that "Unknown Causes" represents effects related to the spill such as cascading ecological effects. In either case,

the results do provide guidance for further research; they suggest that continuing studies should focus on hypotheses “A”, “C”, and “E”.

The necessity for re-evaluating our initial analysis because of unrealistic results in instructive. It reinforces the experience of others who have found that numerical statements of probability given by experts tend to be overly confident (Tversky and Kahneman 1982, Henrion and Fischoff 1986). Our second try produced a result that we felt better reflected the strength of the evidence provided by the experiments.

There is a danger that allowing such reanalysis could result in investigators juggling numbers to arrive at a result that reflected their preconceptions. However, an honest reappraisal of each element in the table is not inappropriate. Most methods for probability elicitation do recommend that assessors return to an earlier phase in the process whenever questioning reveals that the probabilities elicited clearly don’t reflect the expert’s judgement (Kadane et al. 1980, Morgan and Henrion 1990, ch. 7, Laskey 1995). We found the reanalysis of the table caused us to re-examine the basis of our interpretations; rather than reinforcing our preconceptions, it tended to make us change them.

Use of our methodology will make it easier to examine the source of differences in interpretation of a study. For example, a scientist who disagreed with our conclusions might find that the basis of his difference was the weight placed on the blood chemistry results. A sensitivity analysis to alternative interpretations would be easy to perform by replacing the disputed probability with an alternative value to see if this affected the conclusions.

This method is not proposed as a substitute for good experimentation. With scarce, poor quality, and ambiguous data the conclusion reached after applying this method will be that considerable uncertainty remains. However, in such situations this methodology may identify areas of major uncertainty and suggest fruitful lines of investigation. The major benefit of this approach is the explicit documentation and quantification of the unavoidable subjective interpretation of ambiguous results that arise in many ecosystem investigations. In contrast, when strong experimental designs are available that produce clear evidence, subjective interpretation will be minimized and investigators should reach consensus.

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Biomarker Appendices

(BIO)

APPENDIX BIO-01

HEMATOLOGY AND SERUM CHEMISTRY OF SEA OTTERS IN OILED AND UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA, 1996-98[1]

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Introduction

Studies on sea otters (*Enhydra lutris*) following the 1989 *Exxon Valdez* oil spill showed changes in several hematology and serum chemistry values for otters at the rehabilitation centers in 1989 (Rebar et al. 1995, Williams et al. 1995), and for otters caught in the wild in western Prince William Sound (WPWS) in 1990-92 (Rebar et al. 1996; USGS-BRD, unpublished data). Elevated serum enzymes in otters from oiled areas, relative to those from nonoiled areas, were a general finding in all years and although the degree of difference was not large, suggested the possibility of hepatocellular injury or subclinical liver disease. A further observation was an increase in eosinophils in otters from oiled areas, possibly indicating systemic hypersensitivity reactions. Combined with a lack of population increase and low survival rates in oiled areas of WPWS (Ballachey et al. 1994; Chapter 3 Part A, Chapter 3 Part B, Appendix BIO-03), these observations of abnormal blood samples generated concern that lingering effects of acute oil exposure and, possibly, continuing toxic effects of exposure to residual oil, were deleteriously affecting health and survival and limiting recovery of sea otters.

The Nearshore Vertebrate Predator (NVP) study was implemented in 1995 to assess recovery in the nearshore ecosystem, focusing on four top-level predator species inhabiting nearshore areas of Prince William Sound (PWS; Holland-Bartels et al. 1999). One NVP component was to determine whether oil exposure continued, and if so, whether it was associated with adverse toxic effects on health and survival of the predator species (Chapter 2). Sea otters were one of the predator species included in the NVP study (Chapter 3 Part A). Health of sea otters was assessed by examining hematology and serum chemistry profiles and body condition of animals captured in oiled and unoiled areas of WPWS in 1996-98. Herein we report on hematology and serum chemistry data from those animals, with the primary objective of assessing differences between otters in oiled vs. unoiled areas.

Methods

Sea otters were captured in July and August of 1996, 1997 and 1998 at Knight and Naked islands (oiled area), and at Montague Island, using either tangle nets (modified gill nets) or diver-held Wilson traps (Chapter 3 Part A). Otters were anesthetized, weighed, and morphometric measurements taken. A premolar tooth was extracted for age determination (Bodkin et al. 1997), and approximately 35 cc of blood were collected from each otter, from the jugular vein. Approximately 10-15 ml of the blood were drawn from the jugular by vacutainer, with about 3 ml being placed in an EDTA tube and refrigerated pending shipment to the clinical laboratory, and 8-10 ml into 1 or 2 glass tubes and allowed to clot for at least 30 minutes before centrifuging to separate serum. Serum was frozen in several aliquots. Two blood smears on glass slides were made from the whole blood. The rest of the blood sample, about 20-25 ml, was drawn into a 50 ml heparinized syringe for isolation of blood lymphocytes to determine levels of mRNA for the biomarker, cytochrome P4501A (CYP1A; Chapter 2, Appendix BIO-02). Whole blood in the

EDTA tube and blood smears were shipped to Quest Laboratories[2], Portland, OR, as soon as possible after collection. Only samples that arrived at the laboratory within 72 hours of collection were used in the analyses of hematology data. Serum samples were maintained in frozen storage until field work was complete, and then submitted as a batch to Quest Laboratories, Portland. Additional serum samples were shipped to the University of Alaska Fairbanks for haptoglobin analyses (Duffy et al. 1993, Chapter 5).

Stepwise model selection was conducted to determine the magnitude of the area effect on 38 hematology and serum chemistry parameters[3], and one additional measure, CYP1A. The ranks of the blood parameters were used as the responses to control for the non-normality common in the blood variables. We used a combination of forward stepwise selection and backward stepwise selection on each blood parameter starting with a model containing five main effects (sex, year, age, capture type, and area) and all two-way interactions. Variables entered and remained in the model when the p-value for the coefficient of the variable was significantly different from zero based on an F-test at the 0.05 level of significance. All terms in the final model were significantly different from zero, except for the retention of main effects if an interaction was in the model. If area was not present with the resulting model, the area term was added to determine the importance of the area effect on the response (Table 1).

An assumption in the use of covariates to help understand the influence of the area effect is that the covariates are not correlated with area. Sex, age, and capture type were found to be correlated with area in a Chi-square analysis (p-values = 0.005, 0.006, and 0.033 respectively). This imbalance is known to cause confounding and affects power to detect differences. Therefore, some of the effects of area may have been adjusted out while adjusting for the covariates. In this sense, the analysis is conservative, because some blood parameters with a marginally significant area coefficient may in fact have an area difference.

We performed a supplemental analysis of the data, using Akaike's Information Criterion (AIC; Burnham and Anderson 1998), to determine the relative magnitude of the area effect on each blood parameter relative to the four other covariates. For each blood parameter, we fit 31 models, ranging from univariate models of each covariate alone, to the fullest model with all five covariates and all two-way interactions. Models that contained three or more main effects also contained the second-order interactions. Models were weighted based on the AIC values and weights were summed from every model that contained the covariate (Burnham and Anderson

[2]Formerly Corning Clinical Laboratory (CCL), and prior to that, Physicians Medlab Laboratories (PML).

[3]Hematology parameters: WBC, RBC, HB, HCT, MCV, MCH, MCHC, RDW, PLATES, NEU#, LYM#, MON#, EOS#, BAS#. Serum chemistry parameters: GLU, BUN, CREAT, URIC, NA, K, CL, CA, P, TPRT, ALB, GLB, TRIG, CHOL, HDL, VLDL, LDL, TBIL, DBIL, GGT, AP, LDH, AST, ALT. Abbreviations given in Table 1.

1998). Covariates with high values (i.e., that covariate was present in all or most of the top AIC models) are deemed important in the explanation of the blood parameter response (Table 2).

Results

For hematology variables, no significant p-values were obtained for area in the models based on ANOVA of ranked values (Table 1). Only the p-value for WBC, at 0.066, approached significance, with higher mean levels in the unoiled area compared to the oiled (Table 3). Using AIC weights (Table 2), a similar pattern was seen for the hematology variables, with area generally being of low importance in the models. The importance value for WBC was only 0.545, which did not support consideration of an area difference based on the marginally significant p-value. Of the remaining hematology variables, only MCHC had a relatively high importance value (0.826) for area; however, the p-value for area in the MCHC ANOVA was 0.286.

A larger number of significant differences were observed between areas for the serum chemistry variables, with p-values from ANOVA models less than 0.05 for BUN, uric acid, calcium, potassium, total bilirubin, direct bilirubin, GGT, LDH, and haptoglobin (Table 1). Importance values for the area effect on these variables also were generally high (Table 2), with 0.780 for BUN and 0.832 for calcium being the lowest of the group. Importance values for area were also relatively high for albumin (0.996), creatinine (0.992) and total protein (0.809), although the p-values from the ANOVA model did not indicate area to be a significant effect for these variables.

Cytochrome P4501A (CYP1A) was significantly higher in otters from the oiled area, indicated by a p-value < 0.001 (Table 1) and an importance value of 1.000 (Table 2). No other effects in the model were found to be significant or of importance for CYP1A.

The importance values for each covariate based on AIC modeling reaffirm the traditional linear model selection process we conducted. In general, variables with high importance values were included in the final model. For the models that contained a significant area effect, the importance value for area was never below 0.78. There were four variables (ALB, CREAT, MCHC and TPRT) for which the importance value for area was higher than 0.78, but the area effect was not significant in our traditionally selected model (p-values of 0.608, 0.541, 0.285, and 0.283, respectively).

To aid in assessing potential contributions of oil toxicity to differences observed in blood parameters, we compiled selected mean values from previous blood sampling efforts in oiled and unoiled areas (Table 4). Included are data from sea otters captured in western and eastern PWS in 1992, and in western PWS and southeast Alaska in 1991. Consistent trends in means are evident for only two variables: GGT (higher in the oiled area in all three data sets, with $p < 0.001$ in 1996-98 and 1992, and $p = 0.35$ in 1991) and direct bilirubin (higher in the oiled area in all three data sets, but only significantly so in 1996-98, when the area x sex interaction was significant). Differences between the study areas in mean GGT are due not to a general elevation in otters

from the oiled area, but rather to a relatively small proportion of animals from the oiled area with higher GGT values; this pattern was evident in 1996-98 (Figure 1), and in 1991 and 1992. As well as GGT, in the 1992 and 1991 data, serum enzymes AP, AST and ALT are all elevated in the oiled areas relative to the unoiled, although differences are not all statistically significant nor large. However, in the 1996-98 data set, the mean values for AP, AST and ALT are almost identical (Table 4).

Discussion

The combination of ANOVA analyses and information values from the AIC selection procedure identified approximately 10 blood variables for which there is a statistical difference in mean values between the oiled and unoiled areas during 1996-98. However, based on examination of all variables, including those that do not differ between areas, we conclude that (1) the hematology and serum chemistry data for otters in oiled areas do not present a biological pattern which might be associated with oil toxicity, and (2) although mean values for a subset of variables do differ statistically, the absolute levels of these differences are small and do not appear to be biologically meaningful in terms of adverse indicators of health of sea otters.

Comparison of the 1996-98 data with results from 1991 and 1992 supports the first conclusion, as there is no consistent pattern in differences between oiled and unoiled areas over the longer period, with the exception of serum GGT levels. We would expect that if differences observed in the 1996-98 study were attributable to continuing oil exposure, then similar differences would be noted in the previous studies as well, presuming levels of oil contamination were greater in the earlier years. The serum enzymes, AP, AST, ALT and GGT all showed some degree of elevation in oiled areas relative to unoiled in 1991 and 1992, but only for GGT were area differences still noted in the 1996-98 samples. Elevated serum enzymes also were reported for oiled sea otters at rehabilitation centers in 1989 (Rebar et al. 1995, Williams et al. 1995), river otters in oiled areas of WPWS post-spill (Duffy et al. 1994), and mink exposed to petroleum products (Mazet et al. in press). By 1996-98, however, mean values for AP, AST, and ALT are remarkably similar in sea otters from the two areas, which suggests diminishing toxicity associated with residual oil in the environment. Although elevations in GGT persist in the 1996-98 data set, the magnitude of difference between oiled and unoiled areas has declined, relative to that seen in the 1992 data. GGT was positively correlated with AP, AST and ALT levels ($r = 0.36$, 0.61, and 0.76, respectively) in 1996-98, although mean levels of these three enzymes do not differ between areas.

The difference in 1991 and 1992 data, compared to 1996-98, may reflect the value of blood panels to detect acute injury to liver and kidney, which would have been a greater factor in the earlier post-spill years. Presumably, during that time, the proportion of sea otters in the population that had acute, sublethal exposure in 1989 (with significant exposures possibly extending into 1990-91) was relatively high. However, based on ages-at-death data, Monson et al. (Appendix BIO-03) conclude that sea otters in the oiled region had higher mortality rates postspill than prespill. Thus, by the 1996-98 study, there were probably few of these individuals

left in the population, so that blood parameters associated with organ damage had, to a large extent, normalized. The lack of difference in the later years suggests that animals with injury from acute exposure are no longer present, but does not necessarily indicate that chronic exposure, at a relatively low level, is not continuing.

Data comparisons across the different years, however, need to be viewed with caution as the specific study areas were not the same across the three data sets. Unoiled areas in 1991 and 1992 were southeast Alaska and eastern PWS, respectively. The area of WPWS that was considered oiled for purposes of otter capture and sampling in 1991 and 1992 actually encompassed both the oiled (Knight Island) and unoiled (Montague Island) study areas for the 1996-98 sampling. This design was implemented in 1996 to minimize other factors that might differ between populations which were more geographically distinct. In 1991 and 1992, however, few sea otters were actually captured in the area of northern Knight Island (the focus of captures between 1996 and 1998) as otter densities there were so low as to render capture operations very inefficient.

The extent to which the differences among study sites in 1991, 1992 and 1996-98 may be influencing our results is unknown. However, one factor known to differ between WPWS and either eastern PWS or southeast AK is length of occupation, as otters reoccupied the latter two areas more recently than they did WPWS (Jameson et al. 1982, Garshelis and Garshelis 1984), and thus likely have had more plentiful food resources available to them. The differences seen between otters in oiled and unoiled areas in the 1996-98 data may also have been influenced by factors related to otter densities and availability of prey (Chapter 3 Part A, Chapter 3 Part B). Another consideration is variation among animals associated with capture stress. We attempted to control for this by including capture method as an effect in the analyses, as otters caught in traps by divers would generally have less opportunity for physical exertion prior to sedation and sampling. However, the length of time that sea otters spent in tangle nets (which was usually unknown for an individual otter) may have varied. Once deployed, nets are checked for captured otters every 3-4 hours. If sea otters at Knight Island were removed from the nets sooner than at Montague Island, they might be expected to have lower levels of capture stress and, on the average, slightly different blood panels. The significant area difference in LDH, an enzyme that will be elevated in animals following muscle exertion (Bossart and Dierauf 1990), may reflect longer periods in nets for sea otters at Montague Island.

Significant differences were frequently noted for other covariates (age, sex, year and capture method) included in the analytical models. Effects of these covariates generally were as expected based on data from other mammalian species (Duncan and Prasse 1989) and, overall, these results tend to increase our confidence in the accuracy of the data set.

Levels of CYP1A were significantly elevated in oiled areas relative to unoiled, in the 1996-98 data (CYP1A was not assayed in the earlier studies). CYP1A is a biomarker of hydrocarbon exposure (Payne et al. 1986, Stegeman et al. 1992), and higher levels in the oiled area are indicative of ongoing contaminant exposure, most likely to residual EVOS hydrocarbons (Ballachey et al. 1999). Although CYP1A induction in sea otters from the oiled area was quite

variable (over a 300-fold difference from minimum to maximum value), little overlap was seen in CYP1A values between the two areas. A more extensive discussion of the CYP1A results is provided in Ballachey et al. (1999).

The serum enzyme GGT, which was higher in oiled areas in 1996-98 and in 1992, may be a sensitive indicator of liver damage from hydrocarbon exposure in sea otters. Differences between areas were more pronounced in 1992, with mean GGT of 29.11 that year, compared to a mean in 1996-98 of 17.78. By 1996-98, most individuals in the oiled area have "normal" GGT values, with only a subset of the animals exhibiting elevations, rather than a general population increase. A similar pattern of a relatively small proportion of animals with elevated GGT was seen in 1991 and 1992. If GGT is indeed a marker of exposure, and residual oil contamination is patchy in distribution, this might be the expected pattern. However, no relation was detected between CYP1A levels and GGT or any of the other serum enzymes. Perhaps by the 1996-98 study, absolute levels of residual EVOS oil were very low, although sufficient to maintain induction of CYP1A in the general sea otter population in the oiled area. These generally low levels of contamination may not have been sufficient to cause overall population increases in GGT. Rather, it may be that only those few animals which have happened to get greater exposure, possibly for extended periods, show increased GGT levels. Because such exposures may be sporadic (relating to patchiness in oiling of shoreline areas, individual preferences for foraging areas, and perhaps to storm events, when oil in sediments may be released), our measures of CYP1A largely do not reflect them, and thus no relation between the GGT and CYP1A is evident.

In conclusion, sea otter blood data collected during the last decade suggest that differences noted in 1991 and 1992 between otters in oiled and unoiled areas likely resulted from acute exposure with associated organ damage, but that by 1996-98, these differences have diminished in magnitude or disappeared. The majority of sea otters in oiled areas have blood values within a normal range, and only a small proportion of otters exhibit elevated serum enzymes, particularly GGT, which may be a lingering manifestation of oil toxicity. Chronic exposure, which continues through 1998 based on the biomarker CYP1A, may still be affecting recovery of the population but for the majority of animals, residual toxicity is not leading to significant organ damage or altered hematology and serum chemistry parameters in sea otters.

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Table 1. P-values from ANOVA on ranked values of blood variables, sea otter NVP data, 1996-98. Values are from final model selected by forward and backward selection. Only effects that remained in the model have p-values presented. Area was put into each model after model selection. When a significant interaction with area exists[d], the unadjusted area means are given within the levels of the interacting variable.

						Direction of Area Difference	
Variable[e]	Age[a]	Sex	Year	Capture Type[b]	Area[c]	Knight Mean	Montague Mean
ALB	d	.	d	.	0.6079		
ALT	.	.	0.0001	.	0.6265		
AP	0.0000	.	.	.	0.9898		
AST	.	.	0.0434	0.0004	0.9430		
BAS#	.	.	.	.	0.6985		
BUN	0.0000	.	.	.	0.0133	45.430	49.807
CA	d	.	d	.	d	1996: 9.120 1997: 9.144 1998: 8.570	1996: 9.025 1997: 9.329 1998: 8.951
CHOL	.	0.0028	.	.	0.9457		
CL	.	0.0002	0.0000	0.0001	0.6806		
CREAT	d	d	0.0005	d	0.5409		
CYP1A	.	.	.	.	0.0000	27.325	1.503
DBIL	.	d	0.0002	0.0010	d	F: 0.058 M: 0.061	F: 0.532 M: 0.014
EOS#	0.0032	.	0.0193	.	0.8989		
GGT	.	.	0.0000	.	0.0001	17.775	13.966
GLB	0.0007	0.0009	0.0006	.	0.1617		
GLU	.	0.0334	0.0055	.	0.1183		
HAPTOS	d	d	d	.	d	1996: 18.400 1997: 59.296	1996: 42.893 1997: 43.586
HB	0.0000	0.0000	0.0002	.	0.1353		
HCT	0.0000	0.0000	0.0000	.	0.7362		
HDL	d	d	0.0000	.	0.4131		
K	0.0014	.	0.0011	.	0.0021	4.095	4.229
LDH	.	.	.	0.0000	0.0001	334.763	461.560

Table 1 continued - P-values from ANOVA, sea otter blood data.

LDL	.	.	0.0000	0.0029	0.4414		
LYM#	.	0.0000	0.0236	.	0.4700		
MCH	0.0082	.	0.0073	.	0.5246		
MCHC	.	.	d	d	0.2854		
MCV	.	.	d	d	0.5753		
MON#	.	.	.	.	0.9155		
NA	.	0.0000	0.0000	0.0024	0.9048		
NEU#	.	0.0808	.	.	0.1302		
P	0.0000	.	0.0260	.	0.8610		
PLATES	0.0114	0.0215	.	.	0.0939		
RBC	0.0000	.	.	.	0.8918		
RDW	.	0.0001	0.0000	0.0000	0.5345		
TBIL	0.0169	d	0.0000	.	d	F: 0.298 M: 0.304	F: 0.278 M: 0.243
TPRT	.	0.0160	0.0000	.	0.2831		
TRIG	.	0.0000	.	.	0.5876		
URIC	.	0.0055	0.0000	.	0.0000	2.050	2.509
VLDL	.	0.0000	.	.	0.6955		
WBC	.	0.0000	0.0416	.	0.0656		

[a]Age in years.

[b]Two capture methods: tangle net or Wilson trap.

[c]Two areas: Knight Island (oiled) or Montague Island (unoiled).

[d]For covariates involved in an interaction, p-value for significance of covariate is not reported because it depends on the levels of the interacting variable.

[e]Abbreviations and Units: ALB - albumin, g/dl; ALT - alanine aminotransferase, IU/l; AP - alkaline phosphatase, IU/l; AST - aspartate aminotransferase, IU/l; BAS# - basophils, x 10^3/ul; BUN - blood urea nitrogen, mg/dl; CA - calcium, mg/dl; CHOL - cholesterol, mg/dl; CL - chloride, mEq/l; CREAT - creatinine, mg/dl; CYP1A, cytochrome P450 1A, molecules x 10^6 per 100 ng total RNA; DBIL - direct bilirubin, mg/dl; EOS# - eosinophils, x 10^3/ul; GGT - gamma glutamyl transferase, IU/l; GLB - globulin, g/dl; GLU - glucose, mg/dl; HAPTOS - haptoglobin, mg haptoglogin-hemoglobin complex/100 ml; HB - hemoglobin, g/dl; HCT - hematocrit, %; HDL - high density lipoproteins; K - potassium, mEq/l; LDH - lactate dehydrogenase, IU/l; LDL - low density lipoproteins; LYM# - lymphocytes, x 10^3/ul; MCH - mean corpuscular hemoglobin, pg; MCHC - mean corpuscular hemoglobin concentration, g/dl; MCV - mean corpuscular volume, fl; MON# - monocytes, x 10^3/ul; NA - sodium, mEq/l; NEU# - neutrophils, x 10^3/ul; P - phosphorus, mg/dl; PLATES - platelets, x 10^3/ul; RBC - red blood cells, x 10^6/ul; RDW - red cell width; TBIL - total bilirubin, mg/dl; TPRT - total protein, g/dl; TRIG - triglycerides, mg/dl; URIC - uric acid; VLDL - very low density lipoproteins; WBC - white blood cells, x 10^3/ul.

Table 2. Importance values for each covariate, sea otter blood data, 1996-98. Values are calculated as the sum of the AIC weight of each model containing the covariate, from a suite of 31 models run for each variable.

Variable[a]	Age	Sex	Year	Capture Type	Area
ALB	1.000	0.538	1.000	0.255	0.996
ALT	0.548	0.119	1.000	0.395	0.611
AP	1.000	0.221	0.476	0.172	0.238
AST	0.075	0.859	0.980	0.995	0.101
BAS#	0.665	0.077	0.896	0.892	0.718
BUN	1.000	0.046	0.243	0.399	0.780
CA	1.000	0.258	1.000	0.109	0.832
CHOL	0.154	0.990	0.653	0.342	0.096
CL	0.798	0.990	1.000	0.998	0.238
CREAT	0.969	1.000	1.000	0.997	0.992
CYP1A	0.098	0.093	0.299	0.147	1.000
DBIL	0.045	0.989	1.000	0.870	0.972
EOS#	0.963	0.663	0.975	0.137	0.102
GGT	0.166	0.186	1.000	0.354	0.993
GLB	0.987	0.912	0.999	0.099	0.501
GLU	0.086	0.890	0.990	0.420	0.692
HAPTOS	1.000	1.000	0.981	0.040	0.956
HB	1.000	0.996	0.989	0.012	0.072
HCT	1.000	0.998	1.000	0.208	0.015
HDL	0.562	0.606	1.000	0.056	0.350
K	0.816	0.019	0.999	0.561	0.994
LDH	0.466	0.041	0.865	0.998	0.998
LDL	0.119	0.366	1.000	0.950	0.461
LYM#	0.093	1.000	0.966	0.144	0.577
MCH	0.533	0.979	0.998	0.053	0.177
MCHC	0.195	0.087	1.000	1.000	0.826
MCV	0.080	0.976	1.000	0.988	0.059

Table 2 continued -- Importance values for covariates, sea otter blood data.

MON#	0.377	0.242	0.565	0.171	0.165
NA	0.074	0.999	1.000	0.906	0.714
NEU#	0.870	0.578	0.818	0.148	0.691
P	1.000	0.374	0.760	0.157	0.102
PLATES	0.617	0.740	0.881	0.060	0.608
RBC	1.000	0.559	0.551	0.159	0.040
RDW	0.079	0.997	1.000	1.000	0.020
TBIL	0.706	0.985	1.000	0.363	0.954
TPRT	0.242	0.638	1.000	0.356	0.809
TRIG	0.950	1.000	0.508	0.180	0.057
URIC	0.120	0.938	1.000	0.866	1.000
VLDL	0.972	1.000	0.494	0.200	0.054
WBC	0.059	1.000	0.868	0.267	0.545

[a]For abbreviations and units, refer to Table 1.

Table 3. Means (unadjusted) and standard errors of blood variables for sea otters from oiled and unoiled areas of WPWS, captured between 1996-98.

	Oiled			Unoiled		
Variable[a]	N	Mean	Std Error	N	Mean	Std Error
ALB	80	2.76	0.023	91	2.74	0.015
ALT	80	204.34	16.567	91	203.25	12.083
AP	80	132.76	5.824	91	135.85	8.112
AST	80	238.51	23.792	91	234.89	13.569
BAS#	63	39.24	7.989	52	47.46	10.403
BUN	79	45.43	1.108	88	49.81	1.142
CA	80	8.97	0.057	91	9.09	0.05
CHOL	80	139.49	2.773	91	139.16	2.012
CL	80	113.7	0.364	91	113.93	0.292
CREAT	80	0.46	0.014	90	0.44	0.012
CYP1A	71	27.32	5.131	86	1.5	0.231
DBIL	80	0.06	0.006	91	0.05	0.005
EOS#	63	1553.75	124.101	52	1627.85	113.917
GGT	80	17.78	1.226	89	13.97	0.871
GLB	80	3.78	0.055	91	3.83	0.052
GLU	80	141.89	3.766	91	139.96	4.258
HAPTOS	57	37.77	8.010	57	43.25	7.428
HB	63	19.63	0.121	52	19.56	0.131
HCT	63	58.05	0.393	52	57.82	0.575
HDL	79	89.46	2.478	91	86.27	2.711
K	80	4.1	0.035	91	4.23	0.032
LDH	80	334.76	15.125	91	461.56	23.304
LDL	76	39.68	3.274	89	40.67	2.688
LYM#	63	3349.37	177.585	52	4223.81	323.274
MCH	63	40.61	0.253	52	40.68	0.264
MCHC	63	33.85	0.171	52	33.94	0.291
MCV	63	120.16	0.943	52	120.15	1.076
MON#	63	371.9	23.789	52	373.94	29.763
NA	80	152.66	0.546	91	152.45	0.316
NEU#	63	3936.68	154.744	52	4379.17	191.948

Table 3 continued - Area means for sea otter blood variables.

P	80	4.47	0.132	91	4.49	0.149
PLATES	62	267	7.627	51	291.02	8.424
RBC	63	4.85	0.043	52	4.82	0.046
RDW	63	14.43	0.175	52	14.32	0.248
TBIL	80	0.3	0.007	89	0.27	0.008
TPRT	80	6.53	0.063	91	6.57	0.052
TRIG	80	57.91	1.917	91	66.25	2.77
URIC	80	2.05	0.068	88	2.51	0.079
VLDL	76	11.55	0.406	89	13.03	0.546
WBC	63	9.25	0.264	52	10.66	0.369

[a] For abbreviations and units, refer to Table 1.

Table 4. Comparison of mean values of selected sea otter blood variables from 1996-98 (NVP study; Knight vs. Montague Islands) with those from 1992 (western and eastern PWS) and 1991 (western PWS and SE AK)[a].

	Year								
	1996-98			1992-94			1991		
Variable[b]	Oiled (Knight) n = 63 H[c] n = 80 C	Unoiled (Montague) n = 52 H n = 91 C	P-value[d] Area	Oiled (WPWS) n = 34 H n = 35 C	Unoiled (EPWS) n = 49 H n = 53 C	P-value[d] Area	Oiled (WPWS) n = 9 H, C	Unoiled SE AK n = 8 H n = 26 C	P-value[d] Area
WBC	9.25	10.66	*0.066*	9.97	8.84	*0.013*	10.26	9.18	*0.397*
BUN	45.4	49.8	*0.013*	52.1	50.2	*0.397*	46.9	50.1	*0.508*
URIC	2.05	2.51	*0.000*	2.55	2.54	*0.938*	2.67	2.41	*0.294*
K	4.10	4.23	*0.002*	4.11	4.17	*0.411*	4.31	3.93	*0.020*
CA	8.97	9.09	e	8.85	8.73	*0.351*	8.76	8.62	*0.599*
TBIL	0.30	0.27	e	0.57	0.40	*0.080*	0.37	0.49	*0.065*
DBIL	0.06	0.05	e	0.017	0.006	*0.092*	0.011	0.008	*0.761*
GGT	17.78	13.97	*0.000*	29.11	15.68	*0.000*	16.89	14.62	*0.345*
LDH	335	461	*0.000*	483	528	*0.445*	419	374	*0.573*
AP	133	136	*0.990*	93	83	*0.086*	125	96	*0.215*
AST	239	235	*0.943*	344	302	*0.235*	264	203	*0.355*
ALT	204	203	*0.627*	322	236	*0.000*	238	181	*0.076*

[a]Rebar et al. 1995; USGS-BRD unpublished data.
[b]For abbreviations and units, refer to Table 1.
[c]H = Hematology variables (only WBC in this table); C = Chemistry variables.
[d]1996-98 - P-values from ANOVA on ranks; model selection process included covariates of age, sex, year and capture method (see Table 1). 1992 and 1991 - P-values from preliminary analysis -- t-test, two-tailed, on non-transformed data.
[e]Area involved in a significant interaction (see Table 1); because p-value depends on the levels of the interacting variable, it is not reported here.

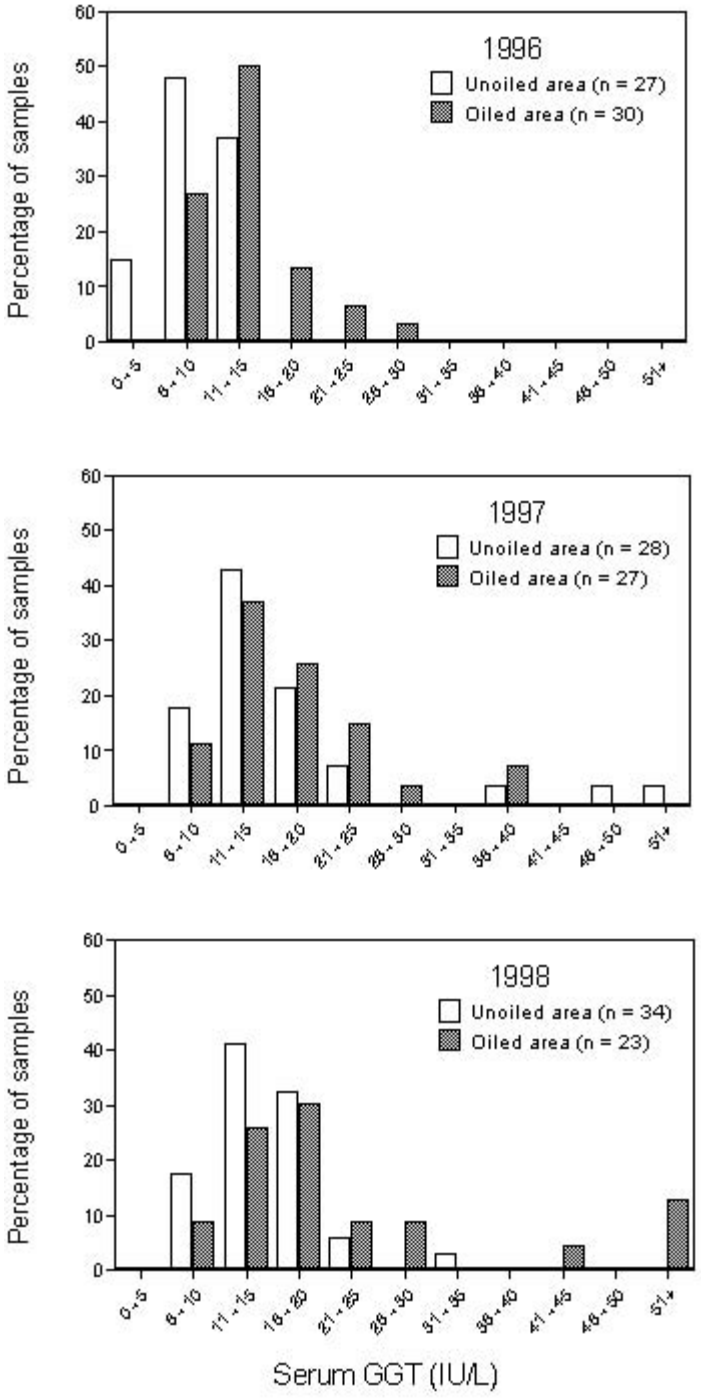

Figure 1. Distribution of serum GGT values in sea otters from oiled and unoiled areas by year.

APPENDIX BIO-02

CYP1A1 Gene Expression in Sea Otters (*Enhydra lutris*): A Quantitative Reverse Transcriptase-Polymerase Chain Reaction to Measure CYP1A mRNA in Peripheral Blood Mononuclear Cells[1]

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ABSTRACT

The nearshore ecosystem of the Prince William Sound serves as a repository for the oil spilled in 1989 from the *Exxon Valdez* tanker. Responses associated with exposure to residual oil may include transcriptional activation of mRNA for cytochrome P450 1A1 (CYP1A1). Here, we report on the isolation, cloning, and sequencing of a PCR product for sea otter (*Enhydra lutris*) CYP1A1. Additionally, we developed a quantitative RT-PCR technique to quantify the level of CYP1A1 expression in sea otter peripheral blood mononuclear cells (PBMC). Using this quantitative RT-PCR method with PBMC isolated from sea otters captured in oiled (n=20) and non-oiled (n=21) areas of the Prince William Sound, we detected a significant difference ($p < 0.001$) in the level of CYP1A1 expression between the two study areas. The mean level of expression in PBMC from sea otters (molecules/100 ng total RNA) was 1.96×10^6 for the oiled area and 0.12×10^6 for the non-oiled area. Analysis of the expression of CYP1A1 in peripheral blood mononuclear cells by RT-PCR represents a sensitive and non-lethal method for evaluating potential exposure to environmental contaminants.

INTRODUCTION

Extensive sections of shoreline in Prince William Sound, Alaska (USA), were contaminated by oil spilled from the tanker *Exxon Valdez* in 1989. Between 8 and 16% of the 10.8 million gallons of crude oil spilled by the T/V *Exxon Valdez* remains buried in marine sediments (1). Such oil is not subject to degradation by marine organisms and remains in a form that is toxic to many vertebrates (2). Moreover, microbial analyses suggest that oil in sediments along oiled shorelines is still present at higher concentrations than in unoiled sites. In 1995, studies were implemented on the effect of residual oil on nearshore vertebrate predators as a potential factor limiting their recovery from spill-related injury (3). One aspect of these studies involves the biochemical responses of individual animals to environmental contaminants.

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[1]In preparation for submission to Archives of Biochemistry and Biophysics

Members of the cytochrome P450 (CYP) family of oxidative metabolizing enzymes are important in the detoxification of environmental contaminants (4-7). Evaluations of the mRNA, protein or catalytic activity of these metabolizing enzymes have been proposed as biomarkers for exposure to a variety of contaminants. Although the CYP enzymes are involved in detoxification reactions, intermediate metabolites of these reactions are frequently more toxic than parent compounds (6,8). The central role of CYP in detoxification makes it a sensitive indicator of xenobiotic exposure compared to other biochemical parameters that are more indicative of stress and cellular damage (6,7). The substrates for CYP enzymes are widespread, ranging from physiologically occurring lipids such as steroids and prostaglandins to biologically and chemically synthesized xenobiotics. Many lipophilic organic xenobiotics such as polyaromatic hydrocarbons (PAHs) are metabolized by the CYP1 gene family (6-9). Specifically, CYPA1 is induced by PAHs (e.g., 3-methylcholanthrene) and coplanar chlorinated hydrocarbons (e.g., 2,3,7,8-tetrachlorodibenzodioxin [TCDD]). There are many reports that have attempted to correlate the level of environmental exposure to contaminants and CYP1A1 mRNA levels, enzyme activity, or CYP1A1 protein concentrations (10-14).

Basal and induced CYP1A1, at low levels, have been detected in a variety of immune tissues including cultured human lymphocytes (15), macrophages from several species (16), murine spleen (17), and human peripheral lymphocytes (18, 19). Transcriptional activation of mRNA for CYP1A1 is one of the most sensitive known responses associated with exposure to the above compounds. As part of an effort to develop biomarkers of contamination for monitoring programs and to determine whether the measurement of CYP1A1 gene induction can be useful in detecting oil exposure in sea otter populations, we have conducted a study of mRNA levels in peripheral blood mononuclear cells of sea otters.

Here we report on a quantitative reverse transcriptase polymerase chain reaction (RT-PCR) method for determining the copy number of cytochrome CYP1A1 mRNA in peripheral blood mononuclear cells of sea otters. By quantitative RT-PCR, we detected a 16-fold increase in CYP1A1 mRNA levels in peripheral blood mononuclear cells from otters in oiled areas as compared to non-oiled areas.

MATERIALS AND METHODS

Animals. Male and female sea otters were captured from oiled and non-oiled areas of western Prince William Sound. Liver was collected from sea otters that died or were euthanized in rehabilitation centers during the initial response of the oil spill. A total of 20 ml of heparinized (preservative free heparin, Sigma Chemical Co., St Louis, MO) blood was collected by venipuncture from each animal for the isolation of peripheral blood mononuclear cells. Liver samples were collected at death and maintained frozen at -70° C.

Materials. TRIzol Reagent was purchased from Life Technologies Inc., Grand Island, NY. Riboprobe® Combination System - T3/T7 RNA Polymerase, Human Genomic DNA, Phi X 174 DNA /Hae III Markers were purchased from Promega, Madison, WI. NuSieve® agarose and Metaphor® agarose were from FMC BioProducts, Rockland, ME. Molony murine leukemia

reverse transcriptase was from Gibco BRL Products, Grand Island, NY. Other reagents were of the highest grade available.

Peripheral blood mononuclear cell isolation. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood by density gradient centrifugation (20). Isolated PBMC were cryopreserved and aliquots were washed and pelleted.

Isolation and Analysis of RNA. Total RNA was isolated from liver using rapid guanidinium-phenol extraction method (21), originally adapted from Chomczynski and Sacchi (22). To examine the quality of RNA extracted, electrophoresis through formaldehyde containing gels was performed as described previously (23). Aliquots of 20 mg native RNA were maintained at -70˚C.

Preparation of Internal Standard. The use of an internal standard that contains target (i.e., sea otter CYP1A1) primer sequences negates tube-to-tube variability in PCR amplification and is essential to quantifying mRNA expression by RT-PCR. We generated recombinant RNA (rcRNA) internal standards as described by Vanden Heuvel et al. (24). Using this method, a rcRNA was generated that upon amplification with sea otter CYP1A1 primers results in a product (221 bp) that is easily resolved from target product (180 bp) following agarose gel electrophoresis.

Cloning CYP1A1 from sea otter. Since sea otter CYP1A1 had not been previously described, we chose primers based on a comparison of several known CYP1A1 cDNA sequences. The CYP1A1 cDNA sequences from sheep (25), human (26), mouse (27), guinea pig (28), hamster (29), and rat (30) were aligned using MegAlign (DNAstar). From this alignment, we chose the following primers, found in a highly conserved area within the sequences listed above:

1A1FP312, 5'-CCACAGAGCTTCTCCTGGC-3';
1A1RP581, 5'-GGGTTCTTCCCCACGGTC-3';

These primers were utilized to amplify sea otter CYP1A1 from heavily oiled animals (see Figure 1). The primers were optimized for Mg, annealing temperature, pH, and number of cycles. The optimized conditions were: 4mM Mg, 54° C annealing temperature, pH 8.8, and 30 cycles.

The PCR products were cloned into T7Blue T-vector according to the manufacture's instructions (Novagen, Madison, WI). Following isolation of plasmid DNA, fluorescence dideoxynucleotide sequencing was performed at the Purdue University DNA Facility. The sequence information was used to obtain more efficient primers specific for sea otter cDNA using PrimerSelect (DNAStar, Madison, WI) as shown below.

Sea otter 1A1FP, 5'-TGGTCAATTTTCTGTTCCTAG-3';
Sea otter 1A1RP, 5'-AGGTCAGCTCAACCTTGAGA-3';

Quantitative Competitive RT-PCR. Competitive PCR was performed essentially as described by Gilliland et al. (31, 32) as modified by Vanden Heuvel et al. (33). For each sample, 8-10 aliquots of total RNA (0.1 mg) were prepared, and a dilution series of the rcRNA internal

standard was spiked into these aliquots. Reverse transcription of RNA was performed in a final volume of 20 ml containing 25 mM Tris-HCl (pH 8.3 at 25°C), 50 mM $(NH_4)_2SO_4$, b-mercaptoethanol, 0.1 mg/ml bovine serum albumin, 5 mM $MgCl_2$, 1 mM of each deoxynucleotide triphosphate, 1 unit RNase inhibitor, 2.5 units M-MLV Reverse Transcriptase (Life Technologies, Inc.), 2.5 mM oligo$(dT)_{16}$, 0.1 mg total RNA, and varying amounts of rcRNA internal standard. The samples were incubated at 45°C for 15 min., and reverse transcriptase was inactivated by heating to 99°C for 5 min. The PCR reaction mixture contained 3 mM $MgCl_2$, 2.5 units Taq polymerase, and 6 pmol of forward and reverse primers. The reactions were heated to 94°C for 3 min. and cycled 30 times through 30-s denaturing step at 94°C, a 30-s annealing step at 54°C, and a 30-s elongation step at 72°C. Following the final cycle, a 5-min. elongation step at 72°C was included.

Aliquots of the PCR reaction were electrophoresed on 2.5% NuSieve® 3:1 agarose (FMC Bio Products, Rockland, ME) gels, and PCR fragments were visualized with ethidium bromide staining. A photographic negative was prepared and densitometry was performed using a LKB Gel Scan II laser densitometer (LKB, Piscataway, NJ). Quantification of the amount of target mRNA present was determined as described by Gilliland et al. (31). Initially, a large internal standard concentration range (i.e., 100-1000 molecules/tube) was examined in order to estimate the concentration of target mRNA in each sample. Once the concentration of the CYP1A1 was determined, a more narrow range of internal standards was used to more precisely determine the levels of CYP1A1 mRNA. The actual number of molecules of CYP1A1 was determined by comparing the ratio of the volume of the internal standard to CYP1A1 mRNA. The ratio of the volume of the internal standard/CYP1A mRNA PCR products were plotted against the amount of internal standard added to individual tubes as previously described (31). Linear regression analysis was used to define the equation for the line through the data points. The amount of CYP1A1 mRNA present for individual animals was defined as the amount of rcRNA present where the volume ratio was equal to 1.

Statistical analysis. We used one-way analysis of variance with Student's test (Statview II, Abacus Concepts, Inc., Berkeley, CA) to compare the differences between the means of two groups.

RESULTS

Cloning of CYP1A1 from sea otter liver.

Since sea otter CYP1A1 had not been previously described, we initially designed primers based on a comparison of several known CYP1A1 cDNA sequences. For this purpose, cDNA was synthesized from RNA fractions purified from liver of heavily oiled sea otters. The CYP1A1 sequence was amplified with PCR forward and reverse primer sets that were optimized for Mg concentration, annealing temperature, and number of cycles. Figure 1 illustrates the ethidium bromide staining of an agarose gel, containing a 310 bp PCR product obtained with sea otter liver RNA samples. The negative control used throughout these studies consisted of PCR reactions performed with RNA samples to which no reverse transcriptase was added. The

absence of detectable signal in these controls provided evidence that the PCR products obtained in the sample lanes were the direct result of cDNA amplification.

We then utilized the pT7Blue T-Vector system for the cloning of the CYP1A1 PCR product isolated from sea otter liver. For T-vector ligation, we used small sample (e.g., 1 ml) of PCR reaction mixture amplified product. The efficiency of ligation was 72-80%. The presence of the appropriate insert was determined using direct colony PCR and colonies with CYP1A1 inserts were used for plasmid DNA isolation and sequencing. The cloned PCR product was purified using Wizard® PCR Preps DNA purification system. After purification, the PCR product showed a clear, distinct band in agarose gel. Forward and reverse primers, specific to sea otter CYP1A1 (see sequences in Materials and Methods), were developed for quantitative RT-PCR.

RT-PCR quantitation of sea otter CYP1A1 mRNA

Sea otter CYP1A1-specific PCR primers sets described above and peripheral blood mononuclear cells isolated from sea otters residing in oiled and non-oiled areas of Prince William Sound were used to develop a quantitative RT-PCR for CYP1A1 mRNA.

To achieve minimum variability in template amplification, we included an internal standard in a competitive RT-PCR reaction utilizing rcRNA templates according to Vanden Heuvel et al. (24). The internal standard was designed such that the PCR product from the mRNA (180 bp) could be easily separated from the rcRNA internal standard (221 bp). Figure 2 represents a typical relation between internal standard and CYP1A1 in competitive RT-PCR. After a series of experiments using a broad range of internal standards concentrations, we then narrowed the dilution of rcRNA internal standard (1 - 5000 molecules/tube) for more precise estimation of CYP1A1 gene expression in sea otter PBMC. Figure 3 is a representative standard curve used to determine the actual molecules of CYP1A1 in a sample. The level of expression of CYP1A1 was determined in PBMC of 21 sea otters from a non-oiled area and 20 otters from an oiled area. These data are presented in Figure 4. In the PBMC from otters in the non-oiled area, the average level of CYP1A1 mRNA was 0.12×10^6 molecules/100 ng total RNA. The average level of CYP1A1 mRNA from the otters in the oiled area was 1.96×10^6 molecules/100 ng total RNA.

DISCUSSION

Detecting and evaluating biological responses that result from environmental contamination are essential steps to determining the significance and duration of the contamination and could help in identifying potential mechanisms or toxicities involved. Biological responses resulting from exposure to contaminants can also be used as biomonitoring tools. The biological response to chemical contaminants are evaluated from the biochemical reaction of an individual animal to complex population and ecosystem interactions (6). Alterations in biochemical systems (e.g., gene expression) are typically more sensitive indicators than those at higher levels of biological organization (e.g., population densities). In this study, we evaluated the biological response of individual animals in an attempt to determine their level of exposure to residual oil.

The CYP1A1 is one of the most studied isozymes in the cytochrome P450 superfamily in regard to its role in metabolizing environmental toxicants and its use as a biomarker of exposure to a variety of environmental toxicants. Nothing is known about the CYP1 gene family in sea otters. This is the first report that the CYP1A gene is present and its respective mRNA is expressed in sea otter liver and PBMC. The use of RT-PCR has several advantages over the more conventional RNA detection procedures. First, this procedure is at least an order of magnitude more sensitive than radioimmunoassay and at least two orders of magnitude more sensitive than Northern or slot blotting for measuring induction of CYP1A1 (33). Second, the evaluation of a circulating population of cells, that can easily be obtained by venapuncture, represents a non-lethal, minimally invasive sampling technique for evaluation.

The CYP1A1 is a key participant in the metabolism of a number of xenobiotics. The reactive metabolites, if not conjugated or detoxified, can cause cell damage including immunotoxicity. The metabolites of CYP1A1 are poor substrates for conjugation and detoxifying enzymes (34). The toxicological significance of the CYP1A1 detected in lymphocytes in this study depends on whether the levels detected are sufficient for xenobiotic activation in immune tissues. The reactive metabolites so formed can induce damage within lymphocytes containing the CYP1A1. Under conditions such as an oil spill, in which unmetabolized toxins may escape the liver, CYP1A1 expression in lymphocytes could be critical to xenobiotic induction of immunosuppression. However, the susceptibility of these immune tissues to toxicant damage is likely to depend on both their activation and detoxification capabilities (35).

Analysis of the expression of P450s in circulating lymphocytes as a marker for exposure to environmental toxicants represents new approach to assess environmental stress. The molecular mechanism of the constitutive expression of CYP1A1 has to be elucidated for a better understanding of the metabolic characteristics of the sea otter. Further cDNA cloning and expression studies of sea otter P450s are required for the clarification of molecular biological properties in this species.

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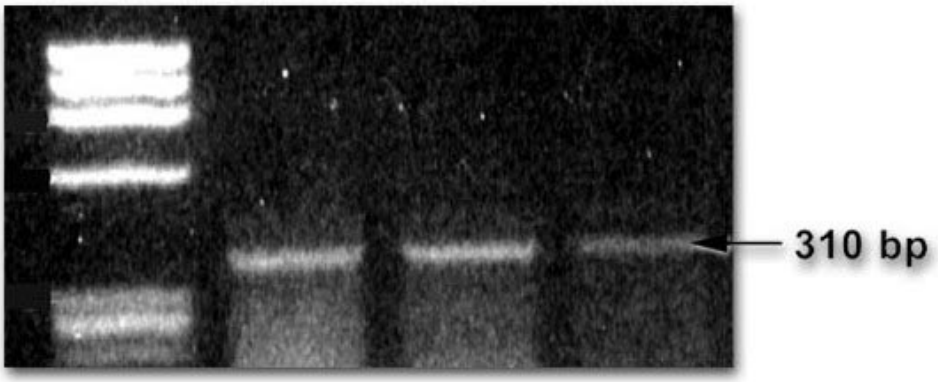

Figure 1. Sea otter (*Enhydra lutris*) CYP1A1 PCR product. Ethidium bromide-stained agarose gel containing PCR products resulting from amplification of sea otter liver CYP1A1 cDNA. Lane 1- molecular weight markers.

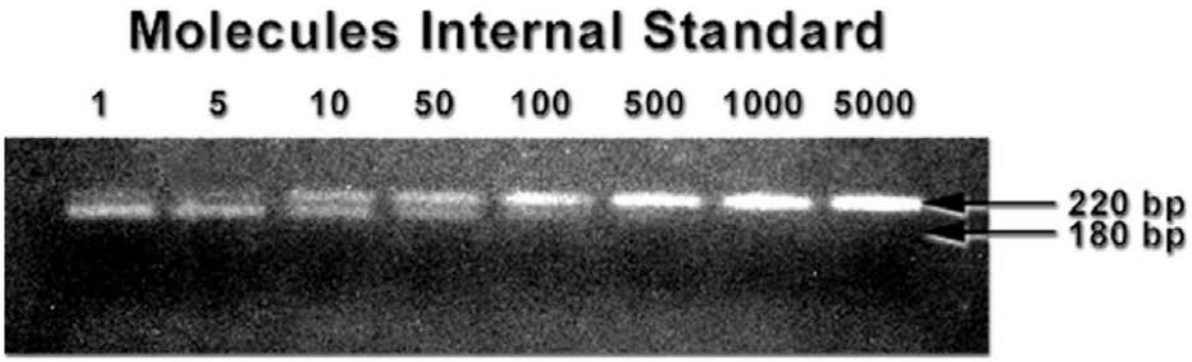

Figure 2. Ethidium bromide stained agarose gel showing quantitation of CYP1A1 mRNA expression in sea otter (*Enhydra lutris*) peripheral blood mononuclear cells. Dilution of rcRNA internal standard (1-5000 molecules/tube) were added to a constant quantity of peripheral blood mononuclear cell RNA. The PCR conditions are described in Materials and Methods.

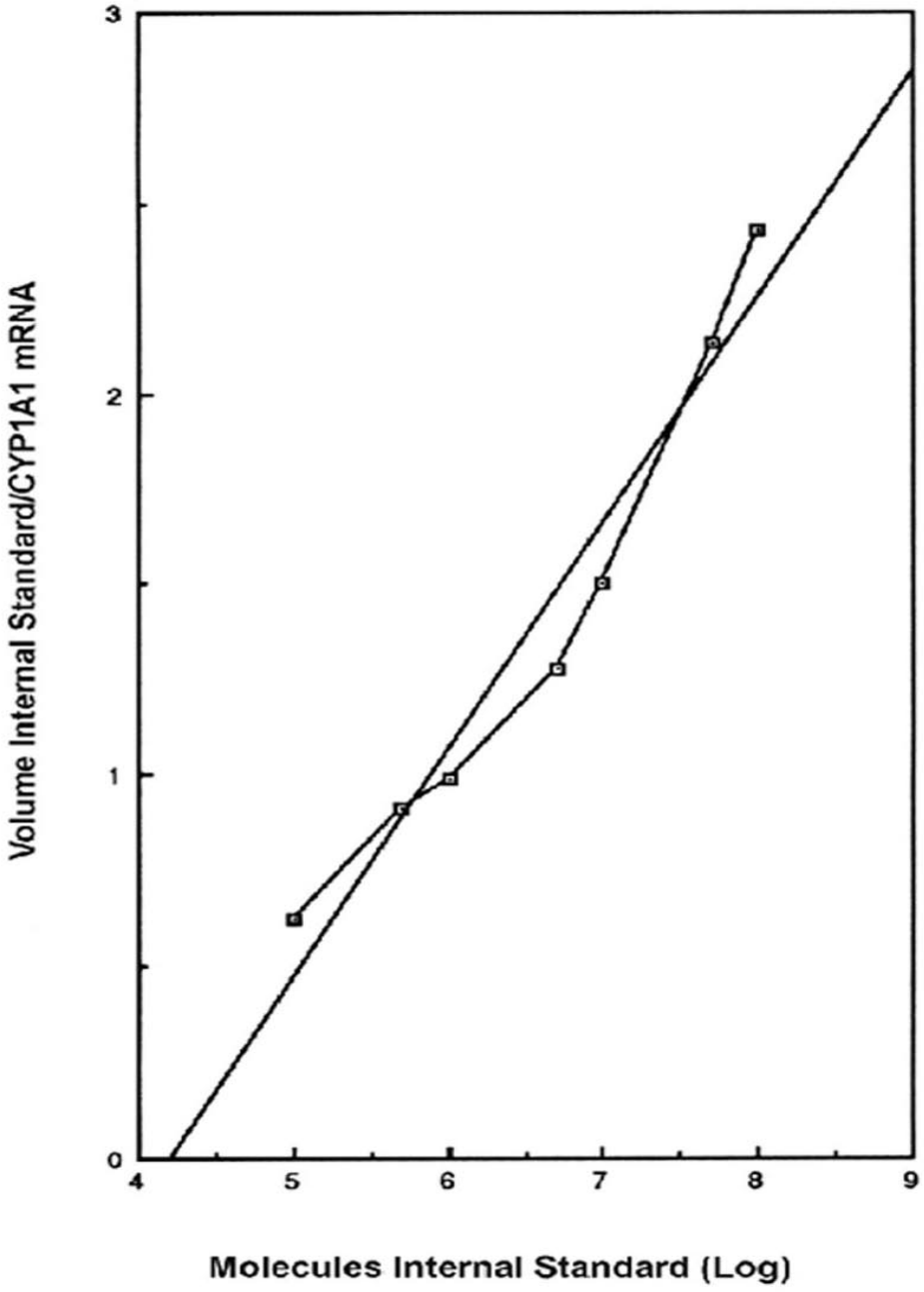

Figure 3. Quantitation of CYP1A1 mRNA expression in sea otter lymphocytes. Dilutions of rcRNA internal standard were added to a constant amount of lymphocyte RNA. The molecules of mRNA are estimated by determining where the volume of the rcRNA spot equals that of the target mRNA. The PCR conditions are described in Materials and Methods.

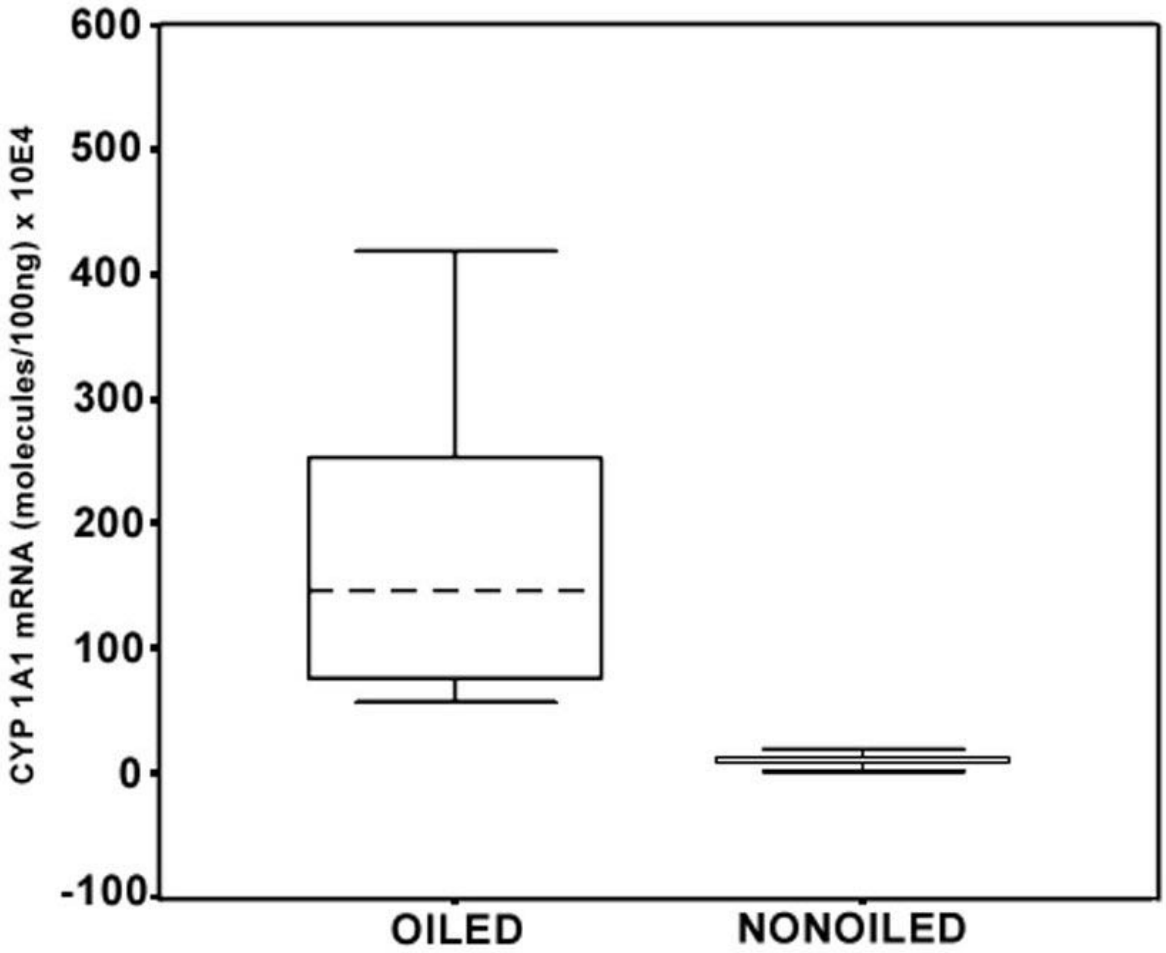

Figure 4. Box plot of CYP1A1 mRNA levels in peripheral blood mononuclear cells, isolated from sea otters (*Enhydra lutris*) in oiled and non-oiled areas, using competitive RT- PCR. The dotted line in each box represents the median, the box encompasses the 5th through 95th confidence limits and the vertical bar indicates the range of measurements.

APPENDIX BIO-03

LONG-TERM IMPACTS OF THE *EXXON VALDEZ* OIL SPILL ON SEA OTTERS, ASSESSED THROUGH AGE-DEPENDENT MORTALITY PATTERNS[1]

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ABSTRACT

We use age distributions of sea otters (*Enhydra lutris*) found dead on the beaches of western Prince William Sound, Alaska, between 1976 and 1998 in conjunction with time-varying demographic models to test for lingering effects from the 1989 *Exxon Valdez* oil spill. Our results show that sea otters in this area had decreased survival rates in the years following the spill and that the effects of the spill on annual survival increased rather than dissipated for older animals. Otters born after the 1989 spill were not as strongly affected as were those alive in March 1989, but do show continuing negative effects though 1998. Population-wide effects of the spill appear to have slowly dissipated through time, due largely to the loss of cohorts alive during the spill. Our results demonstrate that the difficult-to-detect long-term impacts of environmental disasters may still be highly significant and can be rigorously analyzed using a combination of population data, modeling techniques, and statistical analyses.

INTRODUCTION

On 24 March, 1989, the tanker vessel *Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska, spilling an estimated 42 million L of Prudhoe Bay crude oil. Sea otters, a species highly susceptible to oil-related mortality (1-5), occupied the coastal waters affected by the spill. By September 1989, nearly 1,000 dead otters had been recovered in the spill area (6), and total mortality due to the spill was undoubtedly higher (7-9). While acute, short-term effects of the *Exxon Valdez* oil spill (EVOS) on sea otters are indisputable, longer-term effects on this or other species are much more difficult to document. Here, we use a combination of field data, demographic modeling, and maximum likelihood analysis to show that the sea otters of western Prince William Sound (WPWS) have incurred continuing, highly significant effects from the EVOS. Our goal is both to evaluate impacts on this particular population and to illustrate a method that can be adapted to improve assessment of many environmental impacts on populations of long-lived species.

Several lines of evidence suggest that sea otters might have faced oil-related effects long after the spill. Acute pathologies associated with oil exposure in sea otters included lung, liver and kidney damage (10, 11). Sea otters placed in aquaria after the spill had relatively poor survival rates, and at necropsy showed similar patterns of organ damage (T. Williams, pers. com.). These pathologies also resulted in abnormal hematological and serum chemistry values (12). Elevated serum enzymes associated with liver damage were documented in wild sea otters from 1989 to 1992, and again, although to a much lesser extent, in 1996-1998 (unpub. data). From 1996 to 1998 wild otters in oiled areas also had significantly higher induction of cytochrome P4501A (CYP1A), a bioindicator of exposure to aromatic hydrocarbons, than did otters from unoiled areas (13). Thus, individuals surviving initial exposure to oil but remaining in the wild are likely to have experienced initially sublethal pathologies similar to those seen in animals dying immediate after the spill.

Continued exposure to oil persisting in the environment, rather than lingering effects of acute exposure, may also account for some persistent spill effects. Following the spill, an estimated 40% of the oil (16 million L) beached in WPWS (13); by 1992 an estimated 2% of the original oil remained on beaches (14), and oil was still present in sediments on some beaches in 1997 (15). Although most remaining oil residues were deemed non-toxic by the summer of 1991 (16, 17) where oil is protected from weathering toxic components persist and may be mobilized following high energy storms (15, 18). Thus, oiled shorelines provided a reservoir for continued contamination of adjacent intertidal areas and nearshore waters.

While these facts suggest the possibility of lingering spill effects, evaluating this possibility have proven difficult and costly. At the individual level, “clinically ill” individuals are not likely to survive to be sampled, fresh carcasses for post-mortem examination are rarely found, and small sample sizes and high variability in data from live captures results in low statistical power. At the population level, pre-spill survey data from WPWS are available for comparison with post-spill numbers. However, these data are not ideally suited to a straightforward analysis of spill effects, and have proved inconclusive (7-9, 19, 20). Age-at-death data and estimated demographic rates are also available, but again variable sampling efforts and small sample sizes limit the power of simple statistical evaluations.

To overcome these problems, we use time-varying population models in combination with maximum likelihood methods to evaluate alternative hypotheses about changing

demographic rates for otters following the EVOS. We use a simple demographic model with time-varying, age-specific survival rates to predict the observed age distributions of dead otters seen each year following the spill. By modifying survival rates in the model away from pre-spill values and evaluating the fit of different modifications, we can identify the most likely ways in which the spill may have influenced the demography of the population (21).

METHODS

Study Area and Data Collection

Our primary data are the ages of sea otters found dead in WPWS both prior to and following the 1989 EVOS. From 1976 to 1985, the U.S. Fish and Wildlife Service collected sea otter carcasses each spring from Green Island, with an additional collection in 1979 from north-west Montague Island. From April through September 1989, spill response crews collected carcasses from beaches throughout oiled portions of WPWS. In addition, an unknown number of carcasses were recovered offshore within the oil-slick during early spill response efforts (9). Spring beach surveys at Green Island were resumed in 1990 and continued through 1998. In addition, in 1990 and 1991 crews monitoring spill cleanup efforts collected carcasses opportunistically on Naked, Eleanor, Ingot, Knight, Evans, Latouche, Elrington, and Perry islands and numerous smaller islands in the spill area. Monitoring of beaches in 1992 to 1995 was greatly reduced, and few carcasses were recovered other than during spring surveys at Green Island. In 1996 and 1997 opportunistic collections in the northern Knight Island area increased with implementation of a new research project in this area. Spring surveys of beaches in the larger area of oiled WPWS were conducted in 1998 by the U.S. Geological Survey, concurrent with and using similar methods as used at Green Island.

Systematic beach surveys were conducted in April or May soon after snow melt, prior to the regrowth of beach grasses which can conceal carcass remains. Beaches were walked by one or two observers, searching the strand line (the area of debris deposition from the previous winter's storms) and the upper intertidal zone. Observers recorded location, sex (if identifiable), and an age estimate (juvenile or adult) based on tooth wear and closure of skull sutures. Because many carcasses cannot be reliably sexed, we lump all data regardless of sex. The skull was collected when present, and a tooth (preferentially a pre-molar) removed for age analysis. For age estimation, several longitudinal sections of the tooth were decalcified for cementum annuli readings (22). Matson’s Laboratory (Box 308, Milltown, MT 59851) sectioned and aged all teeth.

Sea otters collected in 1989 were judged to be either pre- or post-spill deaths, based on the carcass condition at the time of recovery relative to time since the spill (23). From 1990-1992, we used only carcasses deposited during the previous winter or that spring (i.e., carcass remains had soft tissue and were located on top of previous years layer of vegetation or in intertidal zone) to avoid including pre-spill and spill-year mortalities. After 1992 all recovered carcasses were included.

Data Analysis and Modeling

We first compared the age distributions of otters collected over different time intervals and in different areas. We used two pre-spill time periods (1976-1985 and 1989-pre-spill) and three post-spill periods (1989-post-spill, 1990-1991, and 1992-1998), and two areas: Green Island (the site of systematic pre- and post-spill collections) and the rest of WPWS. To compare age distributions, we used Kolmogorov-Smirnov (K-S) two-sample tests (24). We excluded 0-yr-olds from all analyses since carcasses of the youngest animals are relatively unlikely to persist on beaches (25).

Next we constructed demographic models with survival rates varying from pre-spill estimates ("baseline rates") across both ages and years. We did not alter fecundities, as independent evidence indicates no change in otter reproductive values following the spill (26, 27). Each model was run for nine years, corresponding to the 1990-1998 post-spill years. For each simulation, we compared the predicted age distributions of otters dying in each year with those actually seen in the field, and used maximum likelihood methods to determine the most likely patterns of change. This technique provides a clear way to infer changes in demography from age-at-death data by obviating the need to make assumptions such as constant vital rates or stable age distributions (28,29).

We used a deterministic, two-sex, age-structured matrix model to simulate populations and ran the model with a large number of parameter estimates and model forms to test the robustness of our results. We initialized this model using one of three sets of baseline age- and sex-specific survival estimates from smoothed maximum likelihood analyses of ages-at-death for carcasses collected before and/or immediately after the spill (following methods in 29,30) and one set of fecundity estimates from 1989 carcass data (30,31). We started each simulation with one of two assumptions regarding the age and sex distribution of animals immediately following the spill: either the stable age distribution corresponding to the baseline demographic rates, or the distribution indicated by the presumably age and sex independent mortality patterns generated by the acute effects of the spill (29,30).

To simulate changing survival rates, we created three families of models with differing functions to modify survival rates across ages and years. These three functions are all similar, but span a range of possible forms of variable spill effects across years and ages. We first created models in which the survival rate for each age *i* and sex (male or female) in each year *j* was estimated as the baseline rate for that sex and age multiplied by a Logit function: Modeled $\text{survival}_{i,j}$ = (baseline survival_i) ($\text{Logit}_{i,j}$) where $\text{Logit}_{i,j} = (\exp(fn_{i,j})/(1+\exp(fn_{i,j}))$ and $fn_{i,j}$ = a + b*(i years since spill) + d*(age j) + e*(i years since spill)*(age j).We did not include sex as a factor in altering survivals, assuming that the survival rates of all individuals of a given age were similarly effected.

The Logit function allows quite complicated age and time-specific alterations in survival rates away from base-line estimates. However, it does not allow for survival rates *higher* than those estimated from before the oil spill, as might be predicted due to a release from density-dependent constraints (32). Therefore, we also used two other functions in our models. The first is a Modified Logit function, with each age, sex, and year-specific demographic rate equal to:

$$\text{Modeled survival}_{i,j} = (\text{Logit}_{i,j})^{(\ln(\text{baseline rate})/\ln(½))} \qquad [1]$$

Where $Logit_{i,j}$ is defined as above. This relationship allows each modeled survival rate to vary between {0,1}, both higher and lower than the baseline rate, with the modeled rate equaling the baseline when $Logit_{i,j} = 0.5$. We also used a Linear function to modify survival rates, using the $fn_{i,j}$ function described above:

$$\text{Modeled survival} = \begin{cases} (\text{baseline rate})(fn_{i,j}) & \text{if } 0 \leq fn_{i,j} \leq 1 \\ 0 & \text{if } 0 > fn_{i,j} \\ 1 & \text{if } fn_{i,j} > 1 \end{cases} \quad [2]$$

For each set of model parameter, functions, and initial conditions we found the best fit values and the confidence limits for the four parameters in fn_{ij} using each of six age-at-death data sets: otters collected prior to the spill or otters dying after 1989 and from either: Green Island (the site of the most consistent carcass collections), the rest of WPWS, or all areas. The models predict the relative number of otters dying that were of each age for each of either the sixteen pre-spill years or the nine post-spill years. For each year, this distribution was used as an expectation, and the likelihood of the observed age-distribution of carcasses, given this expectation, was calculated using multinomial probabilities (21, 29). The negative log-likelihoods from each year were then summed to yield a final estimate for each model run (a particular functional form and set of parameter values; 33). Model runs that predicted zero probability of seeing a dead otter of a given age in a year when one was in fact found were rejected outright. In calculating likelihoods, we only considered data on age 1 and older otters. Negative log-likelihoods provide the means to compare models with different parameters and functional forms (using Akaike's Information Criterion, AIC: 32) and to identify the best-fit parameter values and confidence limits on these parameters (using likelihood profiles: 33). For all comparisons, we used relative –log-likelihood values (–log-likelihoods minus constant terms); because our models did not differ in number of free parameters, differences between negative log-likelihood values are equivalent to differences in AICs (with smaller AIC values reflecting greater support for a model). To find best-fit values and confidence limits, we used downhill simplex and parabolic interpolation methods (34).

After identifying the best model forms and most likely parameter values, it is also important to ask if these models generate accurate predictions of the observed carcass age distributions. To determine the goodness of fit between the predicted and observed age distributions, we conducted one-sample K-S tests for each year of age-at-death data from 1990 to 1998 for both the linear and logistic models.

RESULTS

Age Distributions

Because Green Island shores were mostly unoiled or lightly oiled with only localized heavy oiling, we first asked if there is evidence that the demography of otters from Green Island differs from that of the rest WPWS and thus must be considered separately. For none of the time periods did age distributions differ between the two areas (K-S, $p > 0.05$ for all time periods). While we still perform some analyses for the Green Island and WPWS areas separately, these

results give no reason to suspect differences in the two areas in otter demography prior to or following the EVOS.

Next, we asked if age-at-death distributions differed across the five time periods, combining data from both Green Island and WPWS collections. Patterns in these data suggest substantial differences in demography prior to and after the spill. While the 1976-1985 and 1989 pre-spill distributions did not differ from one another (K-S, $p > 0.05$), both were significantly different from the age distributions of direct spill mortalities (post-spill 1989 carcasses) and also from the 1990-1991 distributions (Fig. 1). The 1992-1998 age distribution did not differ significantly from the pre-spill or 1990-1991 distributions, but it was different from the distribution of direct spill deaths. In general, these changes in age distributions suggest a shift in mortality patterns following the spill, with a gradual return towards the pre-spill pattern.

Modeling of Survival Changes

We first checked the reasonableness of our approach by fitting models to pre-spill carcass data. For the best fit models of all three functional forms, the confidence values for the two parameters controlling time effects on survival (b and e) bracketed zero, indicating a lack of temporal changes in survival rates in the pre-spill years (Table 1). Since no shifts in pre-spill demography are likely, this result confirms that our approach is unlikely to give spurious predictions of change. The 95 % confidence limits of the other two parameters (a and d) either encompass zero, only very small values, or are very broad, also supporting the lack of strong differences between the basic age-specific demographic rates and assumptions used in our analyses and those operating prior to the 1989 spill.

Next, we performed a total of 54 model fits, using different combinations of model assumptions and post-spill age distributions. In general the lowest negative log-likelihood (and hence AIC) values resulted from models using an initial stable age distribution, our first set of baseline demographic rates and the logistic or linear functional form. However, the striking result of all these analyses is the consistency of the effects across data-sets and model assumptions. The best-fit parameter values for each functional form predict a consistent, though complex, pattern of demographic change in the nine years following the EVOS (Table 1), regardless of carcass data (Green Island versus the rest of WPWS), initial age distribution, baseline demographic estimates, or functional form. Thus, we report detailed results only from the best-fit model in each family, estimating parameters using the combined WPWS and Green Island data-sets. While the best-fit linear and logistic models are almost equally supported by the data, the modified logistic is substantially less likely (Table 1). Using Akaike weights (35) for the best fit models of each form, the relative likelihoods are 0.40, 0.06 and 0.54 for the logistic, modified logistic and linear model forms, respectively.

The easiest way to convey the influence of the oil spill on predicted otter survivorships is as a proportion of the pre-spill survival rates for a given age in each year following the spill: values greater than one indicate higher survival following the spill, and values lower than one the converse (Fig. 2). Immediately after the spill, young animals are predicted to have suffered the greatest decrease in survivorship, but these effects dissipated rapidly with time (Fig. 2). In contrast, survival of older adults (≥10 yrs old) is initially only slightly reduced, but this effect increased with time, with poorer and poorer performance each year after the spill for a given age group. The best fit models predict that survival of prime reproductive age otters (e.g., age 5) was

reduced by as much as 50% initially and then slowly increased to values near or above pre-spill levels by 1998 (Fig. 2). The predicted effects on the oldest animals (≥15 yrs old) are likely to be somewhat inaccurate due to the small number of older carcasses found to help fit this part of the distribution. To better infer the genesis of these patterns, it is instructive to consider how an otter of a given age at the time of the spill was influenced each year as it aged (Fig. 3). These results suggest that young animals at the time of the spill (e.g. ages 0 and 1) experienced substantially higher mortality rates in the first several years following the spill, but that annual survival improved (relative to pre-spill rates) as they aged. In contrast, animals in their prime reproductive years and older (e.g. ≥5 yrs old) in 1989 have suffered strongly increasing mortality effects as time has passed. Only as these cohorts are lost from the population have demographic rates returned to normal.

While these predicted patterns of change are robust to the range of analyses explored so far, we also ran three additional analyses to gauge their strength and accuracy. First, we added environmental variability in first year survivorship, the demographic rate most likely to show substantial random variability (34; estimated from tagged otters in WPWS in 1990-1991: 36,37), and fit these stochastic simulations to post-spill carcass data (21). The best fit parameter values of these stochastic models are essentially identical to the deterministic results and showed similar confidence limits. Second, to ask if spill effects on otters born after 1989 were likely, we ran models that only modified survivorships of animals that lived through the spill. These altered models resulted in substantially worse fits for all three model functions (increases in AIC = 9.81, 15.32, 10.67 for the best fit logistic, modified logistic, and linear models respectively), directly supporting the conclusion that otters born after 1989 also have experienced spill effects. Third, we modified the basic function controlling alterations of survivorships to include quadratic terms and interactions and fit a suite of these more complicated nested models. Likelihood ratio tests suggested no justification for these more complicated models, and none yielded predictions qualitatively different from those of our simpler models. All these results confirm the robustness of our basic results.

Finally we asked if the predictions of our models accurately reflect our observed age distributions. For the Linear model (the single best model) we find no significant departure in observed carcass age distributions from those predicted until the last two years (K-S one sample tests): in these years, a surplus of older otters result in a significant deviation from the age distributions predicted by either model. For the Logistic model, three years, including the last year, show significantly different distributions; again, a surplus of older otters explained this mismatch in 1998. Overall, these results suggest that the best-fit models do a good job of accurately predicting otter age-at-death distributions, but that the model predications are worst at the end of the data collection period; as we discuss below census data of live otters suggest an explanation for this pattern.

DISCUSSION

Our results lend strong support to the hypothesis that the EVOS has had continuing impacts on the sea otter population of Prince William Sound. In particular, we found no evidence of improved performance for any age-class immediately after the 1989 spill due to a release from density-driven competition (a reasonable scenario if no lingering effects persisted). Rather otters of all ages have shown elevated mortality rates in the nine years following the spill.

These long-term effects are strongest on otters that were four to five years or older during 1989, but the modeling results also suggest that at least through 1996, animals born after the spill were also impacted by the events of 1989. Thus, while lingering effects of acute oil exposure may account for much of the longer-term spill effects, less direct impacts are also likely to have occurred, either due to maternal influences or to continued exposure to oil residues.

While the immediate loss of otters in the aftermath of the spill resulted in a decline in the local population, our results suggest that even more important long-term demographic changes have limited recovery after 1989. In our analyses, we use one population-level effect (age distributions of dead otters) as a tool to infer individual demography. However, the resulting demographic inferences can then be used to predict changes in another population attribute, total numbers. The two best-fit models suggest continuing decline of otters through 1998, while the modified logistic predicts no growth until the mid-nineties, when populations are predicted to have slowly risen (Figure 4).

Direct post-spill boat surveys indicated continued declines in sea otter numbers the first year following the spill, and no subsequent increase in population size in the spill area through at least 1991 (38). In addition, low weanling survival rates were observed in WPWS after the spill (6). Although these findings are consistent with predictions of our models, early boat surveys were not sensitive to small changes in abundance and the lack of pre-spill recruitment data limit inferences from post-spill recruitment patterns. We began more accurate aerial surveys in 1993, and found significant growth in the WPWS sea otter population, particularly since 1995 (27). At first glance, then, from the mid- to late- 1990s, censuses of the live population appear contradictory to the predictions of our two best models (although they match predictions of the Modified Logistic extremely well). However, the models rely on carcass data collected only in oil-effected portions of WPWS, including some of the habitat in WPWS currently supporting the lowest otter densities. In contrast, aerial surveys include large areas of relatively high density, unoiled sea otter habitat. In fact, much of the observed population growth over the past five years has occurred in these unoiled areas, where sea otter densities can be as much as 10 times greater than in the most heavily oiled areas. These differences, combined with our demographic results, which show poor demographic performance but more carcasses of older animals than expected, suggest that oil affected areas may continue to represent a population "sink" that benefits from immigration from healthy segments of the greater WPWS sea otter population.

Several other lines of evidence are consistent with the conclusion that mortality patterns have shown significant long-term effects of the spill and that otter movements account for much of the apparent recovery of oiled areas. Sea otter numbers in the most heavily oiled areas of northern Knight Island have shown no sign of recovery through 1999 (32,37). Lower tagged otter retention rates in this area, compared with those in a unoiled area of Montague Island, suggest sea otters at Knight Island are experienced higher mortality and/or emigration rates even though food resources and body condition of animals there should support some population growth (27,39, but see also 40). Sea otters living in the oiled area have consistently expressed higher levels of CYP1A than those captured in unoiled areas, indicating continued exposure to petroleum hydrocarbons at least through 1998 (13). Similar serological and demographic patterns for harlequin ducks (41, 42), another nearshore predator of benthic invertebrates, also support continuing spill-related effects in oiled areas of WPWS. These similarities suggest that additional species may have suffered analogous consequences to the lingering demographic spill effects we find for otters. However, while our findings document continuing demographic

effects of the EVOS, we also show that these effects have gradually dissipated with time – largely due to the death of the sea otters most impacted by the spill. This suggests that a cautious optimism is warranted concerning the gradual return of the ecological communities of Prince William Sound to pre-spill conditions.

Major anthropogenic "disasters" are usually labeled as such due to their immediate and obvious impacts. However, there is increasing recognition that long-term, large-scale effects of events such as oil spills may actually pose an even greater threat to affected populations and ecosystems. Unfortunately, accurate assessment of these impacts does not yield easily to the simplistic statistical methods usually advocated for environmental impact monitoring (e.g., 43). Here, we have used a more complex mixture of modeling, statistics, and population data to quantify and understand the effects of the EVOS, one of the best studied but also most controversial of recent marine oil disasters. Recognition that such events can have strong, long-term impacts on populations of sea otters and other near-shore species should urge greater caution in short-term assessment of environmental impacts and suggest that greater efforts are needed to understand the community-wide effects of spill events.

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Table 1. Best-fit parameter-values for different models of changing otter demography fit to age distributions of sea otters found dead before or following the *Exxon Valdez* spill. Relative negative log-likelihood values, maximum likelihood parameter estimates and one-dimensional 95 % confidence limits are given for the best model (lowest negative log likelihood) for each model family. All six best-fit models assumed an initial stable age distribution. See text for definitions of parameter effects.

Model Family	Relative –Log-Likelihood	Parameter			
		a (constant)	b (year effect)	d (age effect)	e (interaction)
Fit To Pre-Spill					
Carcasses:					
Logistic	374.55	-47.7348 (-86.4115, 4.1017)	-0.1501 (-2.7285, 0.3162)	0.02285 (0.00545, 17.5616)	0.00097 (-0.00090, 5.7620)
Modified Logistic	371.52	2.38747 (0.82337, 3.58883)	0.10258 (-0.01390, 0.24090)	-0.31686 (-0.44277, -0.17741)	0.00874 (-0.00367, 0.01995)
Linear	369.88	0.00509 (0.00509, 0.38982)	-0.00034 (-0.00034, 0.0000)	0.00018 (0.00018, 0.01785)	-0.00001 (-0.00001, 0)
Fit to Post-Spill					
Carcasses:					
Logistic	503.72	-0.8379 (-2.1982, 0.7026)	0.5133 (0.2035, 0.8375)	0.1798 (0.0812, 0.3179)	-0.0576 (-0.0922, -0.0269)
Modified Logistic	507.55	-1.1747 (-2.2327, 0.2638)	0.5225 (0.3141, 0.7037)	0.06915 (-0.0570, 0.1842)	-0.0706 (-0.0980, -0.0436)
Logistic Logistic					
Linear	503.12	0.2536 (-0.0033, 0.3332)	0.1062 (0.0612, 0.1150)	0.03455 (0.0179, 0.0495)	-0.0107 (-0.0135, -0.0064)

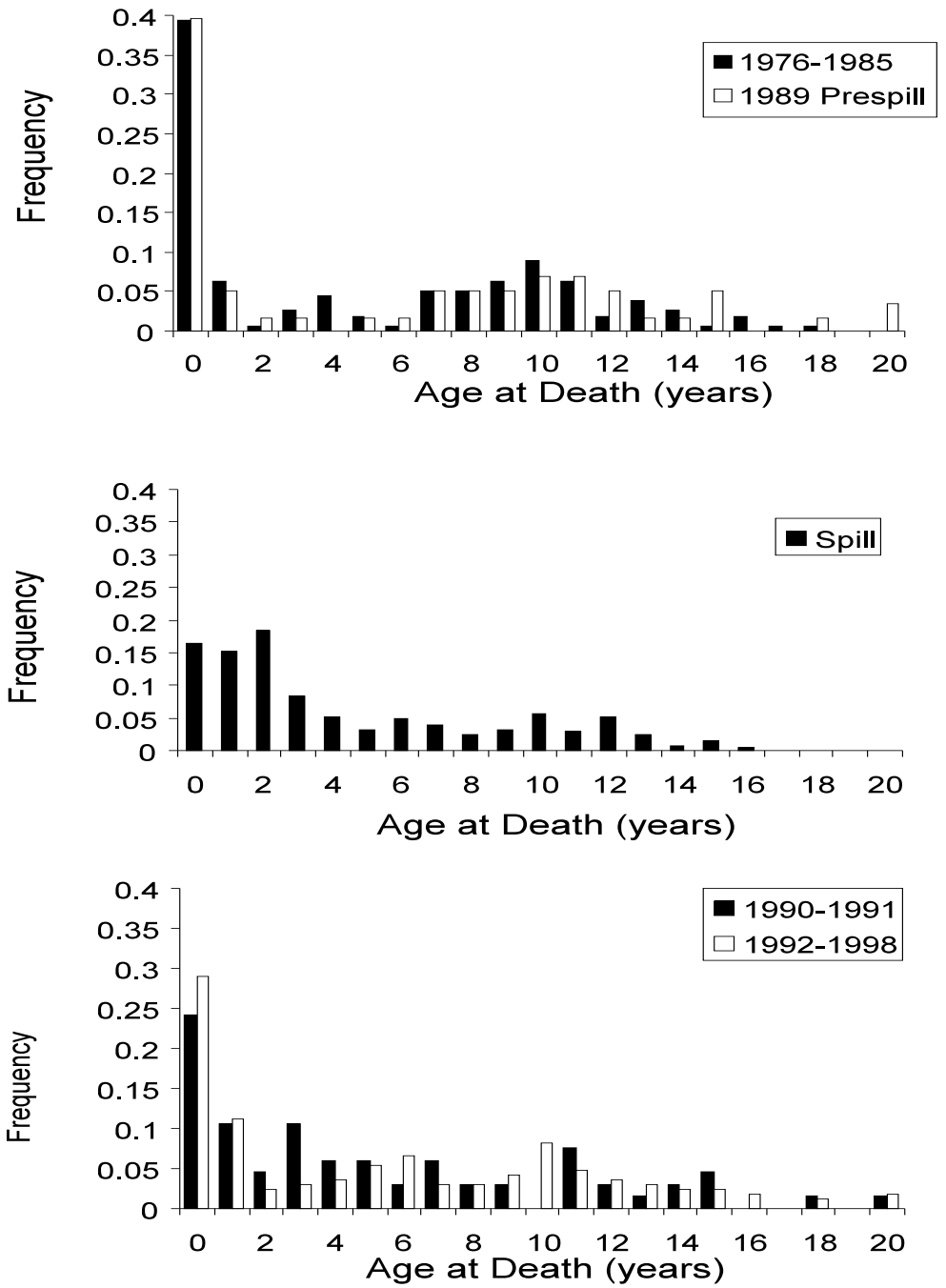

Figure 1. Age distributions of sea otters found dead in western Prince William Sound during five time-periods.

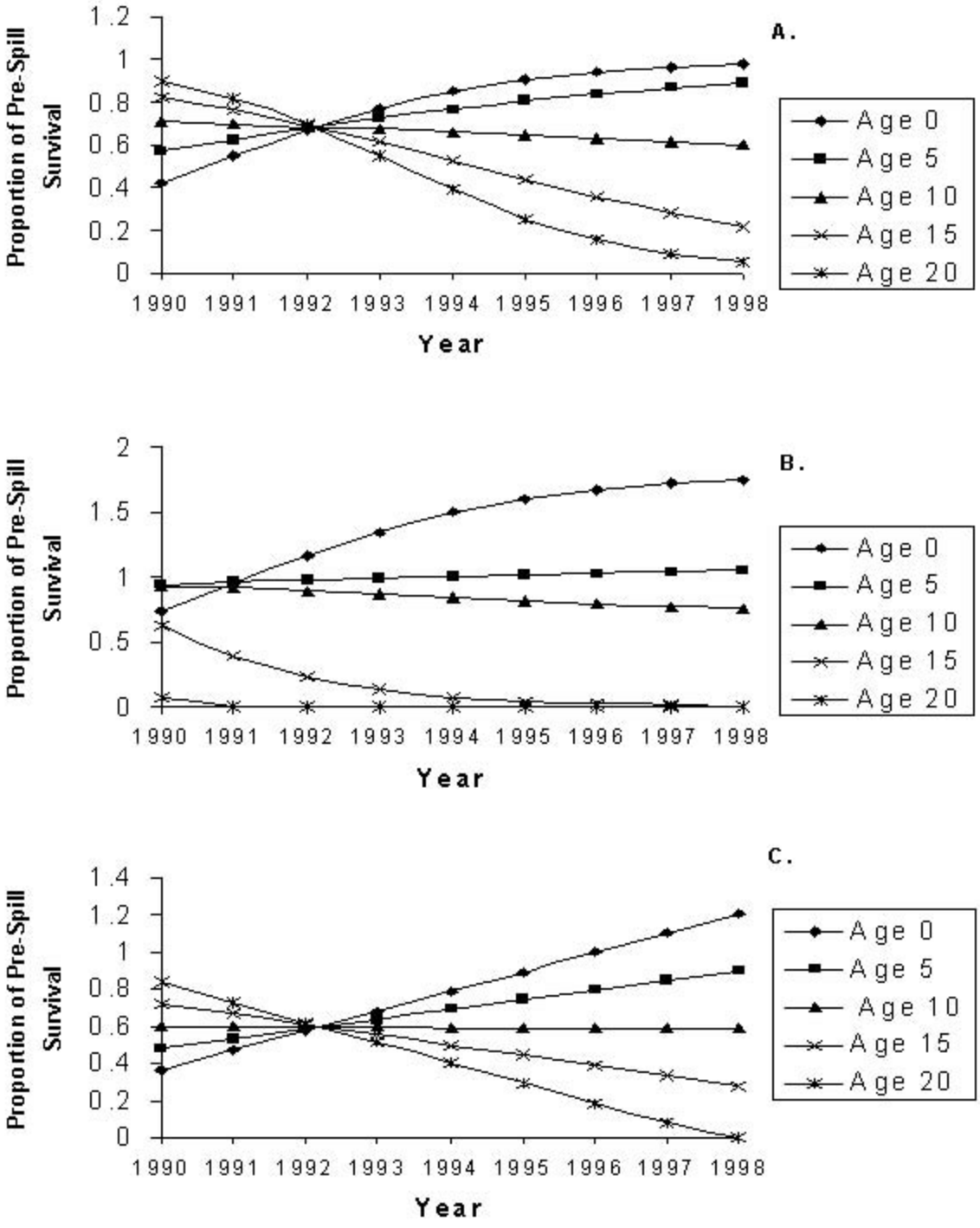

Figure 2. Estimated post-spill effects on age-specific survival rates. The best fit estimates of survival rates are shown as proportions of pre-spill baseline) rates for five representative ages. A. Best-fit results for logistic model; B. Best-fit results for modified logistic model; C. Best-fit results for linear model.

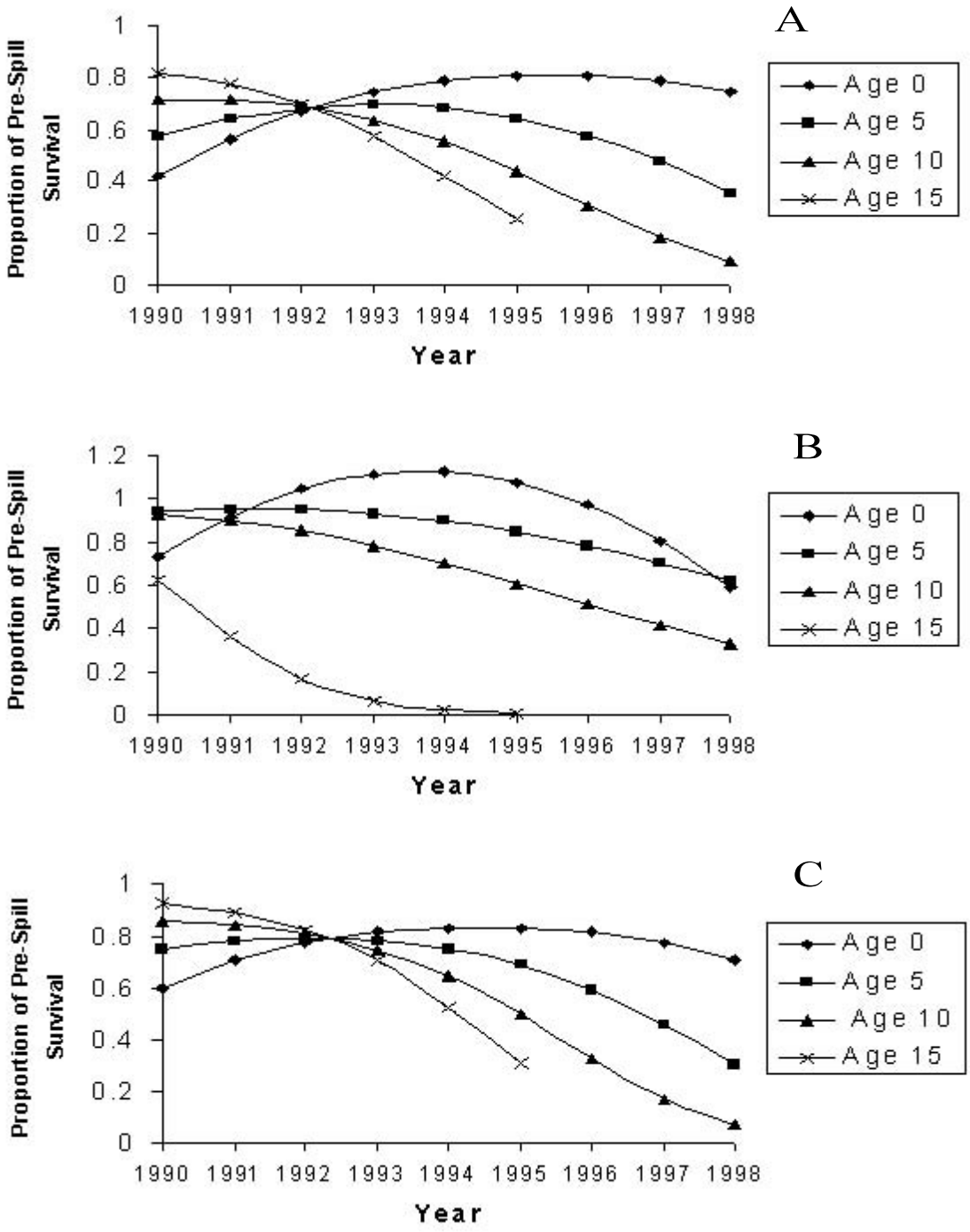

Figure 3. Changing post spill effects for cohorts of otters. Each line represents annual survivals experienced each year for an aging group of otters that were either 0,5,10 or 15 years old at the time of the 1989 spill, expressed as a proportion of pre-spill survival rates. A. Best-fit results for logistic model; B. Best-fit results for modified logistic model; C. Best-fit results for linear model.

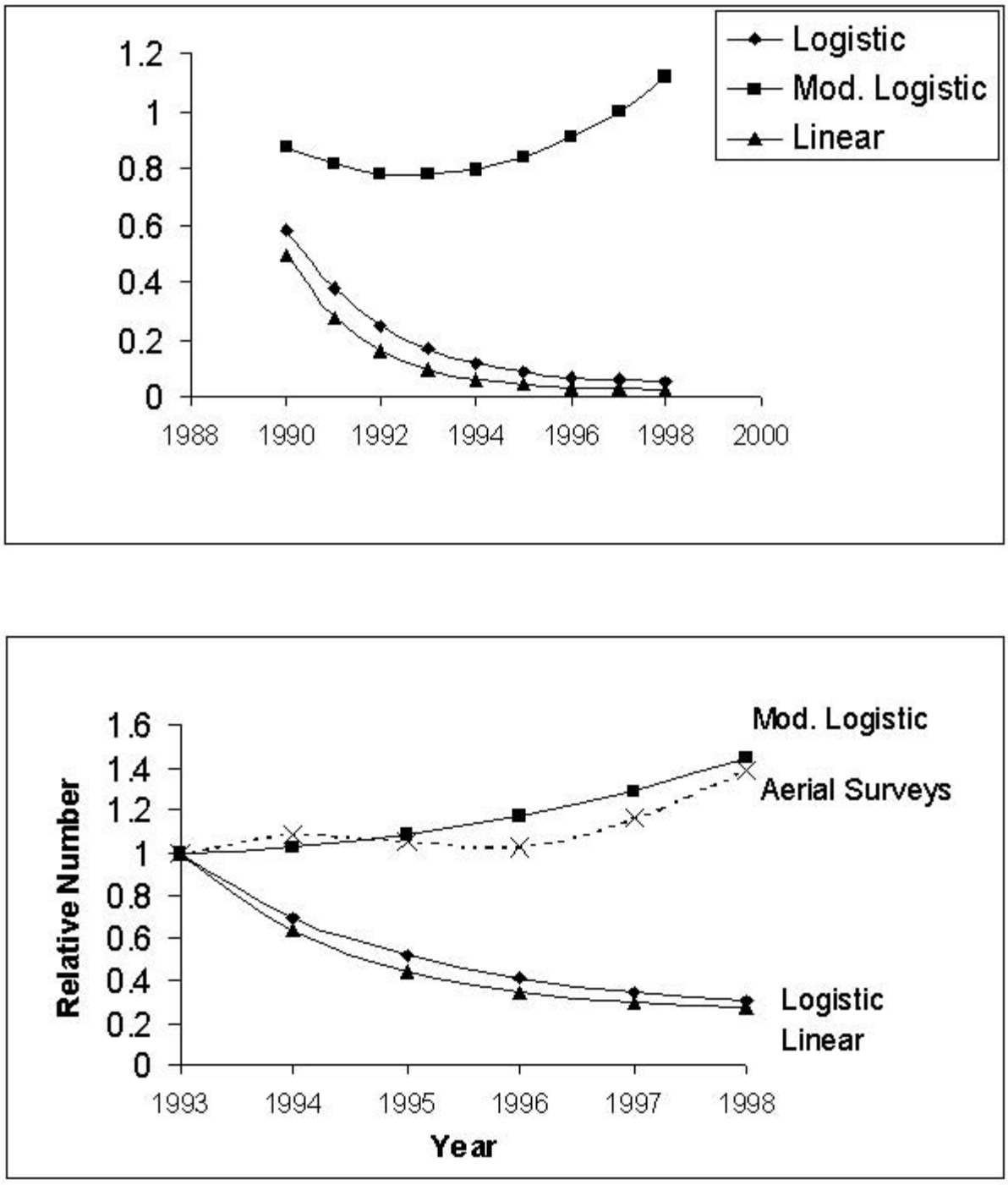

Figure 4. Predicted proportional changes in population size predicted from the three best-fit demographic models (Table 1) and from aerial surveys of WPWS (27).

Sea Otter (*Enhydra lutris*) Appendices

(SO)

APPENDIX SO-01

An aerial survey method to estimate sea otter abundance[1]

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ABSTRACT: Sea otters (*Enhydra lutris*) occur in shallow coastal habitats and can be highly visible on the sea surface. They generally rest in groups and their detection depends on factors that include sea conditions, viewing platform, observer technique and skill, distance, habitat and group size. While visible on the surface, they are difficult to see while diving and may dive in response to an approaching survey platform. We developed and tested an aerial survey method that uses intensive searches within portions of strip transects to adjust for availability and sightability biases. Correction factors are estimated independently for each survey and observer. In tests of our method using shore-based observers, we estimated detection probabilities of 0.52-0.72 in standard strip-transects and 0.96 in intensive searches. We used the survey method in Prince William Sound, Alaska to estimate a sea otter population size of 9,092 (SE = 1422). The new method represents an improvement over various aspects of previous methods, but additional development and testing will be required prior to its broad application.

Keywords: abundance, detection, distribution, disturbance, *Enhydra lutris,* Prince William Sound

1 INTRODUCTION

Conservation and management of sea otters (*Enhydra lutris*) often requires estimates of population abundance. While indices of abundance may be adequate for some purposes, accurate estimates are necessary in some situations (e.g., following an oil spill or managing harvests). Unbiased estimates of abundance are difficult to obtain, largely due to diving behavior which makes sea otters, as well as other marine mammals, undetectable. Estimating that proportion of animals not detected will reduce bias in population estimates.

Several characteristics of sea otters facilitate their detection, compared to most marine mammals. First, sea otters are relatively shallow divers, feeding almost exclusively on benthic prey, resulting in well defined spatial boundaries to their distribution. Foraging depths to 25 m and 40 m have been reported (Wild & Ames 1974, Reidman & Estes 1990) with maximum dive depths estimated from 54 to 100 m (Kenyon 1969, Newby 1975). Although Schneider (1976) observed sea otters as far as 40 km offshore in Bristol Bay, Alaska, he also found that > 90% of his sightings were between the shoreline and the 40 m depth contour. Along coastlines with narrow bathymetric contours, the

[1]Published: 1999. Pages 13–26 *in* G. W. Garner, S. C. Amstrup, J. L. Laake, B. F. J. Manly, L. L. McDonald, and D. G. Robertson, editors. Marine Mammal Survey and Assessment Methods. Balkema Press, The Netherlands.

seaward limit to sea otter distribution may be < 1 km from the shore, allowing shore-based surveys, for which detection probabilities have been estimated (Estes & Jameson 1988).

Dive duration, another factor in marine mammal detection, is relatively short for sea otters, resulting in frequent periods at the surface. Dive times averaged from 25-155 sec among 32 individual sea otters in California (Ralls et al. 1988) and dive times of 10 sea otters in southeast Alaska averaged 62-173 sec (JLB unpub. data). Foraging generally occurs alone (Estes & Jameson 1988) and is distinctly crepuscular, with resting peaks near midday (Kenyon 1969, Estes et al. 1986).

Sea otters often rest in groups that may be more visible than single animals. The sexes are largely segregated with most habitat occupied by adult females and fewer territorial males. Non-territorial males occupy a relatively small portion of habitat (Kenyon 1969, Riedman & Estes 1990). Female group sizes are usually between 1-12 while male aggregations may reach many hundreds of individuals (Riedman & Estes 1990). Where canopy forming kelp beds occur, these habitats tend to be preferred resting areas, possibly resulting in larger, more easily detected groups. However, due to reduced contrast between otters and kelp, detection may be lower in kelp forests.

Although feeding and resting habits can facilitate detection, environmental and observational factors may compromise detection. Their dark pelage can provide good visual contrast with a generally homogenous background, but environmental factors such as sea state, glare, wind speed, and precipitation may make sea otters difficult to see. Detection can also vary with distance, survey platform, number of observers, observer skill, disturbance and search intensity.

Sea otters have previously been counted from shore (Estes & Jameson 1988), small and large vessels (Jameson et al. 1982, Pitcher 1989, Estes 1990) and fixed (Ebert 1968) or rotary wing (Drummer et al. 1990, DeGange et al. 1995) aircraft, or a combination of two or more methods (Estes & Jameson 1983, Geibel & Miller 1984, Jameson et al. 1986). With few exceptions (Estes & Jameson 1988, and Jameson et al. 1986) survey methodologies have not been standardized by search intensity, altitude (for aircraft), number of observers, or environmental conditions, and the proportion of animals detected has not been estimated, biasing results to an unknown extent.

Shore-based surveys have provided the most accurate estimates of nearshore sea otter abundance. Estes & Jameson (1988) estimated an overall probability for sighting sea otters of 94.5% in standardized shore side counts. Theirs was the first study to rigorously evaluate the effect of activity, group size and distance from observer on sea otter detection, and provides a baseline against which other methods can be evaluated. However, because sea otters can occur too far offshore to count from shore and access along most coastlines is limited, shore counts are applicable only to a portion of sea otter habitat.

Aerial surveys are applicable over a broad range of areas. Line transect and strip transect methods are widely used in aerial surveys to estimate population densities (Eberhardt 1978, Burnham et al. 1980, Buckland et al. 1993). The assumption that all animals on the line or strip transects are seen cannot be made with diving mammals, such as the sea otter, and requires estimating detection to reduce bias.

Because of the need for unbiased estimates of sea otter abundance we developed a new aerial survey method that uses intensive searches within strip transects to adjust for availability and sightability biases (detection bias). We report initial test results to determine relations between (1) distance and detection, (2) search intensity and detection (using shore-based observers) and (3) methods of sample selection. We then provide an example of the results of a survey of Prince William Sound, Alaska, incorporating our method and discuss remaining problems.

2 METHODS

2.1 *Preliminary line-transect surveys*

In 1991 we surveyed a series of randomly located line-transects (Buckland et al. 1993) in Western Prince William Sound to test the detection distance of sea otters from the air. In this (and other surveys unless otherwise specified) we flew a Piper PA-18 aircraft at 27 m/s (60 mph) and an altitude of 91 m and the pilot did not aid in observations. The observer recorded the number of individuals and the perpendicular distance to each group of otters detected from one side of the aircraft only. Distances were recorded in 50 m categories based on calibrated wing strut marks.

2.2 *Shore-based evaluation of detection*

In 1991 we used shore-based observers to evaluate effects of altitude, search pattern and search effort on the detection of sea otters in aerial strip-transect surveys and intensive searches within strips. Shore-based observation techniques followed Estes & Jameson (1988). All trials were conducted on areas (search units) without canopy forming kelps large enough to contain a full search pattern, allowing unrestricted observation from an adjacent vantage point on shore, and containing one or more otters immediately prior to arrival of the aircraft.

Shore crews traveled to survey units by skiff, minimizing disturbance to sea otters. Shore crews defined unit boundaries, established an orientation for the aerial search pattern with flagging visible to the pilot and determined the position and activity of each otter within the unit. Shore crews recorded the location, group size, number of pups and activity (swimming, resting or diving) and initiated the trial by radio call to the pilot.

We first tested the effect of altitude (46, 91 and 137 m above sea level) on detection, in sets of three trials. Tested altitudes were randomized in each trial set. All altitude evaluation trials were conducted using a 750 m circle intensive search pattern. We flew along the circumference of a 750 m diameter circle while the aerial observer viewed the circumscribed area. The pilot used a stopwatch, airspeed and minute of turn to define the 750 m diameter circle (128 seconds to complete, 32 seconds through each quadrant). The aerial observer recorded the time, location, group size, number of pups and activity of each sea otter or group of sea otters observed. Groups were defined by a distance $\leq$ one otter length (about 1.5 m) between successive otters. Circling was continued until 5 min had passed without any additional otters being observed.

We next tested the effect of search pattern and search effort on detection using three different intensive search patterns in conjunction with a strip count. Each pattern trial began with a strip count in which the plane flew along one edge of a strip transect while the aerial observer recorded the location, group size, number of pups and activity of each sea otter or group of sea otters observed in the strip. Width of the strip was determined by the aerial observer using distance indicators marked on the wing struts and was either 400 m or 750 m, depending on the subsequent search pattern. The length of the strip was either 400, 750 or 800 m, depending on the search pattern. Immediately following the strip count, the plane began one of three search patterns over the strip that had just been counted. The aircraft was piloted along the circumference of either a 400 or 750 m diameter circle, or a 400 x 800 m oval while the aerial observer viewed the circumscribed area. Selection of the search pattern was made by the shore crew while attempting to obtain an equal number of trials for each pattern. The pilot used techniques analogous to those developed for the 750 m circle to maintain each of the other two search patterns. The aerial observer recorded the circle or oval number, location, group size, number of pups and activity of each additional sea otter

or group of sea otters observed during each pass of the intensive search area. Intensive search patterns were continued until minutes had elapsed without any additional otters being observed. Both air and shore crews recorded the location and behavior of all otters observed outside the boundaries of the unit, changes in sea otter activity over time, and the time the aircraft entered and departed the unit.

At the end of each day, shore and aerial crews compared the mapped locations of all observed otters (for all shore-based trials). For the otters present in each trial we determined the number of otters observed by both crews (b_i), and the number observed only by the shore crew (g_i), in the observation circle or strip. The number of otters in the circle or strip before any response to the approaching aircraft was determined based on shore crew observations prior to the arrival of the aircraft.

Sea otter detection probabilities for the aerial observer were estimated as:

$$\hat{P}_d = \frac{\sum_{i=1}^{r} b_i}{\sum_{i=1}^{r} (b_i + g_i)} , \qquad (1)$$

where r was the number of trials. Detection was also estimated separately for each trial and Kruskal-Wallis tests were used to evaluate differences in detection probabilities between altitudes and patterns. Fisher's exact test for contingency tables was used to evaluate the effect of altitude and pattern on the proportion of trials in which all otters were detected from the air and the proportion of trials in which otters exhibited disturbance behavior, determined by the shore crew. All statistical tests were conducted at the 0.05 significance level.

2.3 *Systematic vs. group initiated intensive search units*

Our shore-based tests suggested we could develop correction factors for adjusting aerial strip-transect surveys, by conducting intensive searches over portions of the strip transects. We refer to the portions of strips on which intensive searches are conducted as intensive search units (ISUs). Correction factors are based on comparing numbers of otters detected during standard strip-transects to numbers detected on the ISU. Precision of estimated correction factors depend on the number of ISUs in which otters are observed. ISUs could be located systematically, but only ISUs with otters could be used to estimate detection. If the probability of detecting each group of otters is independent, then detection probabilities could be estimated for ISUs initiated upon detection of a group, with the estimate only based upon any additional groups present in the ISU. Group initiated ISUs would be usable only if they contained additional groups. Because otter groups tend to occur in clusters, using detection of otters to locate ISUs could result in a higher proportion of usable ISUs. We investigated the relative merits of these 2 approaches for locating ISUs by comparing the proportion of usable ISUs and the estimated strip detection probabilities from samples of systematic and group-initiated ISUs obtained in 1993 and 1996.

Systematic ISUs were located at 2 min intervals along 400 m wide strip transects in Prince William Sound. Group-initiated ISUs were located at each detected otter group separated by more than 800 meters (30 sec) along 400 m wide strip transects, also in Prince William Sound. Observer 1 obtained samples in 1993 and 1996. Observer 2 obtained samples in 1993 only. For each ISU, observers recorded the activity and number of otters with standard strip-transect methodology and the number observed during the ISU. Detection probabilities were estimated based on all detected

otters in each systematically located ISU that contained otters. For group-initiated ISUs, detection probabilities were based on all otters except the initial group in ISUs that contained additional groups. Detection probabilities were estimated according to equation (1). Analyses for effect of observer, year, and type of ISU (systematic vs group initiated) on detection probabilities pooled 1993 and 1996 data and included 150 ISUs. Estimated strip detection was equal to 0 or 1.0 on 73% (109/150) of the ISUs. We categorized detection probabilities as either ≥ 0.5, or < 0.5 for each ISU and used logistic regression to examine differences due to observer, year, and ISU type. We treated observer by year combinations as blocks. If the block effect was significant, we used contrasts to test for observer effects within year (1993) and year effects within observer. Significance tests were based on Wald statistics (Agresti 1990) at $\alpha = 0.05$.

2.4 *1992 distribution survey*

In 1992 we implemented a pilot survey in Western Prince William Sound to determine the spatial distribution of sea otters relative to bathymetric zones. We used this information to define and allocate sampling effort among strata in future surveys. Design for the distributional survey was a series of parallel strip transects, 400 m wide and 1.2 km apart. Each transect was identified by its intersection with the shoreline and an offshore boundary based on shoreline physiography (bays and inlets < 6 km wide were included in the study area regardless of depth), and the 100 m depth contour or a distance of 2 km from the shore, whichever was greater. A GPS in the aircraft was used to locate the endpoints and navigate along each transect. The study area contained 2,404 km^2. Locations and size of each otter group were recorded on a transect map.

2.5 *1994 Prince William Sound survey*

In 1994 we implemented a full survey of Prince William Sound (Figure 1) using group-initiated ISUs to adjust for detection. The survey area was stratified into areas of expected high and low sea otter density based on results of the distributional survey. Sampling effort was allocated to strata in proportion to expected otter densities with approximately 0.18 of the high density stratum sampled and 0.03 of the low density stratum sampled. We flew a Bellanca Scout (a plane similar to the PA-18 used in earlier trials) along systematically located, parallel strip-transects, 400 m wide and 2 km apart (every 5th strip) in the high stratum and 8 km apart (every 20th strip) in the low (Figure 1). The observer searched the 400 m strip between the float and the strut marks, scanning as far forward as conditions allowed. We noted wind, seas, cloud cover, and glare for each transect.

We initiated ISUs at the first sea otter group observed within each 15 minute period of an hour (0-15, 15-30 ...) in the high density stratum and by each group sighted in the low density stratum. Successive ISUs were no closer than 800 m (30 sec) to avoid affecting otters in other ISUs. Intensive searches consisted of 5 concentric circles over the 400 m strip. Circling began at a point indicated by a perpendicular line from the transect edge to the initiating group. The pilot used a stopwatch to time the minimum 30 second spacing between consecutive ISUs and to navigate the circumference of each circle. ISU locations were drawn on the transect map and group size and activity recorded for otters in each ISU. For each group, we recorded the number of otters observed on the strip count and the number observed during the intensive search. Otters that were observed to swim into an ISU post factum were not included. Groups initiating ISUs were not used to calculate detection probabilities.

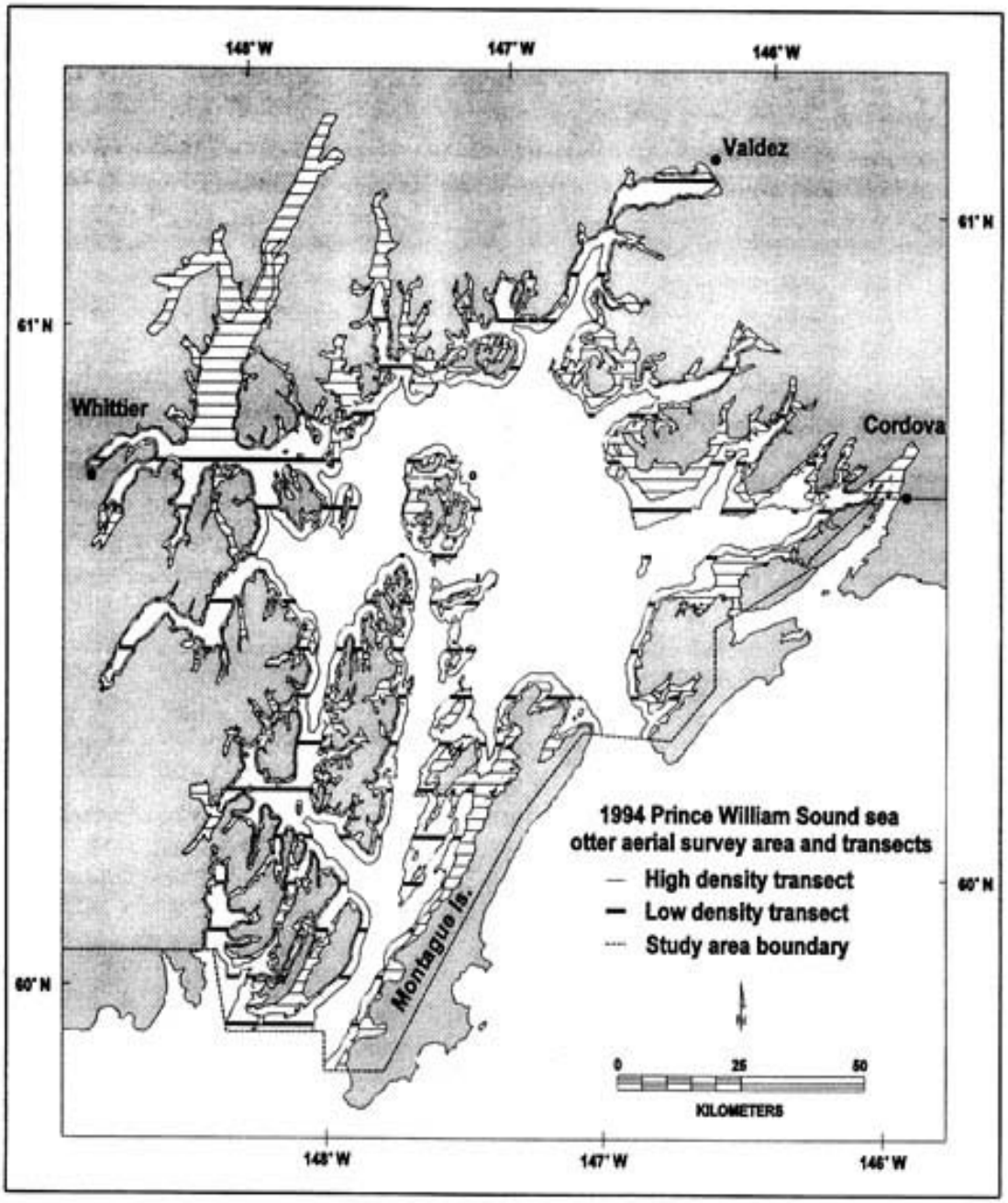

Figure 1. 1994 survey area in Prince William Sound. High and low density transects are 2 and 8 km apart, respectively.

The intensive search method of estimating detection developed here was expected to only be useful for relatively small groups of otters. We assumed groups of 30 or more otters within a 400 m strip would be detected with certainty. Thus, we conceptually divided the population into two portions that are sampled simultaneously and derived separate estimates for the portion that occurred in groups of 30 or less (small groups) and the portion that occurred in groups of more than 30 (large groups). Complete counts, aided by photography (35 mm, 70-210 mm lens), were made of all large

groups detected. These counts were expanded directly based on the proportion of the total area sampled, without any adjustment for detection (i.e., detection was assumed to be 1.0 for this portion of the population). The estimate for the portion of the population occurring in small groups was also expanded based on the portion of the total area sampled but was then adjusted based on the estimated detection of otters in these groups. The overall estimate of the population size was obtained by summing the estimates for these two components of the population.

Two observers were used in 1994, requiring a separate estimate of small group detection for each observer. Each estimate was based only on intensive searches conducted by that observer. For notational convenience, we consider each portion of a stratum surveyed by a different observer to be a separate stratum. The unadjusted population size for stratum j was estimated as:

$$\hat{Y}_{(un)j} = \frac{\sum_{i=1}^{n_j} y_{ij}}{\sum_{i=1}^{n_j} a_{ij}} A_j \qquad (2)$$

$$var(\hat{Y}_{(un)j}) = \frac{A_j^2 (1-f_j) n_j}{\left(\sum_{i=1}^{n_j} a_{ij}\right)^2 (n_j - 1)} \sum_{i=1}^{n_j} \left(y_{ij} - \frac{a_{ij} \sum_{i=1}^{n_j} y_{ij}}{\sum_{i=1}^{n_j} a_{ij}} \right)^2$$

where

n_j = number of surveyed transects in stratum j,
y_{ij} = number of otters detected in strip count on transect i in stratum j, i=1,⋯,n_j,
a_{ij} = area of transect i in stratum j, and
f_j = the sampling fraction, approximated by

$$f_j = \frac{1}{A_j} \sum_{i=1}^{n_j} a_{ij} \; . \qquad (3)$$

the correction factor for observer k was estimated as:

$$\hat{p}_k = \frac{\sum_{i=1}^{t_k} c_i}{\sum_{i=1}^{t_k} s_i}$$

$$var(\hat{p}_k) = \frac{t_k \sum_{i=1}^{t_k} (c_i - \hat{p}_k s_i)^2}{(t_k - 1)\left(\sum_{i=1}^{t_k} s_i\right)^2} \qquad (4)$$

where

s_i = number of otters detected in strip count of ISU i, i=1,…,t_k, and
c_i = total number of otters detected after intensive search of ISU i.

The adjusted population size for stratum j (surveyed by observer k) was estimated as:

$$\hat{Y}_j = \hat{p}_k \hat{Y}_{(un)j}$$

$$var(\hat{Y}_j) = \hat{Y}_{(un)j}^2 var(\hat{p}_j) + \hat{p}_j^2 var(\hat{Y}_{(un)j}) - var(\hat{p}_j) var(\hat{Y}_{(un)j}) . \qquad (5)$$

For the portion of the population in large groups, population size estimates for each stratum were obtained as in (2) with no adjustment for detection. The overall estimates of population size and variance for each stratum were then obtained by summing the respective estimates for otters in small and large groups. Combined estimates of population size and variance for groups of strata were obtained by summing the respective overall stratum estimates. A more detailed protocol for the survey method, including analytical programing is available from the authors.

3 RESULTS

3.1 *Preliminary line-transect surveys*

Preliminary line-transect surveys indicated a region below the plane that was obscured by the aircraft and not visible, and the flight path would have to be offset (64 m horizontal offset at 91 m altitude) from the transect. The detection function appeared to increase with distance from the plane up to a maximum at about 250 m and then decreased with distance beyond that (Figure 2). Few otters were detected at distances beyond 450 m. There was no evidence of an effect of group size on detection, but few groups with more than 2 sea otters were detected in these preliminary surveys (Figure 2).

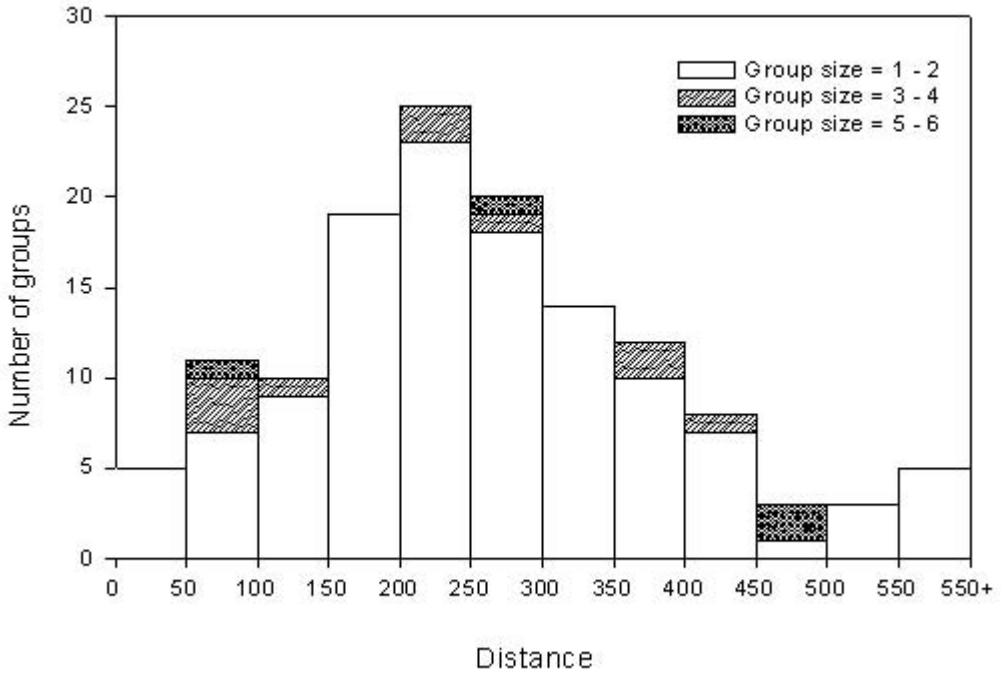

Figure 2. Relation between distance estimated from air and frequency of sea otter sightings using wing strut calibrations in 50 m increments.

3.2 *Shore-based evaluation of detection*

We conducted 98 trials, observing 329 groups of sea otters (741 individuals), in our tests of altitude and search pattern. Intensive searches resulted in detection estimates ≥ 0.90 for all altitudes and patterns investigated (Tables 1 and 2). We detected all otters in over half of the samples (Tables 1 and 2). The type of avoidance behavior observed in boat surveys (Udevitz et al. 1995), in which otters leave the search area before the survey platform arrives, was not observed in response to the aircraft. On 0.08-0.26 of the trials it was apparent that otters were disturbed by the aircraft (Tables 1 and 2), and began diving, swimming out of the area, or swimming erratically within the search area. However, due to the approach speed of the aircraft, otters were unable to leave the survey area before the aircraft arrival.

Detection probability did not differ among trials conducted at 46, 91, or 137 m altitude ($P = 0.72$, Table 1). We would expect detection to decrease at altitudes much greater than those we considered. In general, safety is expected to increase with altitude and we considered 46 m as the minimum altitude safe for this type of survey work. However, at 46 m, disturbance to sea otters within the survey area occurred on 0.23 of our trials, compared to 0.08 at 91 and 137 m altitude (difference not significant, $P = 0.84$, Table 1). We selected an altitude of 91 m for conducting subsequent work because it provides a margin of safety and minimized disturbance without decreasing detection.

Table 1. Detection probabilities (estimated by comparing air to shore observations) at three altitudes in a 750 m diameter search pattern continued for 5 minutes following the last otter sighting.

	Altitude		
	46 m	91 m	137 m
Number of trials	13	12	12
Number of groups	58	43	44
Number of otters	133	104	106
Detection probability	0.92	0.91	0.90
Detection = 1.0[a]	0.62	0.50	0.50
Disturbance [b]	0.23	0.08	0.08

[a] Proportion of samples in which all otters were detected.
[b] Proportion of samples when disturbance by aircraft was detected by shore.

Table 2. Detection probabilities (estimated by comparing air to shore observations) for three search patterns at 91 m continued for 5 minutes following the last otter sighting.

	Search pattern		
	400 m Circle	750 m Circle	800 m Oval
Number of trials	20	19	22
Number of groups	58	40	86
Number of otters	113	72	213
Detection Probability	0.96	0.93	0.90
Detection = 1[a]	0.80	0.79	0.68
Disturbance [b]	0.15	0.26	0.19

[a] Proportion of samples in which all otters were detected.
[b] Proportion of samples when disturbance by aircraft was detected by shore.

We found no differences among the three intensive search patterns evaluated (P=0.64, Table 2). However, with the 400 m circle, the entire ISU remained within the observer's view at all times, making it easier to keep track of which otters and groups had already been detected. With the other two search patterns, the portion of the ISU furthest from the plane was always out of view (although all portions of the ISU were eventually seen each time the plane circled around). Detection probability estimates for initial strip-transect counts ranged from 0.52 to 0.72 (Figure 3). Detection probabilities increased sharply with the first 3 circles or ovals after the strip count (range 0.88 - 0.93) and continued to increase slightly for the next 3 to 4 circles or ovals (Figure 3). No new otters were ever detected after the 7th circle or oval. In the absence of strong differences in detection probabilities, selection of a search pattern could be based on the probability of encountering otters in each search. This probability likely decreases with decreasing the size of the search pattern, thus increasing the number of ISUs necessary to obtain a detection probability estimate with a given level of precision. However, because of decreasing detection probabilities with larger distances from observers (Figure 2) and the need to keep track of otters within ISUs, the 400 m diameter ISU and the corresponding 400 m strip width were selected for use in future work.

The data suggested the most efficient search was three circles or ovals after an initial strip count (Figure 3). Even with intensive searches, however, not all of the otters were detected. Population size estimates based on correction factors derived from these types of intensive counts can be expected to be negatively biased on the order of 0.05-0.10 (Tables 1 and 2).

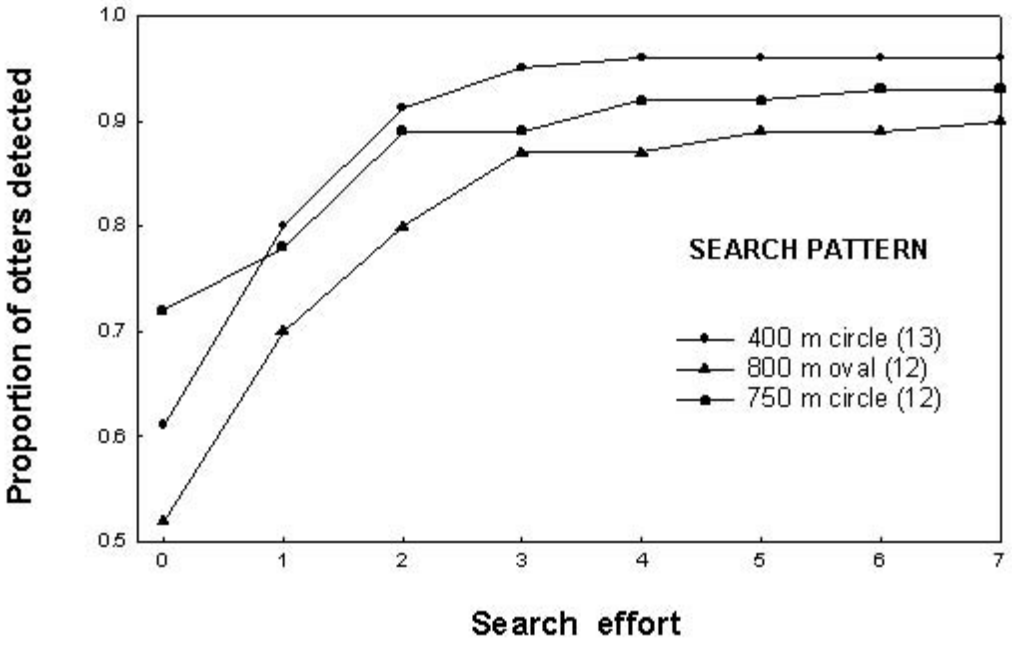

Figure 3. Relation between search effort (number of circles in intensive search) and detection probability for three search patterns.

3.3 *Location of intensive search units*

We estimated detection probabilities from 39 systematically located ISUs and 111 group initiated ISUs. Systematically located ISUs could only be used for estimating detection probabilities if they contained at least one otter; 23% (39/170) met this criteria. Group initiated ISUs could be used for estimating detection probabilities only if they contained more than one group of sea otters, 51% (111/219) met this criteria. Differences in detection probabilities due to type of ISU (P = 0.59) or year (P = 0.65) were not significant, but detection probabilities were significantly different between observers in 1993 (P < 0.01, Table 3).

Table 3. Comparison of detection probabilities obtained from systematically located and group initiated intensive search units (ISUs), by observer and year.

Observer[a]	Year	Type of ISU	Number of ISUs	Ratio estimate of detection probability
2	1993	Group initiated	29	0.18
		Systematic	13	0.41
1	1993	Group initiated	12	0.54
		Systematic	6	0.78
1	1996	Group initiated	70	0.76
		Systematic	20	0.77

[a] Difference between observers significant (P<0.01).

3.4 *1992 distribution survey*

In the 1992 survey, a single observer surveyed 1,936 km of transects (744.4 km^2) in Western Prince William Sound. We found more than 80% of the sea otters in the two near shore bathymetric zones that made up < 35% of the area surveyed (Figure 4). Based on this, we partitioned sea otter habitat in Prince William Sound into two strata. The high density stratum extended 400 m seaward from shore or to the 40 m depth contour, whichever was further from shore. The low density stratum extended from the seaward high density boundary to an offshore boundary based on shoreline physiography, and the 100 m depth contour or a distance of 2 km from shore, whichever was greater. Bays and inlets < 6 km wide were always in the high density stratum.

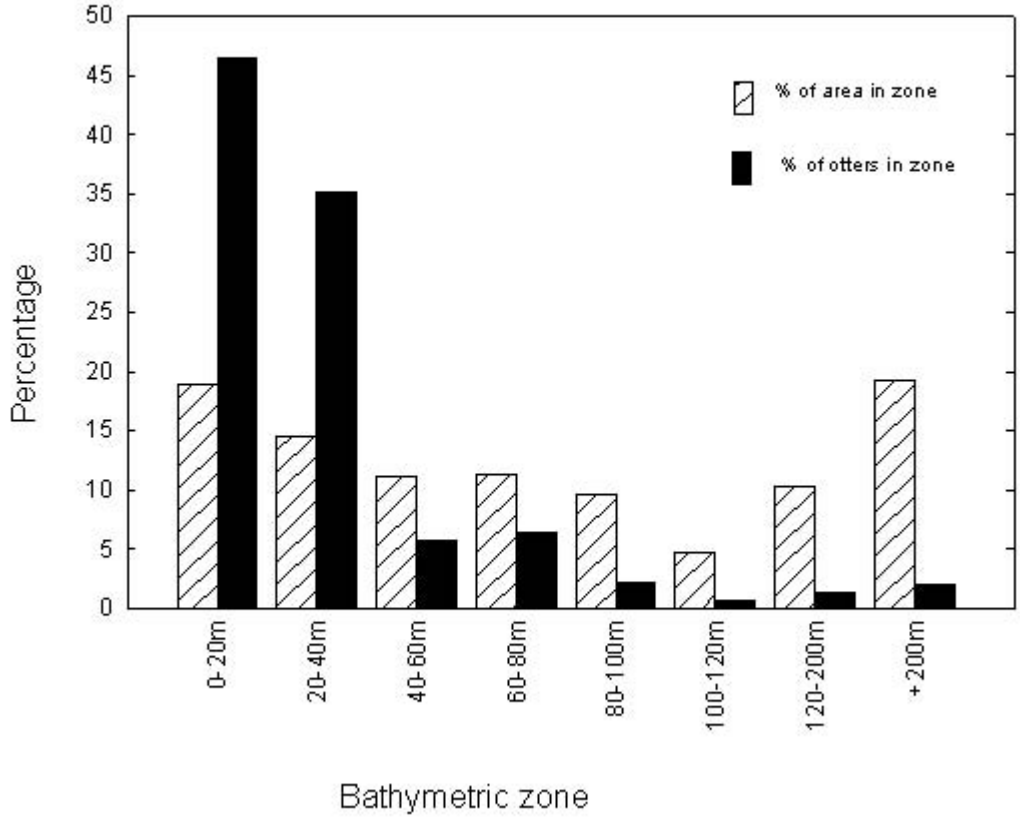

Figure 4. Proportional distribution of sea otters observed in 1992 experimental survey in Western Prince William Sound relative to bathymetric contour intervals.

3.5 *1994 Prince William Sound survey*

The full survey of Prince William Sound was conducted in August 1994 and consisted of 7,120 km^2 (Figure 1). We surveyed 820 km of high stratum transects and 123 km of low stratum transects (Table 4). We counted 888 otters in small groups (size $\leq$ 30). Ninety-seven ISUs with > one group of otters were searched, resulting in individual observer correction factors of 1.39 and 1.92 (Table 4). In addition to the small groups, 131 otters were detected in large groups (size >30). This expanded to a population size estimate of 716 (SE=443) otters occurring in large groups. Combining the

adjusted stratum estimates for small groups (Table 4) and the estimate for large groups gave an estimate of 9,092 sea otters (SE = 1,422) in Prince William Sound. Flight time required to complete the survey was 70 hrs, including transit.

Table 4. Otter counts, unadjusted population size estimates, correction factors and adjusted population size estimates in the 1994 sea otter survey, Prince William Sound, Alaska.

Counts and Unadjusted Estimates

Observer	Stratum	Count[a]	Area[b]	Unadjusted estimate	SE
1	High	221	223	1,209	162
	Low	16	87	532	270
2	High	649	285	3,548	395
	Low	2	43	67	47
	Complete	131	[c]	716	443
	Total	1,042		6,072	674

Correction Factors

Observer	# ISUs	Factor	SE
1	42	1.92	0.20
2	55	1.39	0.08

	Combined Adjusted Estimate	SE
Total	9,092	1,422

[a] Number of otters observed on transects.
[b] Area of surveyed transects (km^2).
[c] Area sampled was the same as high density strata for observer 2. Large groups not observed in other strata, or by observer 1.

4 DISCUSSION

Previous researchers have recognized that some proportion of sea otters in the area surveyed, regardless of the method employed, is not observed, due to diving behavior and sighting error (Geibel & Miller 1984, Estes & Jameson 1988, Udevitz et al. 1995). The result is a bias in the estimate of abundance. This bias can be reduced by estimating the proportion of animals not observed (detection), and using the reciprocal of this proportion as a correction factor. Correction factors may be affected by many variables, including observers, habitat, and survey conditions. Thus, any survey method should incorporate techniques for estimating a correction factor specific for the observers and conditions associated with each application of the method.

We found that detection probabilities in aerial strip counts were low (0.52-0.72), but that intensive searches over selected portions of the strip could provide correction factors to compensate for most of the detection bias. Research conducted in 1993 and 1996 indicated that for a given number of ISUs, the number of usable ISUs could be approximately doubled by initiating searches only when a group was detected. Detection probability estimates based on group initiated ISUs will not be more biased than estimates based on systematically located ISUs if the initiating group is not included in the estimate and if the detection of groups is independent. The assumption of

independence of group detection is common in line transect theory (e.g., Burnham et al. 1980, Quang & Lanctot 1991, Buckland et al. 1993). Our inability to find a difference in estimated detection probabilities from the two methods for locating ISUs is consistent with this assumption. The potential for relations between size and detection of animal groups is well known (Buckland et al. 1993) and the relation has been demonstrated for sea otters in certain cases (Estes & Jameson 1988, Drummer et al. 1990). Our line transect data did not indicate any effect of group size on detection, but the range of observed group sizes (Figure 2) was small. Other studies have found that group size effects were not evident for sea otters when there was little variation in group size or observation distances were relatively short (Drummer et al. 1990, Udevitz et al. 1995). Buckland et al. (1993) suggested that group size effects can usually be eliminated by truncating observation distances. We only apply the ISU technique for estimating detection probabilities of groups with less than 30 individuals and observation distances are truncated at 400 m. Thus, it is unlikely that there would be any strong group size effects on detection in these surveys. In any case, if detection of groups are independent, the size of the initially detected group (or its detection probability) will not affect the estimated detection probabilities in group-initiated ISUs.

Results of the 1994 survey indicate that differences in detection between observers may be large. Difference in detection between observers will not increase the bias of the adjusted population estimate as long as the correction factor for each observer is estimated separately. This can be done, provided each observer can achieve an acceptable level of detection (we suggest >0.90) in the ISUs. The precision of the estimated correction factors will depend on the number of usable ISUs for each observer. To achieve an acceptable level of precision for the adjusted population size estimates, it will be necessary to obtain a sufficient number of usable ISUs for each observer.

It is likely that our ability to detect otters varies with factors we have not tested, including canopy forming kelp beds, and this should be evaluated. Because detection may vary among observers, testing of observers to determine individual relations between search effort and detection for ISUs should be done. Additionally, we conducted shore-based observer trials and surveys only under environmental conditions of calm to light winds, good visibility, and calm seas (Beaufort scale 0-2, rarely 3). Detection probabilities should be tested before applying this method under different environmental conditions.

Further efforts to improve precision and efficiency should include training to increase precision in detection probabilities and assure that all observers detect at least 90% of the otters within ISUs. Precision may be improved by analyzing separately the two components of detection: (1) the probability of detecting a group, and (2) the proportion of the otters detected in a group, given that the group is detected. This separation would allow the use of all ISUs in estimating the second component of the detection probability. Greater overall sampling effort would also increase precision of the population estimate. The precision of the estimates obtained with this survey method is limited by the number of usable ISUs an observer can accumulate during a survey. In areas less than a few hundred km^2 it may be impossible to acquire a sample of ISUs necessary to achieve desired levels of precision, particularly if sea otter densities are low. In such cases, it may be possible to increase precision to an acceptable level by conducting replicate surveys.

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APPENDIX SO-02

CHEMICAL RESTRAINT OF NORTHERN SEA OTTERS: RESULTS OF PAST FIELD STUDIES[1]

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Abstract: Between 1987 and 1997 we chemically immobilized 598 wild sea otters (*Enhydra lutris*) in Alaska for the collection of biological samples or surgical instrumentation. We experienced only one drug-related fatality in this time. Fentanyl in combination with diazepam produced consistent, smooth inductions with minimal need for supplemental anesthetics during procedures lasting 30 to 40 minutes. Reversal with naltrexone or naloxone was rapid and complete, although we observed narcotic recycling in sea otters reversed with naloxone. For surgical procedures, we recommend a fentanyl target dose rate of 0.33 mg/kg of body mass and diazepam at 0.11 mg/kg. For non-surgical sample collection procedures, we recommend fentanyl at 0.22 mg/kg and diazepam at 0.07 mg/kg. We advise use of naltrexone for reversal at a dose equaling twice the total fentanyl administered during processing.

Key words: Anesthesia, azaperone, diazepam, fentanyl, naloxone, naltrexone, sea otter.

INTRODUCTION

Researchers have captured several thousand sea otters throughout their range since the 1950s for translocation, tagging, and collection of biological samples. Capture methods have been well described,[1] and include modified gill nets (also called "tangle nets"), dip nets and diver-operated Wilson traps. Tagging and surgical procedures for the implantation of radiotelemetry transmitters have changed little since they were developed.[4,14] However, chemical immobilization protocols have changed with time. Researchers administered "anti-stress" drugs during translocation projects as early as 1959.[1] Full chemical immobilization protocols were developed for instrumentation and veterinary care.[7,11,13,15] We found that published protocols, although appropriate for clinical settings, gave dosages lower than those which we found necessary for wild, healthy sea otters. Here we describe drug combinations used in Alaska from 1987-97, and recommend dosages for routine biological sampling and surgical instrumentation of wild sea otters.

METHODS

Study area

We captured sea otters along the north Pacific Rim from Vancouver Island, British Columbia to Attu Island, Alaska at the western end of the Aleutian Island chain. Most captures occurred within Prince William Sound in south-central Alaska or at Amchitka Island in the Aleutian chain. We captured otters for the collection of biological samples and, in some cases, for surgical instrumentation.[2,3,9,10]

Capture and immobilization

We employed several capture techniques including tangle nets, dip nets and Wilson traps.[1] We visually estimated total mass of sea otters to calculate the induction dose, which was administered by intramuscular (IM) injection to the hind limb with a hand syringe. Two drug combinations were used: fentanyl citrate (RBI, Natick, Maine, USA) combined with azaperone (Stesnil®, Pitman-Moore, Inc., Washington Crossing, New Jersey, USA), and fentanyl combined with diazepam (Steris Laboratories Inc., Phoenix, Arizona, USA).

Until 1990, we used an equal ratio of fentanyl and azaperone for initial injections. After 1990, azaperone was no longer readily available and we switched to a 3:1 ratio of fentanyl to diazepam for initial injections. We administered Supplemental IM injections of fentanyl as required to maintain an adequate level of anesthesia for the procedures being performed. We also gave supplemental IM or intravenous (IV)

injections of diazepam (0.5-1.5 mg) as needed to control convulsive seizures.

Anesthetized otters were weighed, and actual drug dose calculated. We measured induction time opportunistically (minutes from injection until fully anesthetized) in a subset of 101 animals and "time to first procedure" (TFP) in all animals. Rectal temperature was monitored throughout the period of sedation and handling. We recorded the times of all drug injections, body temperature readings, and tremors or convulsions.

After processing, the otters were reversed with naloxone (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) or naltrexone (Trexonil®, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) at a dose of 1.5-2X the total fentanyl dose administered. Use of naloxone was discontinued in 1992, when naltrexone became available. The reversal agent was given either half IV and half IM, or all IM. The naloxone or naltrexone was drawn up before administering the fentanyl so that it would be immediately ready for use if needed.

Before 1990, we reversed animals with IV and IM injections of naloxone (equal dose by each route) at the water's edge and released them to the water as soon as a conscious "head up" response was observed, generally within 30 seconds of injection. However, naloxone has a shorter half life than fentanyl, and the narcotic can "recycle" if the naloxone wears off before the fentanyl is completely metabolized. Between 1990 and 1992, because of concerns about possible narcotic recycling, we released the otters to floating net pens and observed them for one to two hours after reversal. Because signs of narcotic recycling were observed frequently, we initiated the practice of giving supplemental naloxone at a dose equaling half the initial reversal dose just prior to release from the net pen. After 1992 we accomplished reversal with naltrexone, and because of its longer half-life we found holding and subsequent supplemental doses unnecessary. After this time we gave only IM injections of naltrexone, holding the otter within a capture box until fully alert (usually 1 to 3 minutes), and then released it to the water.

Data analysis

We used logistic regression and the Wald Chi-square statistic to examine four response variables (coded as 1=yes, 2=no): 1) supplemental fentanyl required, 2) tremors or convulsions observed, 3) narcotic recycling observed (only for otters held ≥1 hour post-reversal), and 4) hyper thermic problems observed (defined as >40 ºC). Full models included drug dose rates and drug type (sedative - azaperone vs. diazepam, or reversal - naloxone vs. naltrexone). Obviously, doses rates among drugs administered in combination are highly correlated and thus we used only one drug type dose rate at a

time in each analysis (i.e., one model may include fentanyl dose and sedative type and a second model may include sedative type and sedative dose but no model included both fentanyl dose and sedative dose). We included a drug type*dose interaction term when both were in the model and repeated the analysis separately for each drug type if the interaction was significant. We included body temperature, and capture type (surgical vs. blood sampling only) as covariates in the full model for response variables 1 and 2. Covariates for response variable 3 included handling time and post reversal holding. We included handling time as a covariate for response variable 4 along with capture type and body temperature. We used indicator variables coded as 1 or 0 to represent sedative type, reversal type and capture type in the model. We reduced models using stepwise selection and chose the best fit model based on AIC values (i.e., the best fit model had the lowest AIC value). We present the results of the full and best fit model. We used linear regression analysis to examine the relationship between drug doses and induction times, and Fisher's exact test to compare naloxone and naltrexone recycling rates. We used SAS (version 6.12, SAS Institute Inc. Cary, NC) statistical software for all analyzes. Differences were considered significant at $\alpha \leq 0.05$.

RESULTS

From 1987-97, 265 sea otters were anesthetized for sampling and surgical instrumentation, and 303 sea otters were anesthetized for biological sampling only (Table 1). An additional 30 sea otters were anesthetized for semen collection via electro-ejaculation and required anesthesia similar to surgical levels; these were included in the "surgical" category for analysis. Sea otters handled during rescue efforts at the time of the 1989 *Exxon Valdez* oil spill[11] were not included in this study.

During these studies we had six capture-related mortalities giving an overall loss rate of 1%. Only one death (0.17%) was drug-related, and this involved an animal compromised by injury prior to immobilization.

Drug protocol

Initial surgical drug dosages ranged from 0.16-0.60 mg/kg for fentanyl, 0.16-0.33 mg/kg for azaperone, and 0.07-0.23 mg/kg for diazepam. Initial biological sampling dosages ranged from 0.09-0.38 mg/kg for fentanyl, 0.10-0.44 mg/kg for azaperone, and 0.04-0.17 mg/kg for diazepam. We present mean dose rates actually administered in Table 1. Supplemental fentanyl was required in 45 of 295 (15%) animals undergoing surgical procedures, and 29 of 303 (10%) of animals during sampling procedures. One female and one male never became

adequately immobilized despite several injections totaling approximately 2½ times the estimated required dose, and were reversed and released without processing. Additional diazepam was required during only 15 immobilizations (fentanyl-diazepam combination) for control of convulsive seizures and tremors.

Mean induction time was 9 minutes (SD = 4 min.), and mean TFP was 15 minutes (SD = 4.5 min.) for the subset of 101 otters where both times were recorded. The difference of 6 minutes represents the average time to weigh, measure and secure the otter for processing. For all other anesthetizations, mean TFP was essentially the same ($\bar{x}$ = 14.5 min., SD = 5 min., N = 458) indicating it could be used as an index of induction time. Induction times and TFP were not dose-responsive to any of the anesthetic agents ($R^2 \leq 0.03$ for all drugs). Induction times were similar for azaperone anesthetizations($\bar{x}$ = 9.2 min., SD = 5.1 min.) and diazepam anesthetizations($\bar{x}$ = 8.9 min., SD = 4.0 min.; $t_{(98)}$=0.23, P=0.8).

As would be expected, low initial fentanyl dose lead to a higher probability that supplemental fentanyl would be required, and we required more narcotic for surgical procedures (Table 2, Fig. 1). However, we also required higher doses of fentanyl when used in combination with azaperone as compared with fentanyl-diazepam anaesthetizations (Table 2, Fig. 1).

We observed tremors or convulsions during 71 of 598 (12%) anesthetizations. The probability of tremors or convulsions was significantly less when using diazepam (Table 3), with 47% of otters immobilized with the fentanyl-azaperone combination experiencing tremors or convulsions vs. only 8% for those immobilized with fentanyl-diazepam. Tremors were not related to sedative dose rate or body temperature (Table 3).

Three of 33 otters (9%) reversed with naloxone and held from 30-60 minutes had already shown signs of recycling. An additional 41 of 186 otters (22%) held at least 1 hour after reversal showed signs of narcotic recycling. Recycling was not related to fentanyl dose, but a significant interaction between sedative type and sedative dose was found (Table 4). Thus azaperone and diazepam anaesthetizations were analyzed separately. Twenty of 38 (53%) otters recycled when sedated with azaperone, and the probability of recycling increased with the amount of naloxone or azaperone administered (Table 4; Fig. 2). In contrast, only 20 of 169 (12%) of otters recycled with diazepam, and showed no relationship with diazepam or naloxone dose (Table 4). This suggests azaperone was the primary cause of the dose response seen between recycling rate and azaperone or naloxone dose rate. First signs of recycling usually occurred from one to two hours after narcotic reversal (mean = 80 min., range 8-152 min.).

We monitored 26 otters after reversal with naltrexone, including 14 held between 30-60 minutes and 12 observed for

over an hour (maximum = 3.3 hrs). None showed any signs of renarcotization. Although power is low due to small sample size, the result approached statistical significance when compared with the renarcotization rate of naloxone reversals (*Fisher's exact 1-tailed*, P=0.056 for otters held >1 hr.).

Mean initial body temperature for all captures was 37.5° C (SD = 0.9). During handling, body temperature generally increased with mean changes of +1.2° C and +1.6° C for blood sampling and surgical captures respectively. Elevated temperatures did occur occasionally even with close monitoring and efforts to keep the otters cool. Body temperature of 21 otters surpassed 40° C, at which point they were reversed immediately. We found no relationship between hyperthermia and drug doses or capture type, but the probability of hyperthermia increased with handling time and initial body temperature (Table 5; Fig.3).

DISCUSSION

The most recently published drug protocol for sea otters[11] was developed from experience handling sea otters captured for rehabilitation during the 1989 *Exxon Valdez* oil spill. In the early days after the spill, many otters were severely compromised by exposure to oil and anesthesia was considered risky. Immobilizing these animals, when necessary, was accomplished with low doses of weak narcotics such as meperidine hydrochloride in combination with diazepam. The general health and vigor of animals coming into the rehabilitation facilities increased with time, and more potent drugs were required. Fentanyl, in combination with diazepam (supplies of azaperone were limited), was most commonly used at initial dosages of about 0.1 mg/kg for both fentanyl and diazepam. However, due to prolonged procedures, supplemental doses up to a total of 0.8 mg/kg of fentanyl and 0.2 mg/kg of diazepam were sometimes required.[11] The combination of fentanyl, azaperone and diazepam was also used, and the final recommendation of Sawyer and Williams[11] for the immobilization of sea otters up to 2.5 hours included 0.1 mg/kg of fentanyl, and 0.5 mg/kg of azaperone in combination with 0.1 - 0.5 mg/kg of diazepam. As an alternative to azaperone, they recommend acepromazine at a dose of 0.05 mg/kg.

The protocol recommended by Sawyer and Williams[11] worked well in the clinical setting for sea otters needing to be cleaned, as washing and related handling sometimes continued for several hours. The use of the longer lasting, nonreversible tranquilizers (azaperone or acepromazine) significantly reduced the amount of supplemental narcotic required over these extended periods. But Sawyer and Williams[11] also point out that these same tranquilizers (particularly acepromazine) prolonged recovery times. However, sea otters in the rehabilitation centers could be

reversed and held in a controlled setting, allowing them to be closely monitored during recovery.

Sea otters captured for sampling and instrumentation are generally handled immediately after capture and subjected to procedures lasting less than one hour. The initial reaction of a healthy, wild sea otter to capture includes a vigorous struggle. Animals in a highly excited state may require more drugs for initial induction.[12] Sea otters in our studies required higher doses of fentanyl than were recommended by Sawyer and Williams.[11] In addition, to reduce stress, immediate release was preferred to holding the animals after processing. Thus, use of long lasting tranquilizers is not advisable. We found fentanyl in combination with diazepam alone produced smooth inductions and provided anesthetic effects lasting at least 30 to 40 minutes. Diazepam is now reversible with flumazenil but we have not found this necessary, and in fact believe the residual diazepam actually may help reduce post-reversal stress.

Sea otters tolerated and sometimes required relatively high doses of fentanyl (one adult female required a dose of 0.75 mg/kg before she was adequately immobilized), but generally doses greater than 0.33 mg/kg provide little benefit in terms of improved anesthesia. Less narcotic can be used when biological sampling and tagging are the only purposes of capture. Electro-ejaculation procedures required dosages closer to surgical dosages because of the intense physical stimulation.

Fentanyl is known to have excitatory central nervous system effects.[6] Diazepam has been used to control tremor and seizures in sea otters.[1,11], and we found it more effective than azaperone for this purpose.

Naloxone is an effective narcotic antagonist with a history of use in sea otters.[14,15] However, it has a short half life compared with fentanyl, and others using naloxone have reported recycling, although with more potent narcotics.[5] For an animal such as the sea otter, which spends its life in the water, there is significant potential for narcotic recycling to cause fatalities. Sea otters experiencing the effects of recycling slowed to the point of resting quietly in the water. However, several animals began to roll face down in the water for at least several seconds. At this point supplemental naloxone was given, but without intervention the potential for drowning was clear. For sea otters immobilized with fentanyl and azaperone, and reversed with naloxone, the risk of renarcotization increased with increasing dose of azaperone.

To our knowledge, we did not experience any recycling-related mortalities during our studies. However, prior to 1990, 1 of 45 radio instrumented sea otters, reversed with naloxone and released immediately, disappeared and was not seen again. It is not known if the disappearance of this otter was due to recycling and drowning, death due to

complications from surgery, radio failure, or movement out of the study area. However, had the otter died and the body beached it likely would have been recovered if the radio was functional.

Naltrexone, like naloxone, is a pure antagonist but with a longer half life.[6] We observed no signs of renarcotization in 26 otters observed up to 3 hours post-reversal. Other researchers, however, have observed recycling when using naltrexone in low doses with more potent narcotics.[8] Our recommended naltrexone dose of 1.5-2X the fentanyl administered during processing (usually 0.44 mg/kg - 0.66 mg/kg) is much higher than 0.01 mg/kg recommended by Williams et al[15] for naloxone. However, because naltrexone has little agonistic effect and sea otters appear to tolerate relatively high doses, giving extra to prevent recycling seems prudent. Others have published similar recommendations.[5,8]

Generally, body temperatures of sea otters increased during processing, and monitoring temperature closely is critical to ensure well-being of the otters throughout the handling procedure. Loss of temperature control is common under anesthesia, particularly for an animal like the sea otter which has a dense pelage with extremely good insulating properties. Once an otter is removed from the water, its temperature can rise rapidly, depending primarily on environmental conditions. We have found that keeping otters wet prior to sedation, either by holding them in a net pen or by running water over them when they are held in capture boxes, is key to maintaining normal body temperatures throughout the handling period.

We had only one drug-related mortality, which involved a sea otter with lungs compromised by sea water aspiration. Impaired lung capacity is a known risk factor when using fentanyl,[6] but we did not realize the extent of injury at the time of anesthetization. Over-all, our capture and drug-related mortality rates appear to be well below what is often experienced when handling wild animals.

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Table 1. Mean (and standard deviation) dosages of fentanyl, diazepam and azaperone (mg/kg) administered to sea otters during captures for surgical instrumentation and biological sampling.

		Fentanyl						
		Not supplemented		Supplemented			Diazepam	Azaperone
Procedure	Mean handling time (min)	Dosage mg/kg (SD)	Percent of captures	Initial dosage mg/kg (SD)	Total dosage mg/kg (SD)	Percent of captures	Dosage mg/kg (SD)	Dosage mg/kg (SD)
Surgical *n* = 295	41	0.34 (0.08)	85	0.29 (0.06)	0.39 (0.09)	15	0.10 (0.008)	0.26 (0.02)
Sampling *n* = 303	30	0.23 (0.04)	90	0.21 (0.05)	0.30 (0.09)	10	0.07 (0.008)	0.25 (na)

Table 2. Results of logistic regression modeling the probability of that supplemental fentanyl will be required (insignificant interaction terms not included).

		Results of full model			Best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = supplemental	Intercept	0.03	0.86	367.3	1.00	0.32	365.3
	initial fentanyl	17.17	0.0001		17.17	0.0001	
	sedative type	3.89	0.05		4.03	0.05	
	capture type	18.75	0.0001		18.77	0.0001	
	initial temp.	0.003	0.96		–	–	

Table 3. Results of logistic regression modeling the probability of observing tremor or convulsion (insignificant interaction terms not included).

		Results of full model			Best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = tremor / convulsion	Intercept	0.32	0.57	281.9	0.45	0.50	276.5
	sedative type	3.47	0.06		39.33	0.0001	
	sedative dose	0.44	0.51		—	—	
	capture type	0.20	0.66		—	—	
	body temp.	0.16	0.69		—	—	

Table 4. Results of logistic regression modeling the probability of observing narcotic recycling while using naloxone for reversal. Significant interaction in full model dictated separating diazepam and azaperone anaesthetizations.

		Results of full model			2nd best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = narcotic recycling	Intercept	4.05	0.04	155.5	0.05	0.82	159.7
	initial naloxone dose	5.93	0.01		2.13	0.14	
	sedative type	2.12	0.14		14.23	0.0002	
	sed. type*nal. dose intactio	4.89	**0.03**		—	—	
	handling time	5.85	0.02		4.02	0.04	
	holding time	2.37	0.12		3.36	0.07	
			diazepam			azaperone	
Y = narcotic recycling	Intercept	0.77	0.38	115.5[1]	4.21	0.04	42.9[1]
	initial naloxone dose	0.45	0.50[2]		4.82	0.03[3]	naloxone
	handling time	1.86	0.17		4.38	0.04	
	holding time	0.53	0.47		2.26	0.13	

[1] Data from azaperone and diazepam anaesthetizations separated — AIC values not comparable between models or with full model.
[2] relationship similar using diazepam dose rate P = 0.38, AIC = 115.2.
[3] relationship similar using azaperone dose rate P = 0.03, AIC = 43.4.

Table 5. Results of logistic regression modeling the probability of an otter reaching a hyper thermic (>40 ˚C) state while sedated (insignificant interaction terms not included).

		Results of full model			Best fit model		
	full model variables (X_i)	Wald χ^2	*P*	AIC	Wald χ^2	*P*	AIC
Y = hypothermia	Intercept	54.68	0.0001	164.3	57.123	0.0001	161.5
	fentanyl dose	0.01	0.92		—	—	
	sedative type	0.84	0.36		—	—	
	capture type	2.03	0.15		—	—	
	handling time	2.50	0.11		10.82	0.001	
	initial temp.	53.89	0.0001		56.26	0.0001	

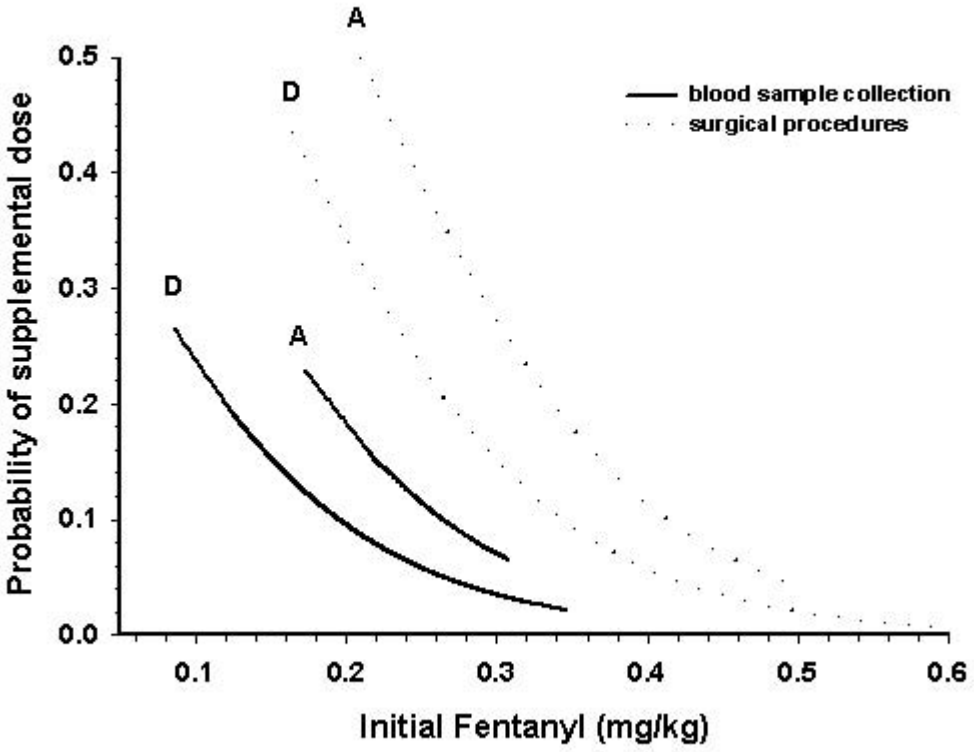

Figure 1. Probability that a sea otter will require supplemental fentanyl during processing in relation to the initial dose of fentanyl administer, the purpose of anesthesia (sample collection only vs. surgical instrumentation) and sedative used (D = diazepam vs. A = azaperone).

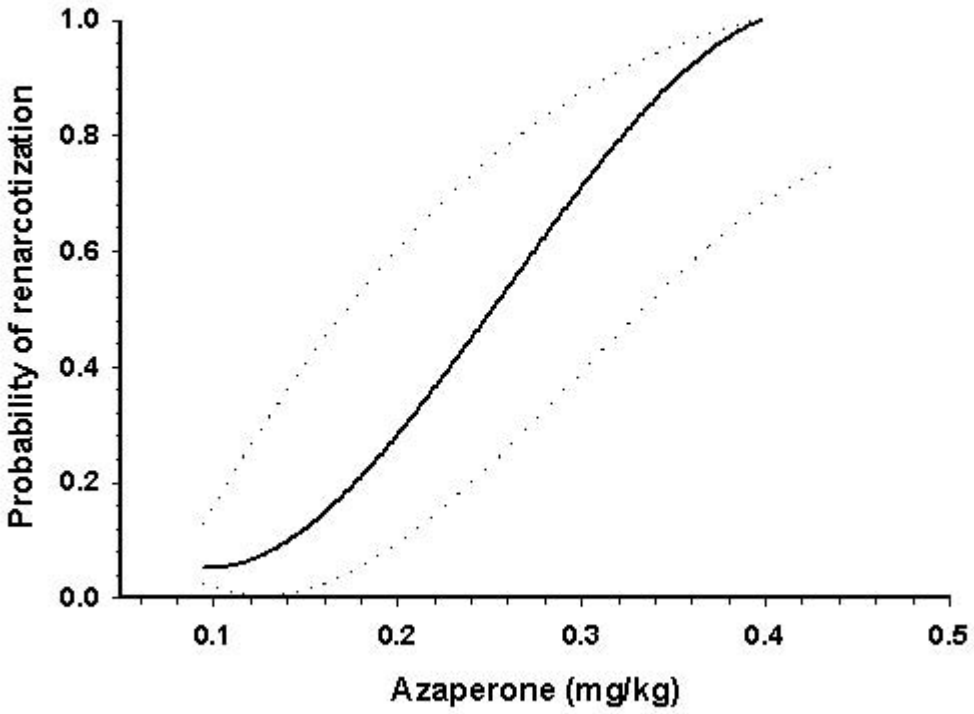

Figure 2. Probability of narcotic recycling for sea otters anesthetized with fentanyl and azaperone, and reversed with naloxone.

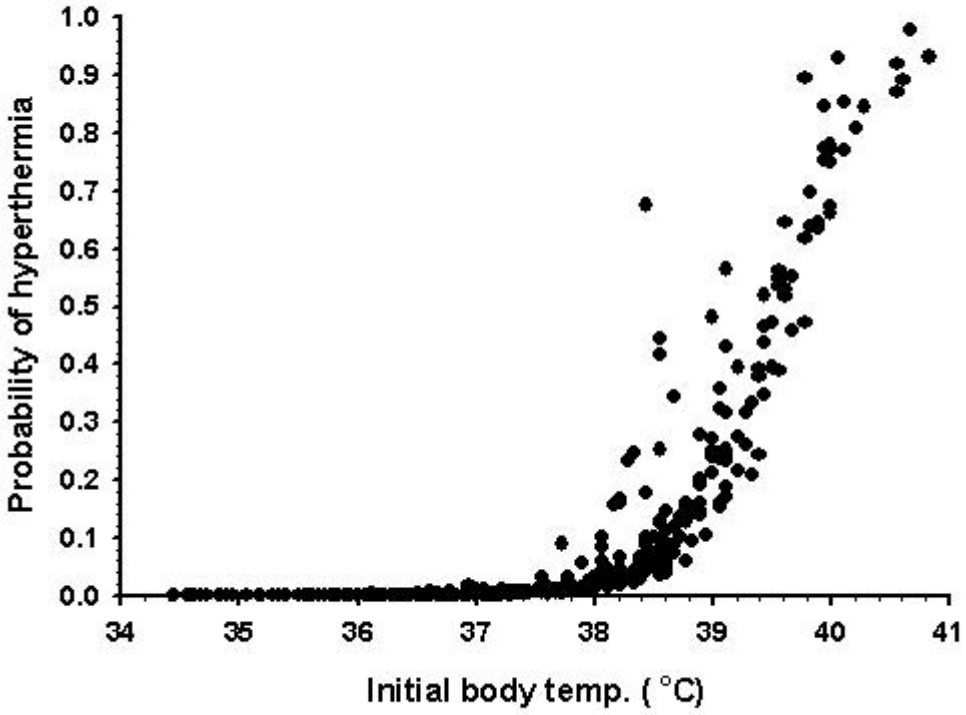

Figure 3. Probability of an anesthetized sea otter reaching a hyper thermic condition (>40 ˚C) in relation to its initial body temperature at the time of drugging.

APPENDIX SO-03

Long-term Changes in the Abundance and Growth of the Pacific Blue Mussel, *Mytilus trossulus*, in a Heavily Oiled Bay in Prince William Sound, Alaska[1]

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Long-term Changes in the Abundance and Growth of the Pacific Blue Mussel, *Mytilus trossulus* in a Heavily Oiled Bay in Prince William Sound, Alaska

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and Susan M. Saupe

Abstract

Since the 1989 *Exxon Valdez* oil spill in Prince William Sound, Alaska, several unrelated studies have reported reduced densities of *Mytilus trossulus* in oiled areas. To examine the long-term changes in mussel populations we combined the results of two projects that conducted research in the heavily oiled Herring Bay to create a data set spanning five years from 1993-1997. Both projects collected mussel samples from similar sites using randomly placed quadrats and carried out growth studies on tagged mussels. Significant differences in mussel density and growth were found between oiled and control sites and between years. Mussel density on oiled sites dropped to their lowest level in 1996-97. Mussel density at the control sites did not change significantly until 1997 when it increased compared to 1996. Mussel length-frequency distributions at oiled sites were more strongly skewed to the right than at control sites in 1994, '95 and '97 indicating that a larger proportion of the population was composed of young individuals at oiled sites than at control sites in those years. Where differences in mussel growth rates were observed over the course of the study, control mussels grew faster than those at oiled sites. Prolonged exposure to residual polynuclear aromatic hydrocarbons may have contributed to the decline of mussel populations at oiled sites in Herring Bay from 1993-1996. However, other factors such as heavy predation by whelks cannot be ruled out.

Keywords: *Mytilus trossulus*, *Exxon Valdez*, oil spill, Prince William Sound, population trends, long-term recovery, growth.

Introduction

After the *Exxon Valdez* oil spill in 1989 several projects studied the response of mussel, *Mytilus trossulus*, populations in Prince William Sound (PWS). These studies found mussels had reduced densities and growth rates in oiled areas. Houghton *et al*. (1993a, 1993b, 1993c, and 1993d) found differences in the abundance of *Mytilus* on oiled and control sites in western PWS resulting from shoreline cleaning during 1991-92. *Mytilus* had reduced density and biomass on sheltered rocky shores in PWS that were oiled by the spill (Highsmith *et al*. 1994). Highsmith *et al*. (1996) found reduced growth at oiled sites in Herring Bay beginning in 1993 that persisted at least until 1995. Babcock *et al*. (1998) observed a significant decline in mussel densities from 1994 to 1996 in both restored and oiled reference (unmanipulated) beds, but this decline was probably not caused by the spill.

In the decade since the *Exxon Valdez* oil spill, no one project has collected the data necessary to examine long-term changes in mussel population dynamics in oiled areas. To extend the time series of data available, we combined data from two projects that carried out research on mussels in the same location. The Herring Bay Restoration studies (HBR) initiated mussel studies during 1993-1995 which examined mussel density and growth in oiled versus non-oiled intertidal areas. The Nearshore Vertebrate Predator (NVP) project studied mussels in three major areas including Herring Bay during 1996-1998 and investigated their role as one of several species of prey that because of their relative scarcity may be limiting the recovery of predators such as sea birds and sea otters in oiled areas (Holland-Bartels 1998). Despite the different objectives of these two projects, comparable data were collected on mussel density and growth in Herring Bay covering the period 1993-1997.

Methods

Location

Herring Bay lies on the NE end of Knight Island near the middle of Prince William Sound, Alaska (Figure 1). The bay's shoreline is categorized as sheltered rocky habitat, and much of it was oiled by the *Exxon Valdez* oil spill. The HBR study sites where data was collected on mussel density, size, and growth were selected based on matched oil and control sections of shoreline. Similar site topography, slope, exposure and presence of comparable mussel beds were considered in site selection. The NVP project site selection was systematic, dividing Herring Bay's shoreline into 200 m segments. Data presented here will be limited to four sites (oil 1 /control 1 and oil 2 /control 2) which were common between the HBR project and the NVP project (Figure 2). An additional third paired site, which was part of the HBR study is not considered here because there was no matching NVP site pair.

Mussel Density and Size-Frequency Distribution

The HBR mussel study began in June 1993. The experimental design consisted of four vertical transects set perpendicular to the shoreline. The position of the first transect was randomly located systematically in the first quarter of the mussel zone. The other

three transects were located by consecutively adding one-fourth of the total mussel zone length to the first transect location. The width of the mussel band along each transect was measured and multiplied by a random number between 0 and 1 (representing a proportion of the width of the mussel zone) to establish the placement of the upper right corner of the sampling quadrat. Quadrat size varied from 0.01 to 0.1m^2 depending on the year. All mussels were removed from the quadrat, bagged and frozen for sorting in the laboratory. Sites were revisited in September 1993, May 1994, September 1994, and May 1995. Quadrats sampled after the first sample period were placed one meter to the left of the previous quadrats. In the laboratory, mussels were thawed and washed in a 0.5 mm sieve. Mussels retained by the sieve were counted and maximum shell length was measured to the nearest millimeter.

The NVP project began in 1996. Each site consisted of a 200 m long shore segment within which ten vertical transects were laid 20 m apart. The first transect to be sampled was placed a random distance between 0 and 20 m from the beginning of the segment. Each transect was laid from the upper limit to the lower limit of the mussel zone perpendicular to the shore. A 500 cm^2 quadrat was positioned a random distance along each transect. All mussels were removed from within the quadrat and handled in the same manner as the HBR study. The shore segments sampled in 1997 were offset 400 m from those sampled in 1996.

Mussel Growth

The HBR growth study removed mussels from the middle of the mussel zone near the sites where density estimates were made, bagged them separately in seawater and returned the mussels to a research vessel where each mussel was tagged or etched with a number on its shell and it's length measured to the nearest 0.05 mm with calipers. Mussels >15 mm were chosen for the growth study. Superglue gel was used to attach an individually numbered polyethylene shellfish tag (Hallprint, Pty. Ltd., Holden Hill, South Australia) to the shell or, alternatively, an engraving tool was used to etch a number directly into the shell. The tagged or etched mussels were stored in a flowing seawater tank until the next low tide, then returned to the sites from which they were removed. Thirty to sixty tagged mussels were placed inside a 1/4 inch, wire mesh cage (20 x 20 x 7 cm) which was attached to the substrate with anchor screws. Growth was measured as the difference in total length between visits. Initial measurements were taken in June 1993 and mussels were collected, measured, and returned to their cages in August 1993, May 1994, August 1994, May 1995, and August 1995. Mussels were caged because of high mortality by predators in an early test deployment. To test for differences in flow rates inside and outside cages, calcium sulfate dissolution cylinders were placed adjacent to each other, one cylinder was caged and the other uncaged. The cages were placed in the intertidal region in areas representing a wide range of flow rates.

In June 1997, the NVP researchers used a method of measuring growth which caused minimal disturbance to the mussel. Mussels were tagged *in situ* in the intertidal region between 1.2 - 2.0 m above mean lower low water. The tagging sites were a systematically selected subset of a series of mussel study sites, each a 200 m length of shore, that were distributed systematically along the entire shoreline of Herring Bay after the first site had been selected randomly. Mussels >15 mm in shell length were

haphazardly chosen to be tagged. Mussels <15 mm in length were too small to be tagged. Mussels were never densely packed at the tagging sites, therefore intraspecific inhibition of growth through overcrowding was not a factor in the mussel growth. Each mussel received the same type of shellfish tag used by the HBR studies and a 2 x 8 mm plastic, reference strip manufactured as ornamental fly-tying ribbon. Superglue gel was used to affix the numbered tag and reference strip to the mussel. Mussels were tagged in place. The reference strip was placed with its axis along the vector of maximum growth and flush with the posterior edge of the mussel's valve. Growth was measured to the nearest 0.1 mm from the posterior edge of the reference strip to the posterior edge of the shell. A 30 x 30 x 5 cm Vexar (high density polyethylene netting) cage with 3.2 mm mesh was bolted around 25 tagged mussels at each site. Twenty-five mussels were also placed directly next to the cage to test for a cage effect. The tagged mussels were revisited during July 1997 and June 1998 to measure growth using the reference strips and to replace lost mussels. At the end of the experiment, July 1998, the mussels were removed and transported to the laboratory for measuring. Growth was measured using calipers to the nearest 0.1 mm as the difference in length from the umbo to the end of the reference strip and to the posterior edge of the valve.

Statistical Analysis

Three-way analysis of variance was used to test for differences in mean mussel densities between years, oil and control treatments and individual sites. An F-test was used for planned comparisons of density between oiled and unoiled treatments in each year. The Tukey-Kramer *post hoc* test for differences in mean mussel density between years assuming equal variances and with unequal sample sizes was used to test for between-year differences in mussel density (treatments combined). Size-frequency data were grouped into 2 mm size classes and tested for differences between oil and control site pairs 1 and 2 from 1993-1997 using the two-sample Kolmogorov-Smirnov test on percent cumulative size-frequency data. The mussels used in the length-frequency analysis were removed from the quadrats for measurement. These mussels because of their close proximity to one another might not necessarily be expected to grow independently. However, our growth data showed highly variable growth between side-by-side individuals reaching differences as great as 4 mm mo^{-1} (unpublished data) and indicating that the length-frequency data was based on independent measurements. Moreover, because of the large number of individuals required for size-frequency analysis it would have been impractical to select each mussel randomly. Mussels <2 mm were enumerated differently by the two projects and were not included in the density data but were lumped as a size class for the size-frequency data.

Annual growth rates for the HBR studies (1993/94 and 1994/95) were analyzed using two-sample t-tests for comparisons of oiled sites with control sites. The NVP project annual growth data (1997/1998) were analyzed using a two-way analysis of variance between sites and between oil and control treatments. Welch's approximate t-test (for unequal variances and sample sizes) with Satterthwaite's adjusted degrees of freedom was used to test for differences in growth between oiled and control sites of each site pair (Day and Quinn 1989).

Results

Mussel Density

Mussel density showed significant differences between treatments (oil verses control) and years for mussels >2 mm (Table 1) (Figure 3). The highest mussel density on oiled sites occurred in 1994 and the lowest in 1996-97. Significantly greater densities of mussels on oiled sites compared to control sites occurred only in 1994 (two-tailed F-test, $p < 0.01$). The ANOVA revealed only one a significant interaction, year by treatment (Table 1). Therefore between-year differences in mussel density were compared separately at control sites and oiled sites. Densities of mussels on control sites showed no significant between-year differences from 1993 to 1996 (Tukey-Kramer test, $p > 0.05$). Density increased between 1996 and 1997 at control sites (Tukey-Kramer test, $p < 0.05$). Density at control sites was significantly greater than at oiled sites in 1997 (two-tailed F-test, $p < 0.05$). Mean mussel density tended to decrease at oiled sites after 1994, but between-year differences were not significant until 1996. Mussel density at oiled sites in both 1996 and 1997 was less than that at oiled sites in 1994 (Tukey-Kramer tests, $p < 0.01$).

Mussel Size-Frequency Distribution

Percent cumulative length-frequency curves between matched pair oil and control sites were significantly different during 1994, 1995, and 1997 (Table 2) (Figure 4). These differences were consistent in direction over all three years, and showed a greater concentration of individuals among smaller length classes at the oiled sites. The density of mussels >20 mm at all sites was lowest during 1995 and 1996 but all sites experienced strong recruitment of mussels (<5 mm in shell length) during 1996 (Figures 5, 6). The heaviest recruitment of mussels occurred on control site 2 during 1997, the last year of sampling.

Mussel Growth

The HBR studies found significantly higher mussel growth rates on control 2 than oil 2 during 1994/95 (two sample t-test, $p < 0.001$). At site pair 1 the oiled site did not differ from the control in the growth year 1993/94 or 1994/1995. The NVP mussel data for the 1997/98 growth year showed significantly greater growth at control sites 1 and 2 combined than at the oiled sites (Table 3, Figure 7). For that same growth year, further analysis showed that for both site pairs, oil/control 1 and oil/control 2, there was significantly greater growth at the control site than at the oiled site (Welch's approximate t-test with Satterthwaite's adjusted degrees of freedom; oil/control 1, $p< 0.01$, oil/control 2, $p< 0.001$). The mean growth rates obtained by the NVP project were two to three times greater than those found by the HBR studies (Figure 7). This suggests that some aspect of the HBR growth measurement methodology perhaps related to the removal of individuals twice a year may have introduced more stress in the tagged mussels and thereby reduced mussel growth rates compared to those obtained by the NVP project. Caution should be taken when interpreting the low mean growth rate of oiled site 2 during 1997/98 because

of the small sample size (n = 5), owing to heavy mortality of the tagged mussels at the site.

The calcium sulphate dissolution cylinders used by the HBR studies to test for caging effects revealed 4.94 ± 2.71 % (n = 6) less dissolution for caged cylinders. This indicates that there may have been a reduced volume of water, and thus particulates, that flowed past mussels in the cages verses mussels outside the cages. However, the NVP project showed no significant differences in growth between caged mussels and those outside cages (Two-way ANOVA, p = 0.188 with significant heteroscedasticity; Mann-Whitney U-test p = 0.521).

Discussion

Several studies found reduced populations of oiled mussels at various times in western Prince William Sound after the *Exxon Valdez* oil spill (Highsmith *et al.* 1994 and 1996; Houghton *et al*. 1993 and 1994; Babcock *et al*. 1998). Even though the combined data sets for the HBR studies and the NVP project were relatively small and represent only one bay in Prince William Sound, we were able to detect a downward trend in oiled mussel populations after 1994. The most pronounced result was in the reduction of individuals in all size classes between 1994 and 1996-97. Mussel densities reached their lowest point on oiled sites in 1996-97. Recruitment by, mussels <2 mm and the growth of individuals into larger size-classes was evident in 1996 and 1997.

Oiled sites experienced a decline in mussel density after 1994. Although density at control sites was less than that at oiled sites in 1994 a decline comparable to that at oiled sites did not occur at the control sites. Density increased between 1996 and 1997 at control sites. No such increase was observed at oiled sites between 1996 and 1997. Localized oiling and clean-up history on each site may have accounted for the difference in these results. One to two months after the *Exxon Valdez* oil spill the highest concentrations of total polynuclear aromatic hydrocarbons (PAH) were found in mussels suspended in the water column of Herring Bay compared to all other oiled areas (Short and Harris, 1996). Mussels exposed to increasing concentrations of crude oil from 0.12 mg 1^{-1} of 6 mg 1^{-1} showed reduced scope for growth (Gilfillan, 1975). Widdows *et al*. (1987) reported that an order of magnitude increase in mussel tissue concentration of two and three-ring aromatic hydrocarbons can account for an approximately 50% reduction in the growth potential of *Mytilus edulis*. Babcock *et al*. (1998) reported mean total PAH levels in Herring Bay in 1995 were 3,845 μg/g (wet wt.) in surface sediments of mussel beds and 4,191 ng/g (dry tissue wt.) in mussels. This indicates that PAHs were still bioavailable at sites in Herring Bay seven years after the spill, although concentrations were relatively low. Nevertheless, Thomas *et al.* (1999) found that oil-exposed mussels sampled in Herring Bay during 1996 had a significantly lower lethal tolerance (LT_{50}) for air survival than reference groups.

Physical characteristics could in part explain the decline of Herring Bay's mussel population. The HBR studies reported inherent differences between matched oil and control sites due to significantly lower sea surface temperature and salinity on control sites (van Tamelen and Stekoll 1996). It was also discovered using satellite photos during the winter months of 1989 and 1990 that the control sites can experience ice scour (van Tamelen and Stekoll 1996). The combination of temperature, salinity, and ice scour could

explain the lower densities of mussels found on control sites during 1994. During 1996 Prince William Sound experienced a strong El Niño, with sea surface temperatures frequenting 15°-17°C (unpublished data C-LAB Mid-sound buoy, Univ. of Alaska, Institute of Marine Science). This warming event was not catastrophic for *Mytilus trossulus* which has been reported to survive in temperatures ranging from –2°-16°C in the Port of Valdez (Blanchard and Feder 1997) but it may have contributed to higher than normal mortalities resulting in the lowest mussel density recorded on oiled sites in 1996-97. An indirect impact of a warming event may be a change in predation pressure. Sanford (1999) found that a slight decrease in water temperature dramatically reduced the effects of the sea star *Pisaster ochraceaus* on its principal prey *Mytilus californianus* and *Mytilus trossulus*.

Predation may have acted differentially on oil and control sites in Herring Bay. Two species of *Nucella*, *N. lima* and *N. lamellosa*, inhabit the intertidal region in Herring Bay. The HBR studies and the NVP project reported whelks and asteroids were the main predators of *Mytilus trossulus* in Herring Bay. Carroll and Highsmith (1996) conducting research in Kachemak Bay, Alaska, after a lethal winter freeze event, found the whelk, *Nucella lima*, was able to eliminate 60-90% of the mussels at a given site in one season. With *Nucella* present, the mussel population was significantly reduced for over three years. There is evidence of similar forces acting on the mussel population in Herring Bay. The HBR studies reported *Nucella* densities on separate population dynamic sites were approximately 5-10 individuals/m^2 between 1991-1995 (Highsmith *et al*. 1996). Ebert and Lees (1996) found reduced recapture rates in tagged *N. lamellosa* at oiled sites compared to unoiled sites in 1991-92 in Prince William Sound. Ebert and Lees (1996) did not measure *Nucella* density, therefore it is difficult to compare their recapture rate data with the *Nucella* density data of Highsmith et al. (1996) and the present study. Researchers observations also suggest local aggregations of *Nucella* at our mussel study sites in September 1994 (S. Saupe, personal observation). The NVP project reported a mean *Nucella* density of 1.2/m^2 throughout Herring Bay during 1996 and 2.4/m^2 in 1997 (Holland-Bartels *et al*. 1998). The higher densities of *Nucella* from 1992-1995 and their observed aggregations at our mussel study sites in 1994 suggest predation may have been a factor in the reduction of mussels on control sites compared to oiled sites in Herring Bay.

Conclusions

Mussels at oiled sites in Herring Bay during 1993-1997 had not recovered, possibly from a combination of disturbance events. Some residual effect of the oil spill in Herring Bay in combination with predation and possibly warming of sea surface temperatures may have been responsible for the continued decline in mussel abundance at oiled sites for more than eight years after the spill. Although similar decreases in mussel density between 1993 and 1997 have been reported by other investigators over the same period of time as our study, we do not know the complete geographical extent of this trend. *Mytilus trossulus* is the third most abundant taxon found in Prince William Sound's intertidal region (Lindstrom 1999) and a major food source for sea birds and sea otters. A long-term reduction of mussels could have a detrimental effect on their higher order consumers.

Acknowledgments

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Table 1. Three-way analysis of variance of *Mytilus trossulus* densities (no./m sq.) between years (1993-1997), sites and treatments (oiled [O], control [C]) for Herring Bay.

	DF	MS	F Value	P
Year	4	7.6×10^{7}	3.554	0.009
O/C	1	1.2×10^{8}	5.583	0.020
Site	1	1.4×10^{6}	0.076	0.797
Year * O & C	4	1.0×10^{8}	4.665	0.002
Year * Site	4	4.1×10^{6}	0.188	0.944
O & C * Site	1	1.8×10^{6}	0.087	0.769
Year * O & C * Site	4	3.0×10^{6}	0.141	0.967
error	106	2.1×10^{7}		

Table 2. Two-sample Kolmogorov-Smirnov statistics of *Mytilus trossulus* percent cumulative length-frequency data for oil and control (O/C) site pairs 1 and 2.

	O/C 1		O/C 2	
Year	D	P	D	P
1993	0.0581	ns	0.1401	ns
1994	0.4089	<0.001	0.0847	<0.001
1995	0.2142	<0.05	0.2781	<0.01
1996	0.0172	ns	0.2727	ns
1997	0.1936	<0.05	0.6493	<0.001

Table 3. Two-way analysis of variance of *Mytilus trossulus* 1997/98 annual growth rates (mm/mo) on oiled and control site pairs 1 and 2 in Herring Bay. O/C = oiled/control treatments.

	DF	MS	F Value	P
Site	1	9.997	19.039	<0.001
O/C	1	18.228	34.716	<0.001
Site * Oil	1	3.817	7.270	0.008
error	110	0.525		

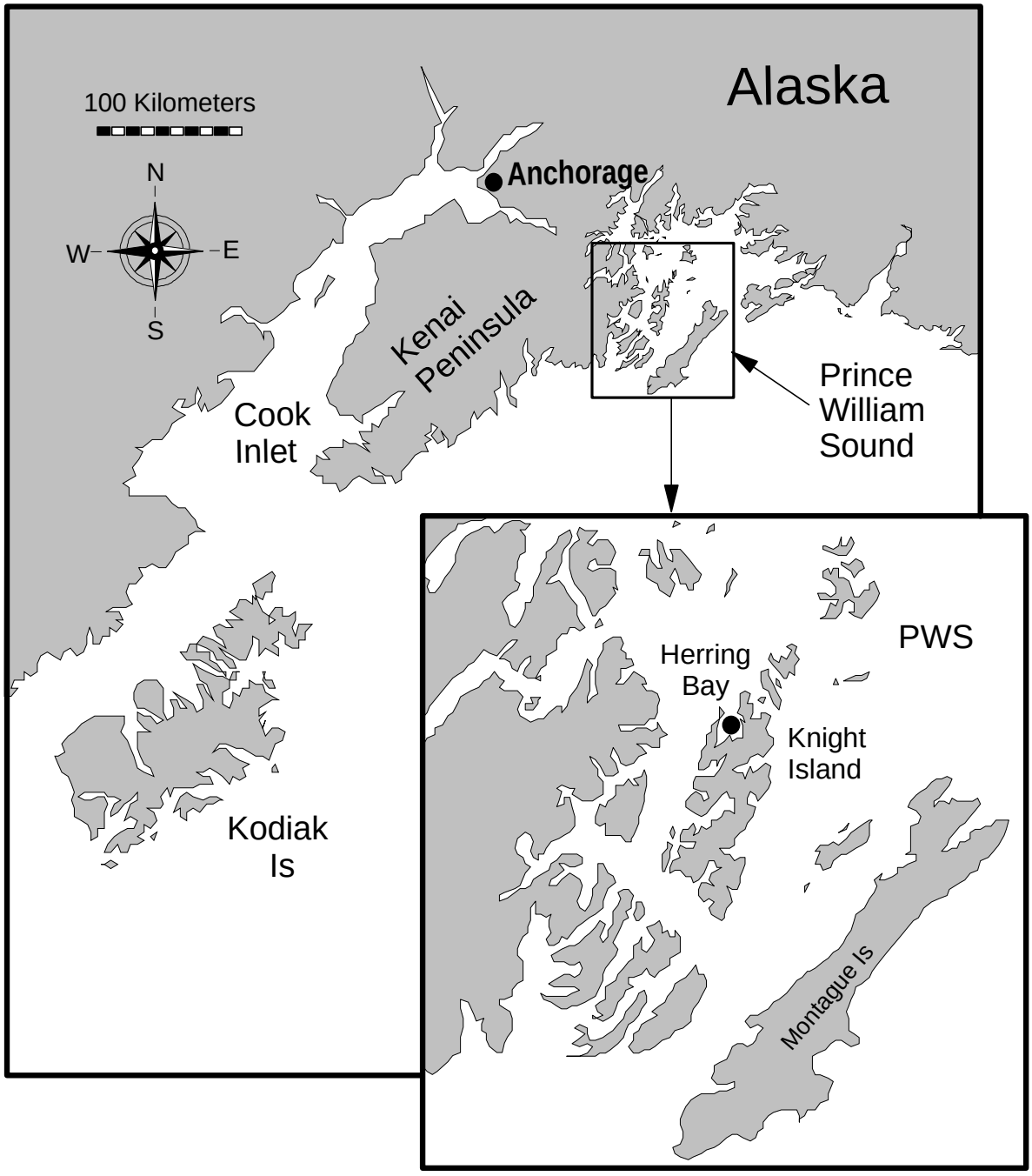

Figure 1. Map showing the location of Prince William Sound, Alaska, and Herring Bay.

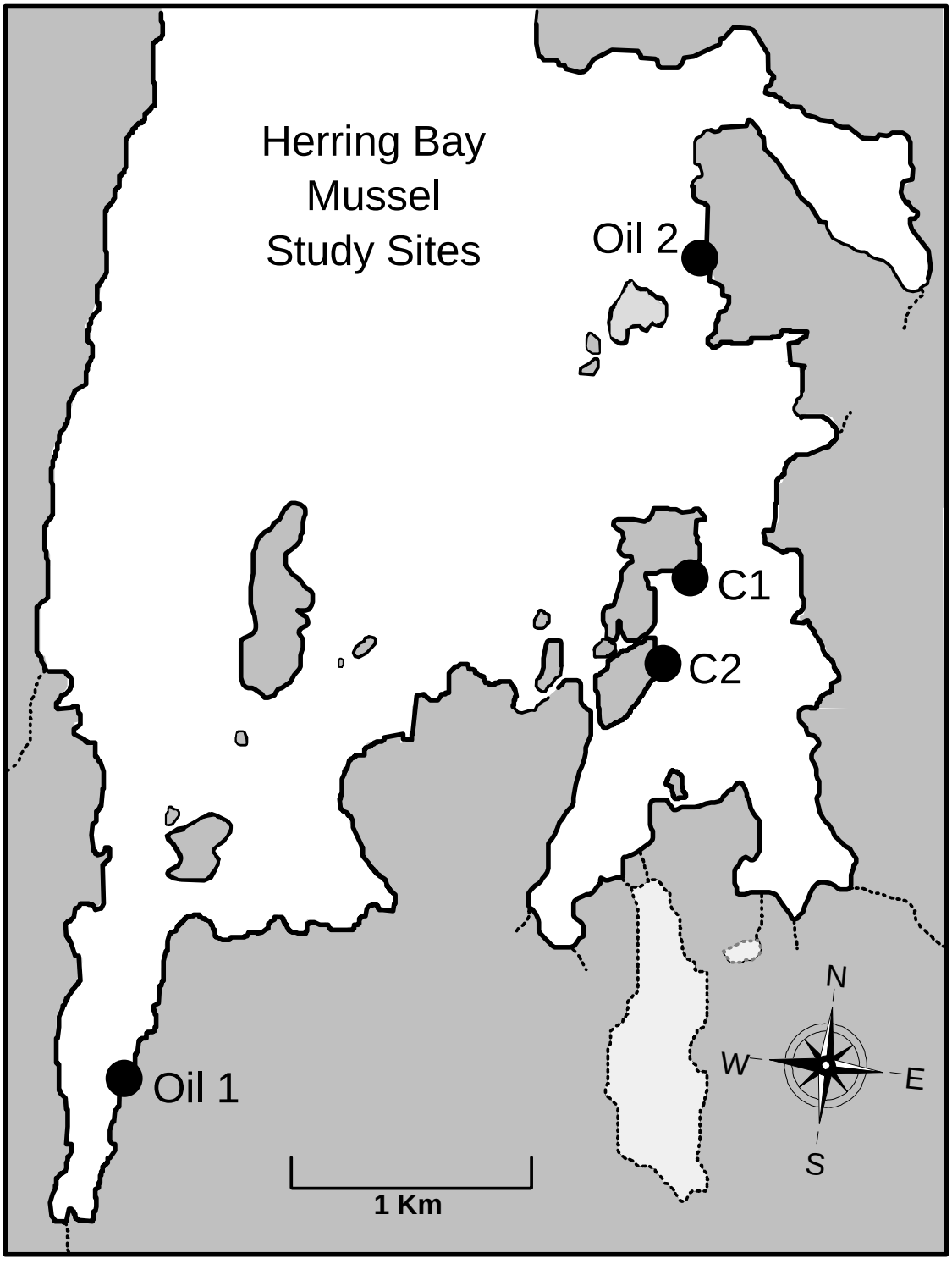

Figure 2. Map of oil and control site pairs 1 and 2 in Herring Bay, Prince William Sound, Alaska.

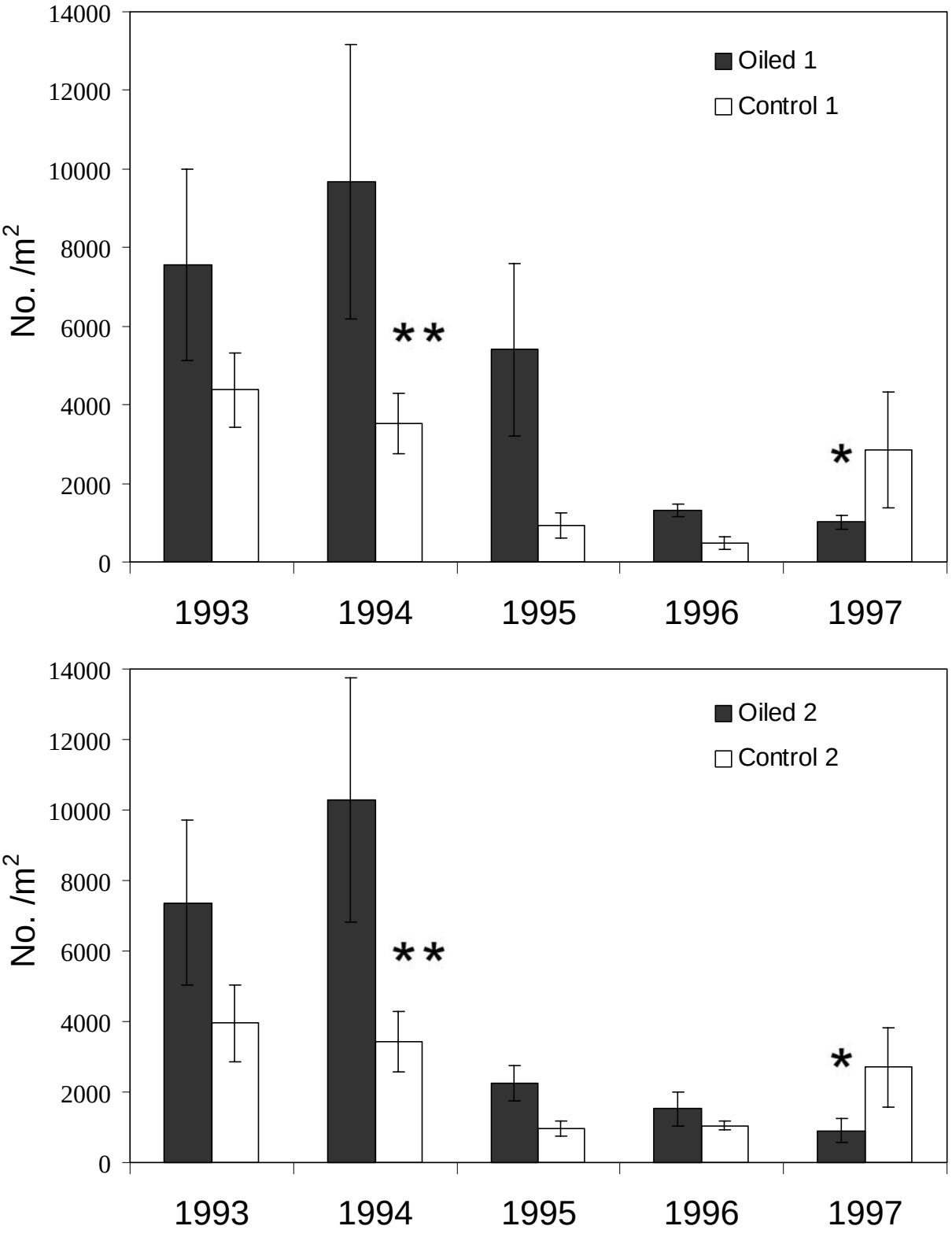

Figure 3. *Mytilus trossulus* densities (no./m^2) between 1993 and 1997 for oiled and control site pairs 1 and 2 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project. The asterisk indicates significance between oiled and control sites 1 and 2 combined (**, $p < 0.01$;*, $p < 0.05$).

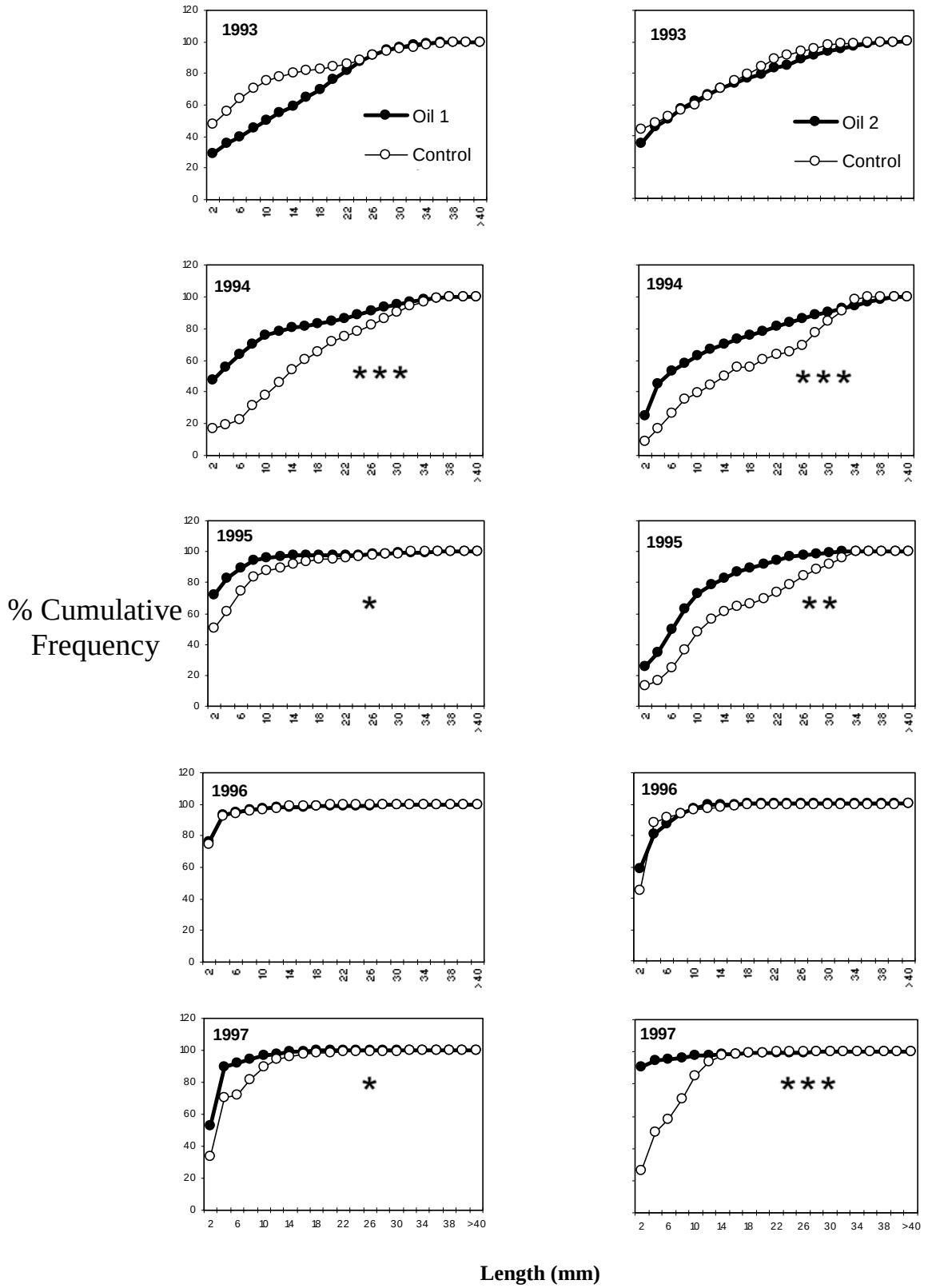

Figure 4. Percent cumulative size-frequency curves of *Mytilus trossulus* for oil and control site pairs 1 and 2 from 1993-1997 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project (***, $p < 0.001$;**, $p < 0.01$;*, $p < 0.05$).

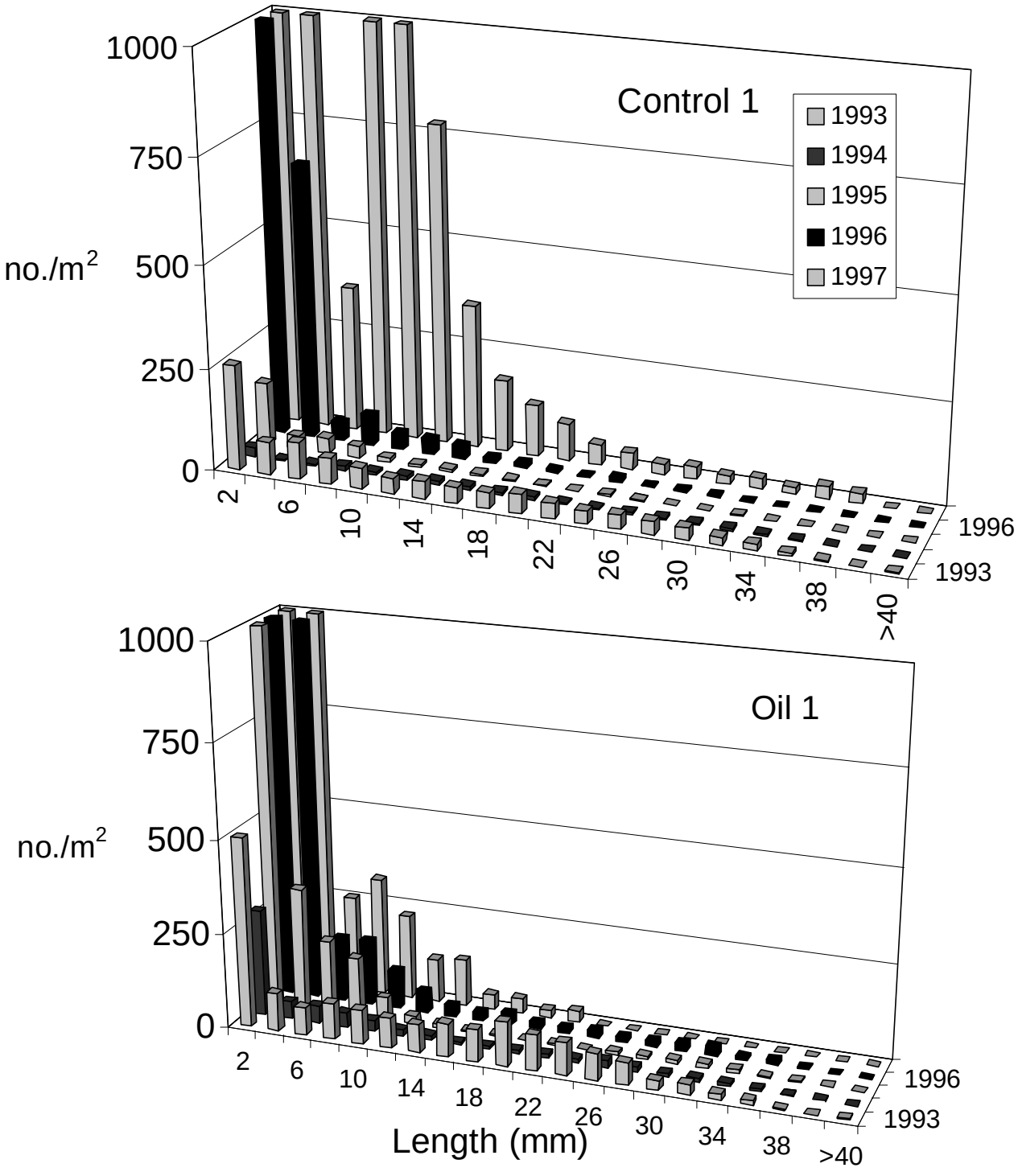

Figure 5. Size-frequency distribution of *Mytilus trossulus* for oil and control site pair 1 from 1993-1997 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project.

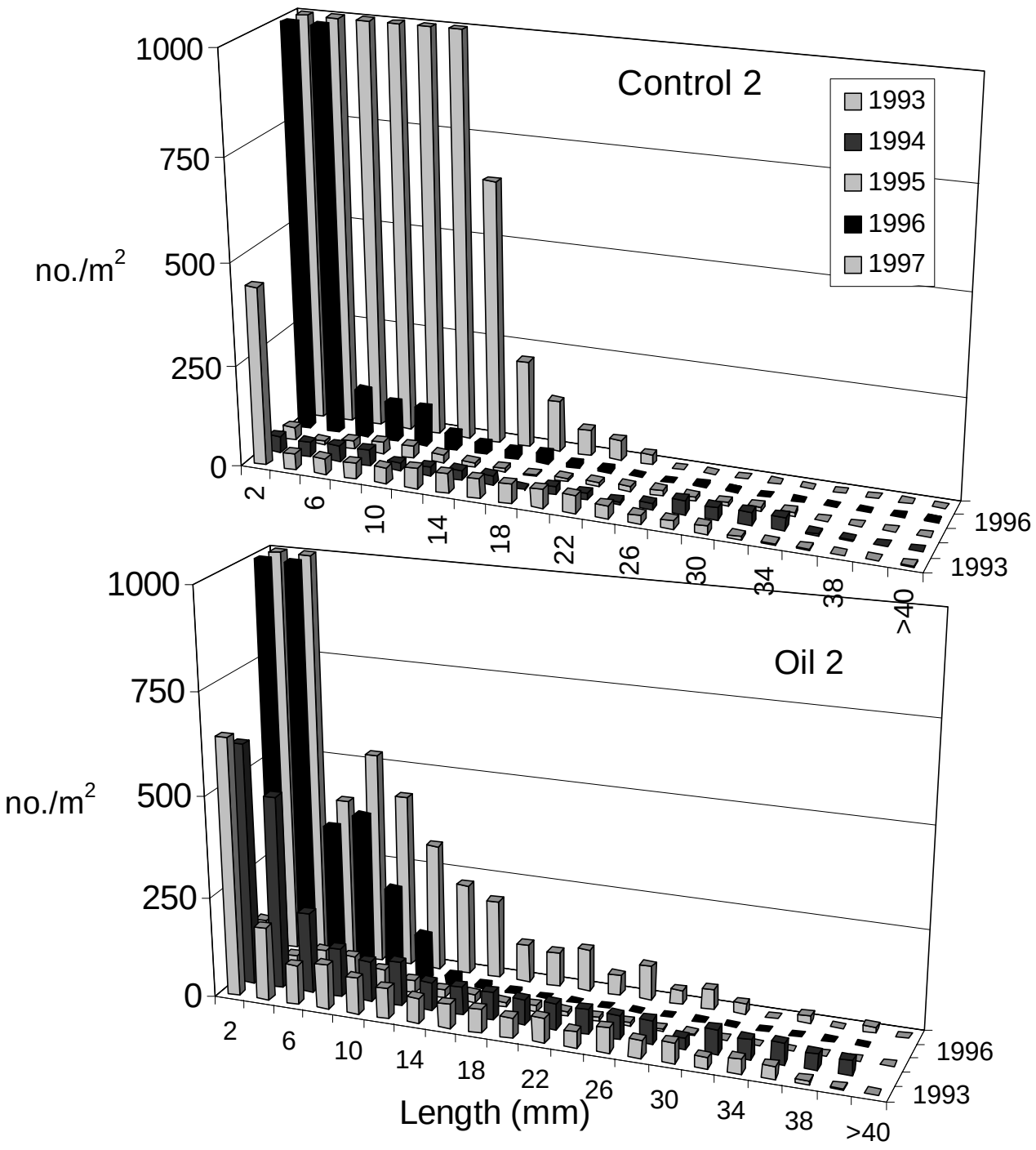

Figure 6. Size-frequency distribution of *Mytilus trossulus* for oil and control site pair 2 from 1993-1997 in Herring Bay. 1993-1995 data were from the HBR studies and 1996 and 1997 data from the NVP project.

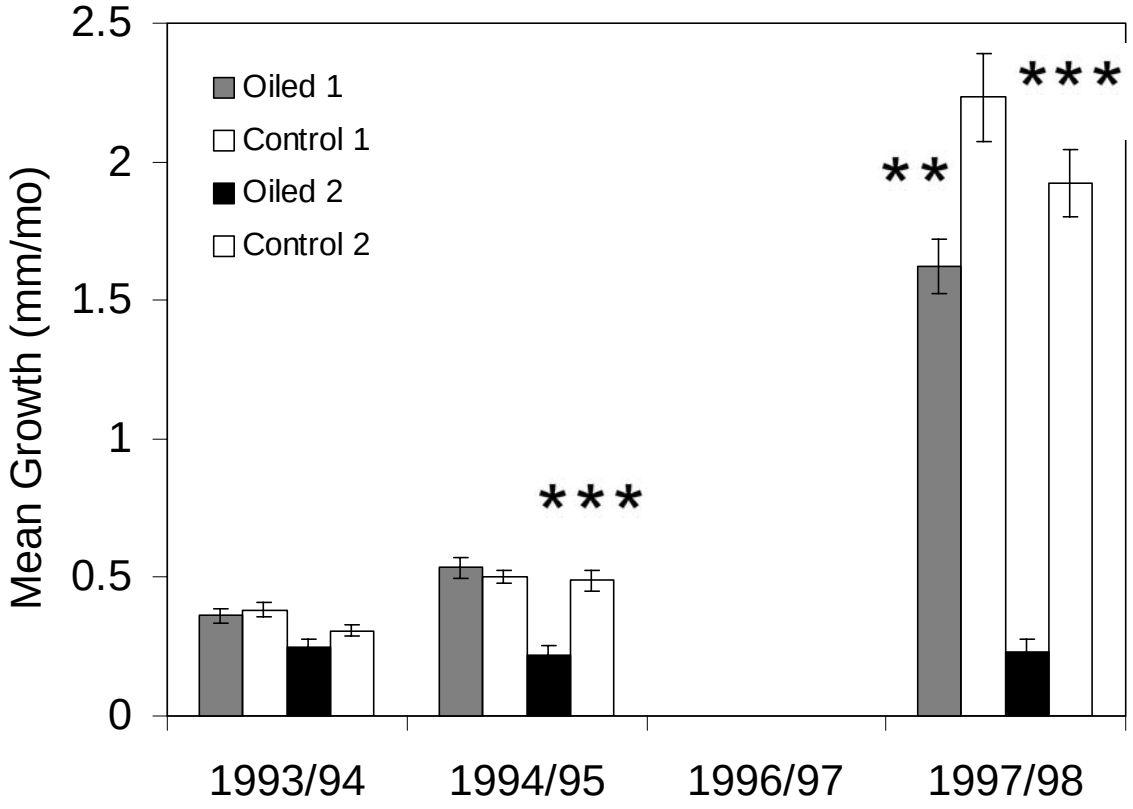

Figure 7. Mean annual growth (mm/mo) of *Mytilus trossulus* for oiled and control site pairs 1 and 2 in Herring Bay (***, $p < 0.001$;**, $p < 0.01$). Growth years 1993/94 and 1994/95 data were from the HBR studies. Growth year 1997/98 data were from the NVP project.

APPENDIX SO-04

Comparison of Age-length and Growth-increment General Growth Models of the Schnute Type in the Pacific Blue Mussel, Mytilus trossulus Gould[1]

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Comparison of Age-length and Growth-increment General Models of the Schnute Type in the Pacific Blue Mussel, Mytilus trossulus

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Abstract

Models of Mytilus growth, based mostly on length-at-age data, have typically taken the form exemplified by the von Bertalanffy or Gompertz formulations. We examined growth in the Pacific Blue Mussel, Mytilus trossulus, in Prince William Sound, Alaska. Mussels were tagged with individually numbered, plastic tags and a plastic reference marker was glued at the posterior edge of the shell. The mussels were tagged at 13 sites in July 1997 and were collected in July 1998. Age was determined from surface growth rings on the shell, and shell length at maximum annulus was measured. Annual deposition of the growth rings was verified through radial sections of mussel valves, aided by acetate peels, in conjunction with in situ annual growth measurements. Growth-increment was measured from the reference marker to the posterior edge of the shell. Growth was modeled with the Schnute general growth model for age-length data which provides a convenient analytical method for selecting among all previously published growth models. An analog of the Schnute model designed for mark-recapture data (ie growth-increment data) was used to model growth measured in situ on the tagged mussels, as well as growth between the outermost annuli on mussel shells. Bootstrap confidence intervals were obtained for all parameters of the model and for model predicted lengths at each annulus. Confidence intervals of the between-annuli growth-increment model overlapped those of the age-length model at all annuli when growth over the entire range of ages in the population was estimated. Differences in growth model parameters between the age-length model and the mark-recapture analog could be accounted for solely by inherent differences in age-based versus length-based models. Growth estimates generated from between-annuli measurements were equivalent to growth estimates obtained from mark-recapture measurements of annual growth.

Keywords: Growth, Mytilus trossulus, Pacific Blue Mussel, Schnute Model, von Bertalanffy Model

1. Introduction

Accurate models of individual growth are fundamental to reliable estimates of secondary production. Growth is often estimated by relating the length of an individual to its age, which in bivalve mollusks is usually determined by examination of disturbances in the valves caused by changes in seasonal, tidal or circadian accretionary growth. Aging bivalves has usually involved counting rings on the shell surface produced during periods of greatly reduced or suspended growth, usually during winter months at higher latitudes (Haskin, 1954; Lubinsky, 1958; Seed, 1969; Andrews, 1972; Theisen 1973; Seed and Richardson, 1990). However, in mussels it is often necessary to examine growth lines in radial sections of the shell to obtain accurate and reliable estimates of age (Lutz, 1976; Thompson, 1984; Anwar et al., 1990). Improved resolution of growth lines can be achieved by inspection of acetate peels of shell sections (Rhoads and Pannella 1970).

Mytilus growth, usually measured as an increase in shell length with age, has typically been modeled with the von Bertalanffy or Gompertz formulations (Seed and Suchanek, 1992). These formulations assume that growth is determinate and, therefore, ceases at some fixed adult size. Asymptotic growth may not always be realized in the life span of Mytilus (Seed, 1980; Gardner and Thomas, 1987). Schnute (1981) proposed a general size-at-age growth model that incorporates the von Bertalanffy and Gompertz formulations as well as many others as submodels. The model has four (or fewer) parameters, the estimates of which are almost invariably statistically stable. Special cases of the model include not only asymptotic growth, but also linear, quadratic, or exponential growth. A model analogous to the Schnute model proposed by Baker et al. (1991) allows the use of growth increment data from mark-recapture studies to model growth if one of the parameters, usually the starting age, is specified beforehand. Although it would seem of value to employ growth-increment models to validate age-length estimates of growth, Francis (1988) cautions that age-length models are age-based whereas growth-increment models are length-based, and therefore the two techniques describe different population parameters. However, comparison of growth models that use between-annuli, growth-increment measurements with those that use growth-increment measurements from actual mark-recapture data could circumvent this problem. Once validated, between-annuli growth-increment measurements can serve as a surrogate for growth-increment measurements from mark-recapture methodology and can therefore reduce field time. Mark-recapture methods require the expenditure of time and resources to mark individuals, suffer from the risk of data loss from the mortality of marked individuals or tag loss, and require at least two visits to the field site over the course of at least one year to monitor growth. The annulus growth-increment method requires only one visit to the field when mussels are collected or measured in the field. The method also allows for the comparison of historical growth rates using growth-increment data from geologically preserved assemblages of shells.

We report here the results of a comparison of the length-at-age and growth-increment versions of the Schnute model applied to the same collection of mussels (Mytilus trossulus Gould 1850) from Prince William Sound, bordering the Northern Gulf of Alaska. We address the problem of using models based on mark-recapture data to validate age-length estimates of growth with the aid of a growth-increment model based on interannular distances on mussel shells. To our knowledge this is the first attempt to model growth in any

invertebrate by comparing size-at-age and size-increment versions of the same general growth model in the same population.

2. Methods

2.1 Tagging

Mussels, Mytilus trossulus, were tagged in situ in the intertidal region on the shores of Montague Island and Knight Island bordering Montague Strait, Prince William Sound, Alaska (Figure 1). Tagging sites were a systematically selected subset of a series of mussel study sites, each a 200-m length of shore, that were distributed systematically along the shoreline of the two islands after the first site had been selected randomly. Mussels were tagged between 1.2 and 2.0 m above mean lower low water at eight sites on Montague Island and five sites in Bay of Isles, Knight Island. To minimize the stress on the mussels associated with the common practice of removing them from the substrate, transporting them to a central location for tagging and then returning them to the site of collection and caging them until they reattach themselves to the substrate, and to avoid caging the mussels for a long period of time (which may affect growth), mussels were tagged, in place with minimal disturbance and were not caged. An individually numbered, flexible, polyethylene shellfish tag (Hallprint Pty. Ltd., Holden Hill, South Australia) was glued to a pre-dried spot on one valve of each mussel using superglue in gel form. The spot on the shell was usually dried by sequentially wiping with an alcohol wipe and a dry towel. If necessary, compressed air in a can was gently blown on the spot after it was swabbed with the alcohol wipe. A reference strip consisting of a 2 x 8 mm length of plastic, ornamental, fly-tying ribbon was also glued to the same valve. The reference strip was glued in place with its long axis along the vector of maximum growth of the valve and its beveled posterior edge sloped toward and flush with the posterior edge of the valve.

The mussels were collected approximately one year after they were tagged and were transported to the laboratory where they were shucked and the valves separated. Tag growth-increment, defined as the difference between length at collection (distance from umbo to posterior edge of valve along the vector of maximum growth) and length at tagging (distance from umbo to posterior edge of reference strip), was measured to the nearest 0.1 mm on the tagged valve of 110 surviving mussels. The mussels ranged in initial length from 16.5 to 43.4 mm. We tagged no mussels less than 16 mm in maximum shell length because of the mechanical difficulty of gluing tags and reference strips to small mussels. Mussels exceeding 40 mm in shell length were rare in our study area.

2.2 Aging and between-annuli length-increments

To relate the rings on the surface of the shell of Mytilus trossulus to the growth lines that are formed annually in the nacreous layer of the shell (Lutz, 1976) we made radial sections of the valves of 90 Mytilus collected in our study areas on Montague Island and Knight Island in 1996. As broad a size range of mussels as possible was selected haphazardly from samples of mussels collected at four to six sites chosen at systematic intervals along the shore within each study area. The mussel shells were prepared for aging using a method modified after Rhoads and Pannella (1970). All tissue was removed from the mussel shell, and the valve with the least erosion was selected for aging. The valve was washed with

detergent to remove any lipid residue, dipped briefly in a 1.0 % HCL-water solution, rinsed immediately with freshwater, and dried. Each valve was imbedded in a block of epoxy resin (Epofix; Struers, Westlake, Ohio). A radial section of the imbedded valve was made using a precision cut-off machine (Accutom-2) with a diamond blade. The section was made perpendicular to the surface of the valve along a plane that bisected the umbo and the posterior edge of the valve (the vector of maximum growth). The cut valve surface was wet-polished sequentially with 3600, 6000 and 12000 grit polishing paper on a grinding wheel and then buffed with 0.05 μm Alumina buffing powder on a felt buffing cloth on the wheel. The polished surface was etched with a 1 % HCL-water solution for 105 sec., then immediately blotted dry with paper wipers. The etched shell surface was flooded with acetone and a piece of sheet acetate 0.5 mm thick was applied to the etched surface. The acetate peel was removed from the shell surface after 6.0 min. and examined for growth lines under a compound microscope with phase contrast optics.

The increased resolution of the growth lines from the acetate peels allowed growth lines visible in the umbo to be followed through the nacreous and prismatic layers to the surface of the valve where they emerged as annuli (Figure 2). The strength of and the distance between the surface annuli varied with site, tidal height and age of mussel. In heavily eroded shells, erosion in the umbonal region rendered identification of the first annulus difficult. However, remnants of the surface annulus were usually present at the margins of the valve (Figure 2).

After we developed our aging technique by establishing the relationship between the annually-formed nacreous growth lines and surface annuli in the 90 sectioned shells, we aged the untagged valves of the 110 tagged mussels using surface annuli alone. Three workers independently aged all 110 untagged valves. Each mussel was aged and the length to the last annulus was measured to the nearest 0.1 mm with a digital caliper. To avoid confusion arising from the variation in ages of juvenile mussels at the start of their first winter, age was expressed as number of annuli instead of years. We also measured the difference between length to the last annulus and length to the next to the last annulus (termed here annulus growth-increment) on the untagged valve. Studies of bivalve growth often use data that include a series of length measurements to consecutive annuli for each bivalve. With this approach the successive measurements on each shell are not independent and cannot be treated statistically unless a technique for repeated measures is used. To ensure independence in each data set here, only one measurement was made on each mussel in each version of the growth model.

To compensate for the lack of tagged mussels less than 16 mm in shell length, we randomly selected 18 mussels from the 110 tagged survivors to measure growth in the first two years of life. In nine of these mussels we measured the distance from the umbo to the first annulus for the age-length analysis and the distance between the first and second annulus for the annulus growth-increment analysis. Similarly, in the remaining nine mussels we measured the distance from the umbo to the second annulus for the age-length analysis and the distance between the second and third annulus for the annulus growth-increment analysis. We present models fitted to data with and without these substituted measurements.

2.3 Growth models

2.3.1 Age-length model

Schnute's (1981) general growth model with four parameters was fit to the age-length data. The Schnute age-length model takes the form

$$Y(t) = \left[y_1^b + (y_2^b - y_1^b) \frac{1 - e^{-a(t-\tau_1)}}{1 - e^{-a(\tau_2-\tau_1)}} \right]^{1/b}$$

under case 1 where Y(t) is length Y at age t, y_1 and y_2 are lengths at ages τ_1 and τ_2. Parameters τ_1 and τ_2 are ages that are chosen and fixed by the biologist, usually among the youngest and oldest ages of observed animals. Parameters a and b define the shape of the curve and are unequal to zero under case 1. The parameters, a, b, y_1, and y_2 (or a subset of these in the submodels) are found by minimization of the sum of squares (S)

$$S = \sum_{i=1}^{n} [\hat{Y}_i - Y_i(y_1, y_2, a, b)]^2$$

based on an additive error assumption and where $\hat{Y}_i$ is the observed length at age t_i and Y_i is the length at age t_i predicted by the model. The multiplicative error structure is usually selected if variability about a fitted growth curve increases (Quinn and Deriso 1999) with increasing length. An additive error assumption was used here, because variability in the response did not consistently increase with the predictor. In fact, variability in the growth increment data often tended to decrease with mussel length. Baker et al. (1991) found that parameter and standard error estimates for their data were not greatly affected by the choice between additive and multiplicative error structures. They also questioned the stability of the multiplicative model after experiencing difficulties obtaining consistent parameter and standard error estimates.

Cases 2, 3, and 4 are submodels of case 1 with 3, 3, and 2 parameters, respectively, that define situations where b = 0, a = 0, and a = b = 0 (see Appendix A for equations). The case 1 model with a > 0 and b fixed at 1 reduces to the von Bertalanffy model with a equal to the von Bertalanffy parameter k. The case 2 model with a > 0 is equivalent to the Gompertz model (see Schnute, 1981 for a discussion of these and other submodels). Initially, the four parameter case 1 model is fit to the data. If the parameters appear to approach limiting values that correspond to another case, then the appropriate submodel is selected with the aid of an F-test of the variance ratio

$$F = \frac{\left(\frac{RSS_j - RSS_i}{df_j - df_i} \right)}{R} MS_i$$

which is approximately F-distributed when the sample size is large (Schnute 1981). Two submodels, case I and case j, have residual sums of squares RSS_i and RSS_j with df_i and df_j

degrees of freedom. RMS_i is the residual mean square of case I, where case I is the submodel with the greater number of parameters. We rejected the null hypothesis that the submodels were the same at the $p = 0.05$ level if the F-statistic was greater than $F_{0.05}(df_i - df_I, df_i)$. If two submodels had the same number of free parameters, and if both submodels were determined by the F-test to be more parsimonious (ie. had fewer parameters) than the four parameter case 1 model, then the submodel with the smallest residual sum of squares was chosen.

2.3.2 Growth-increment model

Baker et al. (1991) developed a growth model for mark-recapture data analogous to the Schnute (1981) model that incorporates size at marking, length of time at large, and size at recapture. Because absolute age is not included in the data set, the model can only predict size at relative age. The biologist must supply one parameter, usually size at initial age (y_1), decreasing the number of estimated parameters in the Schnute (1981) four-parameter model to three. Here we obtained this parameter by taking the mean y_1 from the age-length results. The case 1 analog to the Schnute model takes the form

$$Y_r = \left[Y_m^b e^{-a(t_r - t_m)} + (y_2^b - y_1^b e^{-a(\tau_2 - \tau_1)}) \frac{1 - e^{-a(t_r - t_m)}}{1 - e^{-a(\tau_2 - \tau_1)}} \right]^{1/b}$$

where Y_m and Y_r are sizes at t_m, age at marking, and t_r, age at recapture, respectively (cases 2, 3, and 4 are given in Appendix B). The parameters, a, b, y_1, y_2, τ_1, and τ_2 are the same as those in the Schnute (1981) age-length growth model. Standard errors and confidence limits were calculated using bootstrap methods (Efron and Tibshirani, 1986).

3. Results

3.1 Precision of age estimation

Aging of the tagged mussels revealed individuals with up to 12 annuli (11+ yr olds) ranging in shell length at last annulus from 16.8 mm to 43.2 mm (Figure 3). The discrepancy in annulus count between agers was one or fewer in 77.2% of the mussels aged, and complete agreement between at least 2 two agers in 85.4% (Table 1). Mean length at annulus differed between agers by less than one standard deviation in almost all cases (Table 2).

Nearly all of the 110 mussels that were tagged and left in situ for one year clearly showed exactly one annulus in the region of new shell growth posterior to the reference marker. Only one mussel showed no distinguishable annulus in the region of new shell growth. Nine mussels showed a single ‘check’, or non-annular shell disturbance in addition to the annulus. These checks could be distinguished from annuli by a discontinuity or a fading-out of the check toward the edge of the valve.

3.2 Age-length model

The case 2 Schnute age-length submodel for the age at length to last annulus (F-test; mussels with three or more annuli) was selected for all three agers in the absence of very small mussels with only one or two annuli in the original group of tagged mussels (Tables 3 and 4). However, the von Bertalanffy submodel (with parameter b fixed at 1 and parameter a free) was also determined by the F-test to be more parsimonious than the case 1 model for all three agers. The RSS's of the von Bertalanffy submodels were greater than the case 2 submodels by only about 0.1% or less, and the RSS 95% confidence intervals were almost coincident. For these data sets the two submodels produce such similar fits that the choice between them based on the RSS was somewhat arbitrary. The 95% confidence intervals for age-length curves overlapped each other at all annuli (Figure 4). When length measurements to the first and second annuli substituted from a randomly selected subset of the tagged mussels were added to the model, the von Bertalanffy submodel was selected for all three agers (Tables 4 and 5). For consistency, all curves presented in Figures 4, 7 and 8 are constructed from the von Bertalanffy submodel.

3.3 Growth-increments

Shell growth in the tagged mussels ranged from 0.4 to 12.8 mm over one year. Growth tended to decrease with increasing initial length (Figure 5). No growth-increment data were available for mussels <16 mm in initial length because none were tagged (Figure 6). The origin of the growth-increment curve was defined by the parameter $\bar{y}_1$, which was the mean y_1 from the appropriate age-length curves. Hence, the growth-increment curve was the predicted length at age relative to the initial length $\bar{y}_1$ at age τ_1. The growth-increment model was applied to both tag growth-increment and annulus growth-increment data. Case 3 of the Schnute model was selected for the tag growth-increment data. The von Bertalanffy submodel was also determined by the F-test to be more parsimonious than the case 1 model, and the RSS was only 0.5% greater than the RSS of the case 3 submodel. Without the augmentation of first and second year growth, the von Bertalanffy submodel was selected for the annulus growth-increment data from one ager, the case 3 submodel was selected for the second ager, and the case 2 submodel was selected for the third ager. The RSS's for the von Bertalanffy fit to the data of the second and third ager were less than 1% more than both other submodels (Tables 4, 6 and 7).

When the growth-increment analog of the Schnute model was fit to the first and second year augmented annulus growth-increment data the von Bertalanffy submodel with $b = 1$ was the best fit for two agers, and the case 1 Schnute model with four parameters was the best fit for the remaining ager (Table 8). Although the fit of the von Bertalanffy submodel to the data of one ager was not more parsimonious than the case 1 model (F-test), the von Bertalanffy RSS was less than one SE greater than the case 1 RSS.

3.4 Model comparisons

Schnute growth curves, fit to age-length data from annuli three to eight for the three agers, fell significantly below the curve generated from tag growth-increment data (Figure 4). The 95% confidence intervals for age-length curves overlapped each other at all annuli but

overlapped those of the tag growth-increment curve only at annulus four. When we fit the Schnute model analog for mark-recapture data to the annulus growth-increment data, the growth curve was much closer to that of the tag growth-increment model (Figure 7). In fact, confidence intervals for the tag growth-increment and annulus growth-increment models overlapped at all five annuli. Comparison of the age-length and annulus growth-increment curves using the augmented data sets revealed that the initial slope of the age-length curve was steeper than that of the annulus growth-increment curve, but that growth in older mussels slowed more rapidly under the age-length model (Figure 8).

4. Discussion

By comparing age-length and growth-increment data from the same group of mussels, we were able to distinguish differences in growth estimates owed to growth estimation technique and aging bias as well as eliminate differences owed to sampling bias. Agreement between annulus growth-increment and tag growth-increment data (Figure 7) indicated that the deposition of surface rings on the shells of Mytilus trossulus at our study sites in Prince William Sound occurred with approximately annual periodicity. The appearance of one new annulus in tagged mussels left in situ for one year, also supports this interpretation. This relationship was validated only for the size range of mussels tagged (16.5 - 43.4 mm), although there is no obvious reason why there would be any difference for mussels outside this size range. The correspondence between growth layers in the shell, which were found to be annually deposited in Mytilus edulis by Lutz (1976), and surface annuli is also consistent with the interpretation of annual deposition of surface rings (Figure 2). This implies that most of the differences between the age-length and tag growth-increment curves can be attributed to differences in the growth estimation techniques. If growth rate varies with age (and it seems reasonable to assume that older mussels grow more slowly than equally-sized, younger mussels), then size-specific growth rate estimates will be influenced by year-class strength. Effectively, a growth curve based on length-increment data will be weighted by year-class strength for year-classes with overlapping size ranges. Such a model should generate a less biased prediction of growth based on the length of an individual from the same population than a model constructed from age-length data, because growth rate at a given length will be weighted properly to reflect the age distribution of the population. Indirectly, this type of model could also provide a less biased estimate of age from length (if y_1 is chosen correctly), because factors such as year-class strength that could influence the probability of an individual of a given size being a certain age would be incorporated into the model. Alternatively, to avoid bias when estimating age from length with age-length data, the growth model used must be structured to have length as the predictor and age as the response. Conventional growth curves constructed from age-length data will not be affected by year-class strength, but instead will be influenced by size-selective mortality as described by Lee's phenomenon (Lee, 1912; Ricker, 1958). Hence, these models should generate less biased estimates of length from age than curves constructed from growth-increment data. Age (really τ_1 + time-at-large) is still an appropriate independent variable in a mark-recapture growth model because time is under the control of the investigator.

The annulus growth-increment curves allow us to compare the tag growth-increment data to growth data obtained from surface annuli, and to avoid the difficulties discussed above. The only difference between the annulus growth-increment curve and the tag growth-

increment curve should have been caused by the difference in the environmental regime in the time periods during which growth occurred. It is clear from Figure 7 that these curves were in fact very close. Annulus growth-increment data are also more powerful for studying temporal and year-class effects on growth than age-length data, because the growth estimates are based on a more restricted period of time.

If age is a better predictor of growth rate than length, we should expect growth rate to be more sensitive to changes in age than changes in length. In Figure 8 changes in slope of the annulus-increment curves represent changes in growth rate with mussel length, but changes in slope of the age-length curves represent changes in growth rate with mussel age. The greater degree of curvature in the age-length curves may reflect the greater sensitivity in growth rate to age than to length. At annuli one through three the growth rate from the age-length model was greater than that from the annulus growth-increment model, but the reverse was true for greater ages. This difference should occur if growth rate estimates for older individuals from the annulus growth-increment model have an upward bias owing to the presence of young, large, fast-growing mussels in the population, while growth rate estimates for younger individuals have a downward bias caused by the presence of old, small, slow-growing mussels.

The Schnute model provides an added level of information about the general shape of the growth curve because it does not constrain the curve by assumptions like asymptotic size, thereby allowing the curve to conform more closely to the actual data. Conventional parameters such as l_∞ or t_0 can still be calculated if they exist (see Schnute 1981 for formulae). Comparisons between submodels can be made more directly. Our initial choice of the Schnute growth model over the von Bertalanffy submodel, for example, seems justified because it allowed us to compare the fit of many other submodels and to reach a final decision based on the character of the data itself. Selection of the von Bertalanffy submodel for the age-length and annulus growth-increment data in most cases where $\tau_1 = 1$ as well as the relatively good fit for the remaining data sets suggests that the use of this model to construct growth curves may be generally appropriate for mussels (Tables 4, 5 and 8).

5. Conclusion

This study revealed that the growth rings on the outer surface of the shell of Mytilus trossulus in Prince William Sound, Alaska were deposited annually. The Schnute general growth model for age-length data provided a convenient analytical method for selecting among all previously published growth models. An analog of the Schnute model designed for mark-recapture data (ie growth-increment data) was useful for modeling growth of tagged mussels measured in situ in Prince William Sound, as well as growth between the outermost annuli on mussel shells. The von Bertalanffy submodel was the most appropriate general model for our data. This result supports the common tendency by previous workers to use the von Bertalanffy model for mussel growth. Comparison between age-length and tag growth-increment models, with the entire range of ages in the population considered, revealed that the accumulation of positive and negative differences in growth rate tended to balance out over the lifetime of the mussel resulting in similar length predictions of both models for the older individuals. Differences in growth model parameters between the age-length model and the mark-recapture analog could be attributed solely to inherent differences in the estimation techniques. These discrepancies can be avoided by replacing age-length data with annulus

growth-increment data, which generates growth estimates equivalent to estimates from mark-recapture data, to construct growth curves.

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Table 1. Level of agreement and discrepancy in number of annuli counted by three agers on 110 tagged mussels, Mytilus trossulus, from Prince William Sound.

Category	No. of Mussels	% of Total
Level of Agreement[1]		
3	27	24.5
2	67	60.9
0	16	14.5
Discrepancy between agers[2]		
0	27	24.5
1	58	52.7
2	21	19.1
3	3	2.7
4	1	0.9

[1]Number of agers (of three total) agreeing on the number of annuli on each mussel shell after independent counts.
[2]Number of annuli by which agers differ on each mussel shell.

Table 2. Mean length (mm) at annulus of tagged mussels, Mytilus trossulus, from Prince William Sound. Mean length to last annulus is shown for mussels with three or more annuli. Mean length to first or second annulus of a randomly selected subset of older mussels is shown for annuli 1 and 2. SD, standard deviation; n, number of mussels.

Annulus	Ager 1			Ager 2			Ager 3		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
1	3.6	1.3	9	4.5	1.6	9	5.1	2.3	9
2	10.6	3.0	9	12.2	2.2	9	11.4	3.7	9
3	20.5	2.4	6	22.2	2.4	6	21.2	3.7	4
4	23.7	4.0	12	22.6	4.4	15	23.9	3.9	12
5	26.6	3.6	22	27.6	3.8	30	27.1	4.8	33
6	29.0	4.4	36	29.3	4.1	37	29.1	4.4	41
7	30.2	5.6	19	34.1	6.5	14	32.9	4.5	13
8	34.5	3.0	9	34.3	4.4	3	31.2	2.5	3

Table 3. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for Schnute case 2 (b = 0) and the von Bertalanffy submodel (LVB1; b = 1) parameters of age-length growth model of tagged mussels, Mytilus trossulus, from Prince William Sound. All tagged mussels had three or more annuli. $\tau_1 = 3$, $\tau_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Ager 1				Ager 2				Ager 3			
	Value	SE	LL	UL	Value	SE	LL	UL	Value	SE	LL	UL
Case 2												
SS	1969	279	1512	2406	1829	233	1404	2178	2127	263	1655	2526
a	0.27	0.10	0.12	0.45	0.25	0.09	0.11	0.42	0.21	0.12	0.03	0.44
y_1	20.4	1.3	18.3	22.5	20.4	1.2	18.2	22.3	21.3	1.4	18.7	23.5
y_2	30.8	0.52	29.9	31.6	32.0	0.58	31.0	32.9	31.7	0.63	30.6	32.7
LVB1												
SS	1972	273	1462	2375	1836	234	1418	2190	2128	261	1646	2513
a	0.20	0.09	0.07	0.37	0.16	0.09	0.04	0.33	0.13	0.12	-0.04	0.34
y_1	20.3	1.34	17.9	22.4	20.3	1.30	18.0	22.2	21.2	1.53	18.6	23.6
y_2	30.8	0.50	29.9	31.5	31.8	0.55	31.0	32.8	31.6	0.63	30.5	32.6

Table 4. Statistical tests (F- test) of the best submodel (case) by ager of the Schnute age-length and analogous annulus growth-increment models, and the best case of the tag growth-increment model for tagged mussels, Mytilus trossulus, from Prince William Sound. LVB1, von Bertalanffy submodel (b = 1); τ_1, age at beginning.

Ager	Model	Case j / Case i	df_j	df_i	F	P
1	Age-length, $\tau_1 = 3$	2/1	107	106	0.07	>0.05
	Age-length, $\tau_1 = 1$	LVB1/1	107	106	0.03	>0.05
	Annulus Growth-incr., $\tau_1 = 3$	LVB1/1	108	107	0.77	>0.05
	Annulus Growth-incr., $\tau_1 = 1$	2/1	108	107	4.38	<0.05
2	Age-length, $\tau_1 = 3$	2/1	106	105	1.06	>0.05
	Age-length, $\tau_1 = 1$	LVB1/1	106	105	0.15	>0.05
	Annulus Growth-incr., $\tau_1 = 3$	3/1	107	106	0.18	>0.05
	Annulus Growth-incr., $\tau_1 = 1$	LVB1/1	107	106	3.88	>0.05
3	Age-length, $\tau_1 = 3$	2/1	107	106	0.09	>0.05
	Age-length, $\tau_1 = 1$	LVB1/1	107	106	0.04	>0.05
	Annulus Growth-incr., $\tau_1 = 3$	2/1	108	107	0.20	>0.05
	Annulus Growth-incr., $\tau_1 = 1$	LVB1/1	108	107	2.52	>0.05
-	Tag Growth-increment, $\tau_1 = 3$	3/1	108	107	0.19	>0.05

Table 5. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for Schnute case 1 parameters with b = 1 (von Bertalanffy submodel) of the age-length growth model of tagged mussels, Mytilus trossulus, from Prince William Sound. Length to first or second annulus estimated from a randomly selected subset of 18 mussels with three or more annuli. $\tau_1 = 1$, $\tau_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Value	SE	LL	UL
Ager 1				
SS	1764	285	1267	2193
a	0.28	0.04	0.22	0.35
y_1	3.5	1.2	1.5	5.3
y_2	30.9	0.47	30.1	31.7
Ager 2				
SS	1482	211	1122	1804
a	0.23	0.04	0.17	0.30
y_1	4.7	1.1	2.8	6.4
y_2	32.0	0.52	31.1	32.8
Ager 3				
SS	1934	252	1478	2314
a	0.24	0.05	0.17	0.33
y_1	4.8	1.2	2.8	6.8
y_2	31.9	0.60	30.9	32.9

Table 6. Parameter value and bootstrap estimates of standard error and 95% confidence intervals of case 3 ($\underline{a} = 0$) and the von Bertalanffy submodel (LVB1; b = 1) of the tag growth-increment model of tagged mussels, Mytilus trossulus, from Prince William Sound. $\bar{y}1$ = mean $\underline{y}_1$ of age-length models (see Table 3), $\underline{\tau}_1 = 3$, $\underline{\tau}_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Value	SE	LL	UL
Case 3				
SS	465	68.0	350	572
$\underline{b}$	2.6	0.37	2.0	3.2
$\bar{y}_1$	20.7	-	-	-
$\underline{y}_2$	35.4	0.67	34.3	36.6
LVB1				
SS	468	75.6	340	588
$\underline{a}$	0.21	0.04	0.14	0.29
$\bar{y}_1$	20.7	-	-	-
$\underline{y}_2$	35.6	0.62	34.6	36.6

Table 7. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for annulus growth-increment (growth between next to the last and last annulus) growth model of tagged mussels, Mytilus trossulus, in Prince William Sound. The model that best fit the data of each ager was: Ager 1, von Bertalanffy submodel (b = 1); Ager 2, case 3 ($\underline{a} = 0$); Ager 3, case 2 ($\underline{b} = 0$). $\bar{\underline{y}}_1$ = mean $\underline{y}_1$ of age-length models (see Table 3), $\underline{\tau}_1 = 3$, $\underline{\tau}_2 = 7$. SE, standard error; LL, lower limit: UL, upper limit.

Parameter	Value	SE	LL	UL
Ager 1				
SS	366	45.4	288	438
$\underline{a}$	0.21	0.03	0.16	0.27
$\underline{b}$	1.0	-	-	-
$\bar{\underline{y}}_1$	20.7	-	-	-
$\underline{y}_2$	34.8	0.59	33.8	35.8
Ager 2				
SS	343	44.3	267	413
$\underline{a}$	0	-	-	-
$\underline{b}$	2.0	0.2	1.7	2.4
$\bar{\underline{y}}_1$	20.7	-	-	-
$\underline{y}_2$	35.2	0.66	34.1	36.2
Ager 3				
SS	339	43.2	264	408
$\underline{a}$	0.27	0.03	0.22	0.33
$\underline{b}$	0	-	-	-
$\bar{\underline{y}}_1$	20.7	-	-	-
$\underline{y}_2$	34.7	0.58	33.8	35.6

Table 8. Parameter value and bootstrap estimates of standard error and 95% confidence intervals by ager for the Schnute case 1 submodel [von Bertalanffy submodel ($b = 1$) for agers 2 and 3] of the annulus growth-increment model of tagged mussels, Mytilus trossulus, from Prince William Sound. Length to first and second annuli estimated from a randomly selected subset of mussels with three or more annuli. $\tau_1 = 1$, $\tau_2 = 7$. SE, standard error; LL, lower limit; UL, upper limit.

Parameter	Value	SE	LL	UL
Ager 1				
SS	363	45.6	283	430
a	0.30	0.06	0.20	0.41
b	0.41	0.20	0.07	0.72
y_1	4.3	-	-	-
y_2	32.9	0.69	31.7	34.0
Ager 2				
SS	399	50.5	312	478
a	0.14	0.03	0.10	0.19
b	1.0	-	-	-
y_1	4.3	-	-	-
y_2	33.0	0.81	31.7	34.3
Ager 3				
SS	424	59.6	322	521
a	0.15	0.03	0.11	0.19
b	1.0	0	-	-
y_1	4.3	-	-	-
y_2	32.2	0.83	30.7	33.5

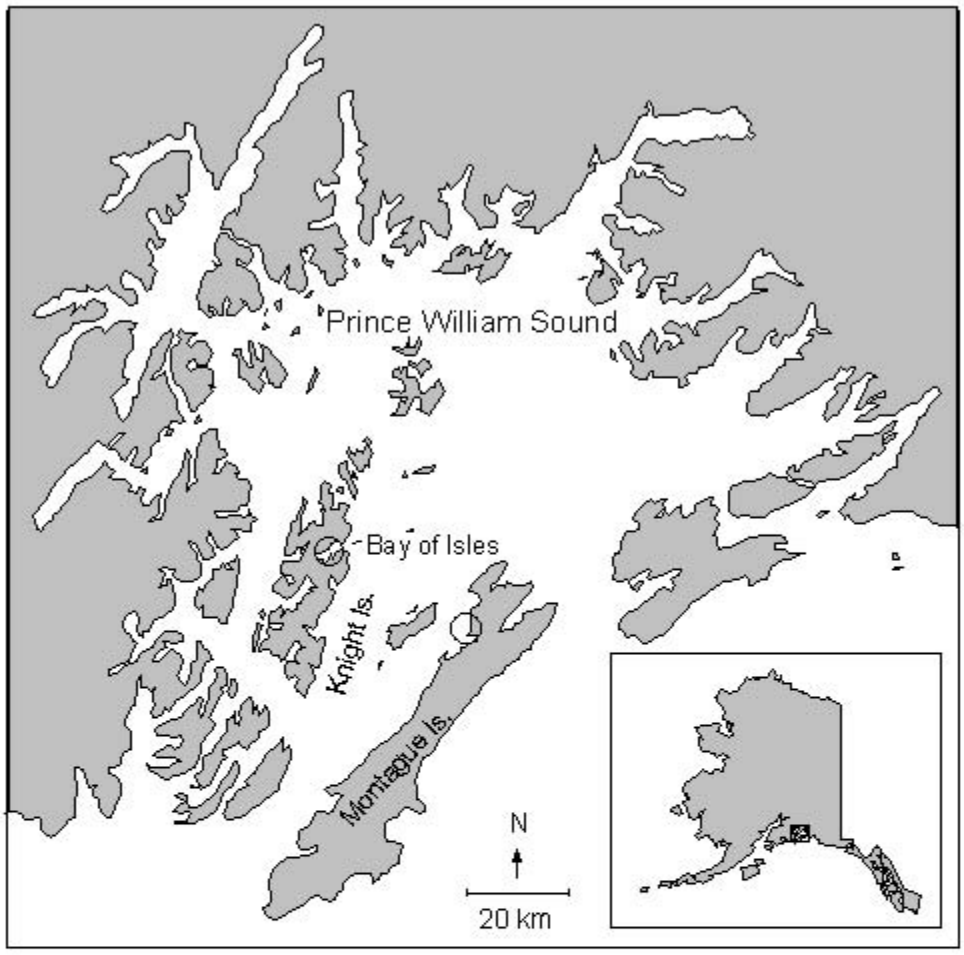

Figure 1. Map of Prince William Sound, Alaska showing study areas (circled).

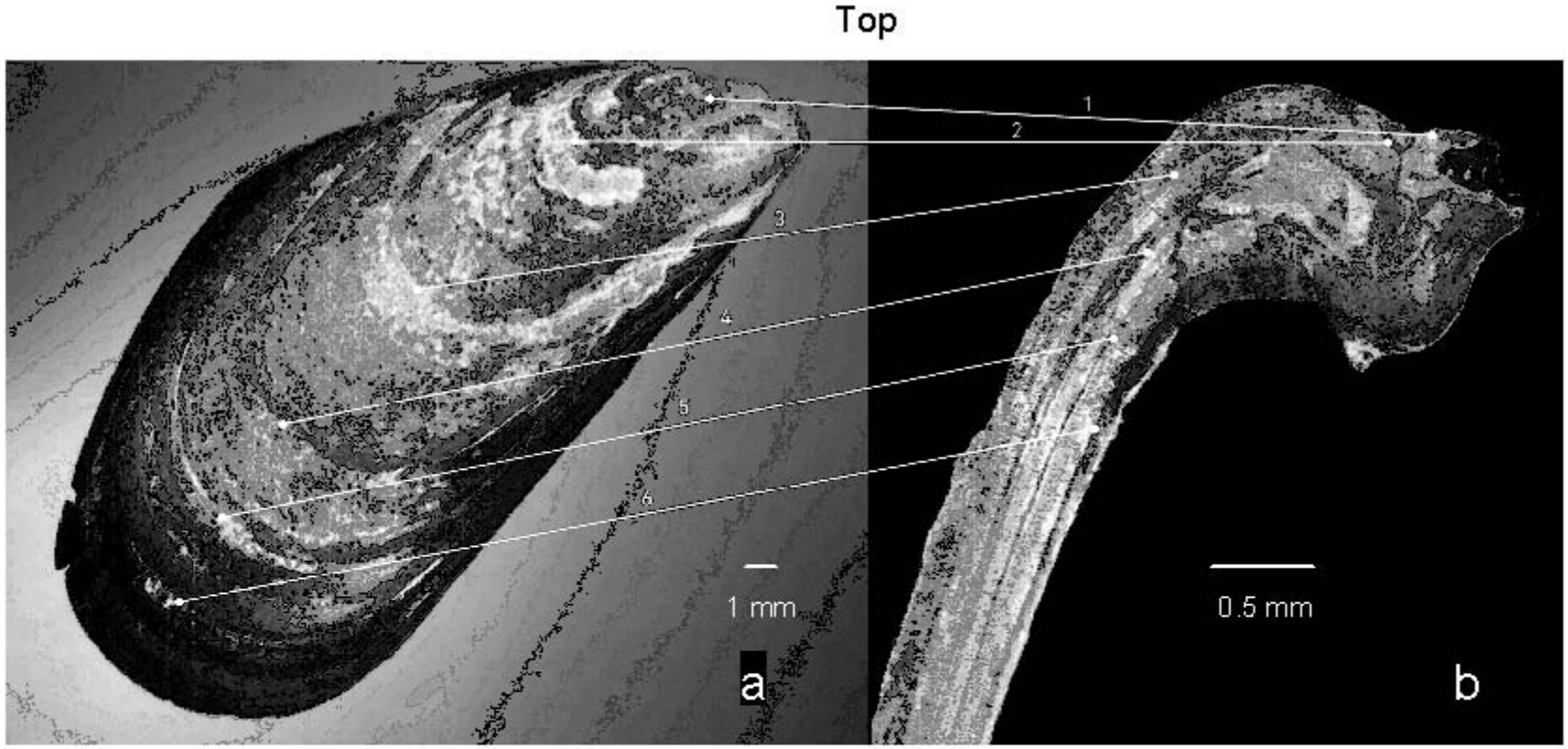

Figure 2. Right valve (a) and acetate peel (b) of radial section through umbonal region of corresponding left valve of a 29.4 mm mussel, Mytilus trossulus, from Prince William Sound. Numbered lines connect annual rings on the valve surface with corresponding growth lines in the inner nacreous layer of the sectioned valve.

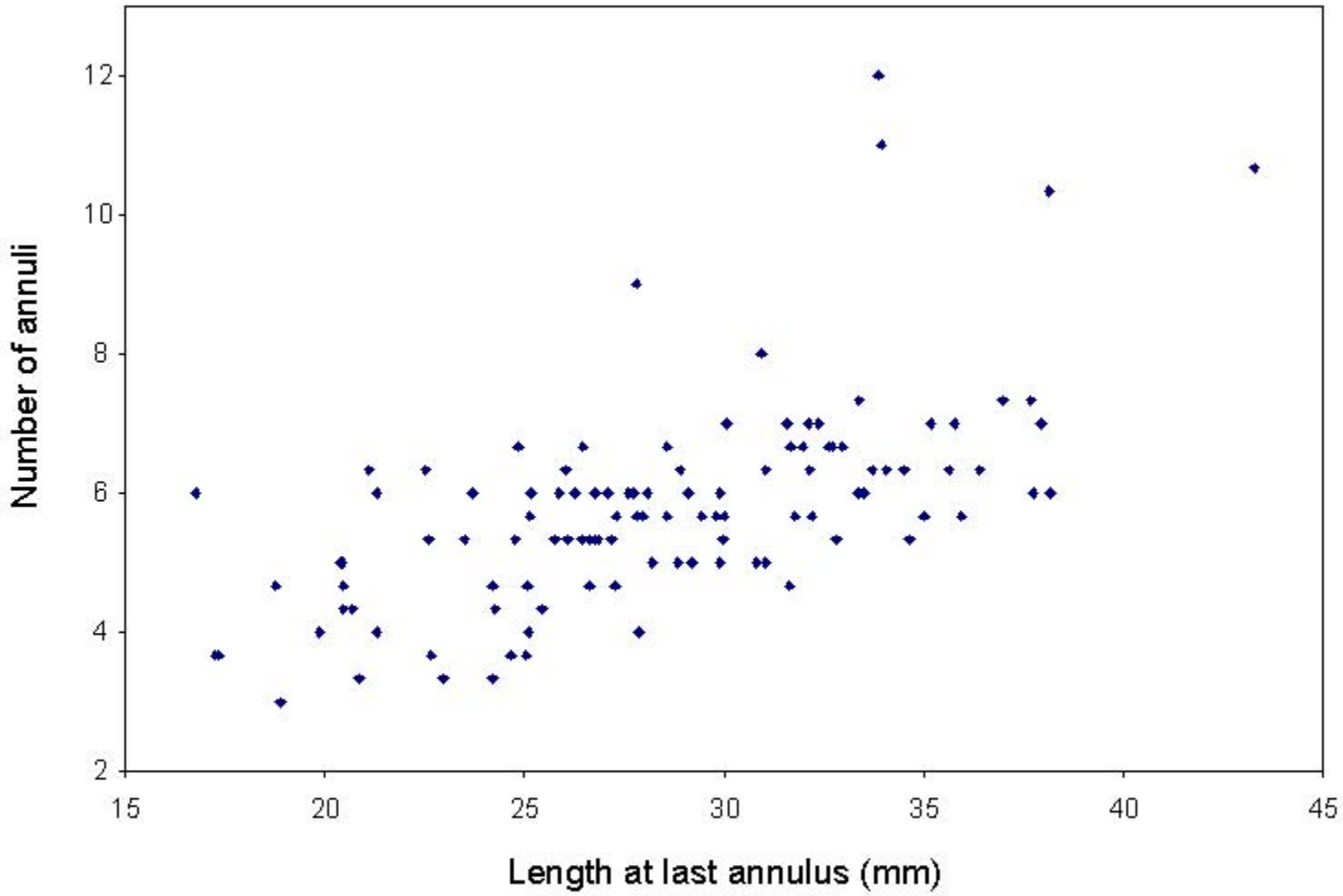

Figure 3. Number of annuli versus shell length at last annulus for 110 Mytilus trossulus tagged at Montague Island and Knight Island in Prince William Sound, Alaska. Each point is the mean of independent observations by three agers of number of annuli and length at last annulus for each mussel.

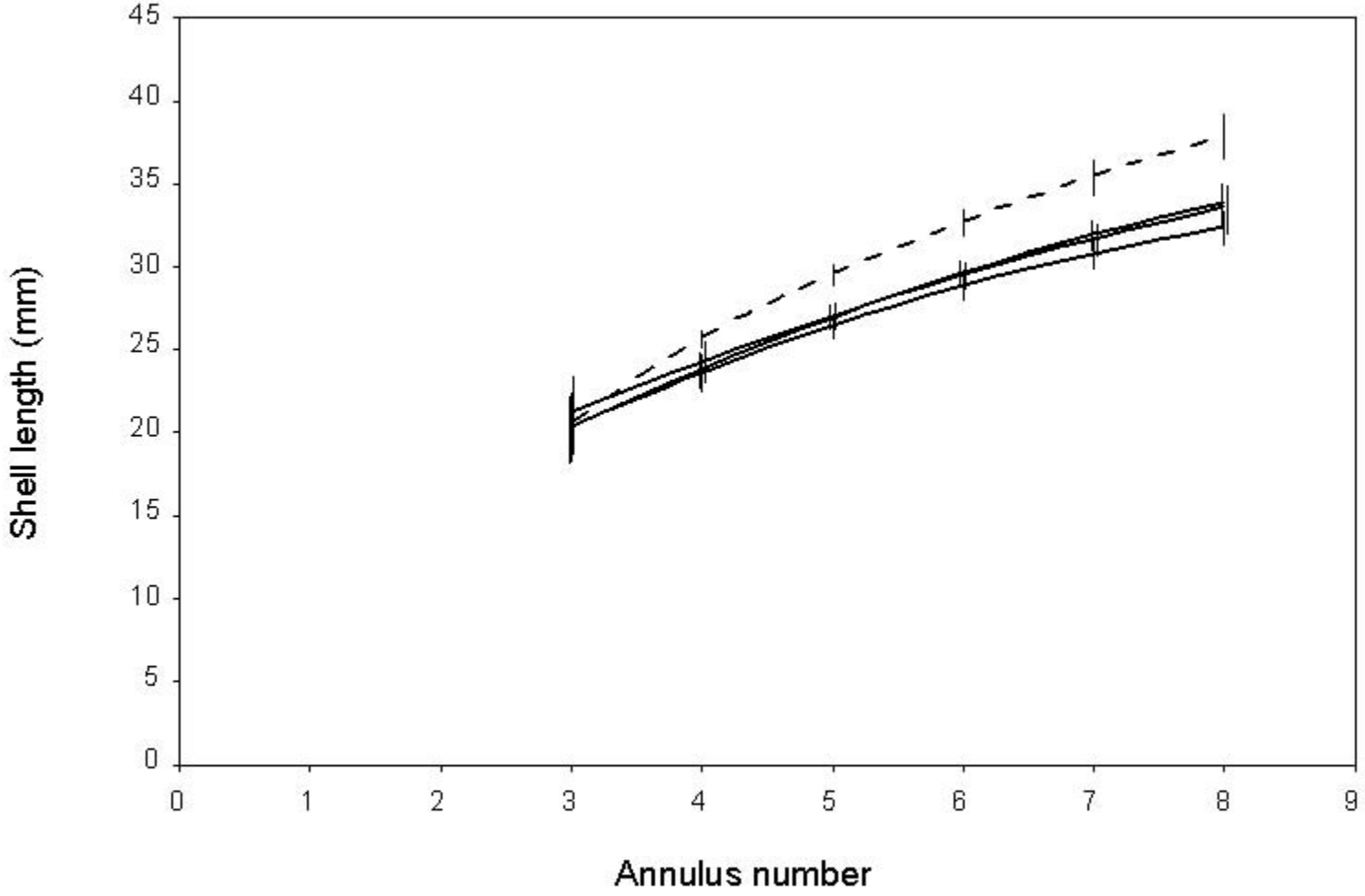

Figure 4. Curves of growth of 110 tagged Mytilus trossulus at Montague Island and Knight Island in Prince William Sound, Alaska depicting growth from age-length data estimated by three observers and modeled by the Schnute equations (solid lines) and from tag growth-increment data modeled by the Schnute analog growth equation for mark-recapture data (dashed line; see Tables 2 and 5 for parameter values). Vertical bars are bootstrap estimates of 95% confidence intervals.

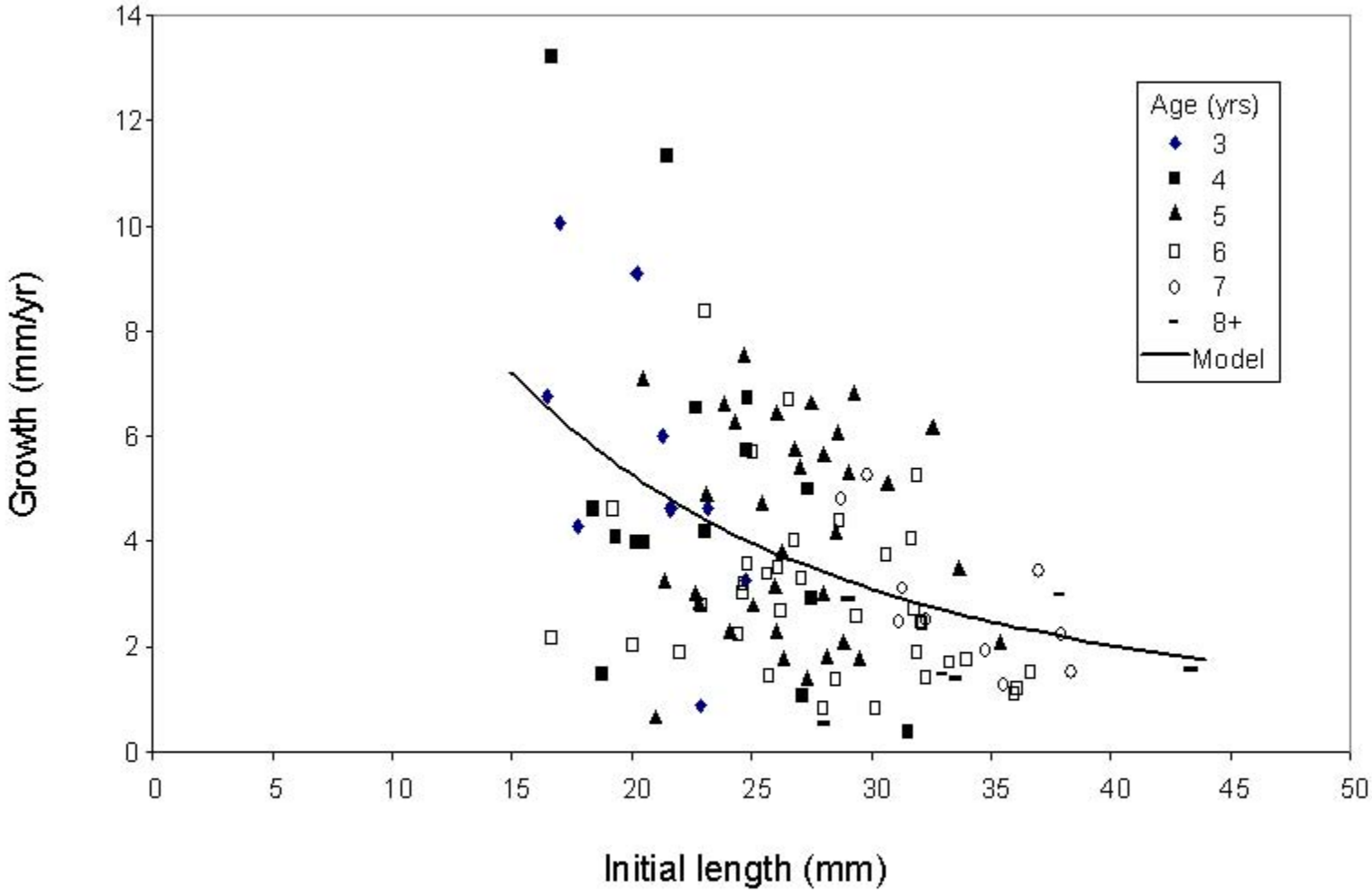

Figure 5. Increase in shell length measured one year after tagging versus initial shell length of 110 Mytilus trossulus aged 3 to 8+ yrs at Montague Island and Knight Island in Prince William Sound, Alaska.

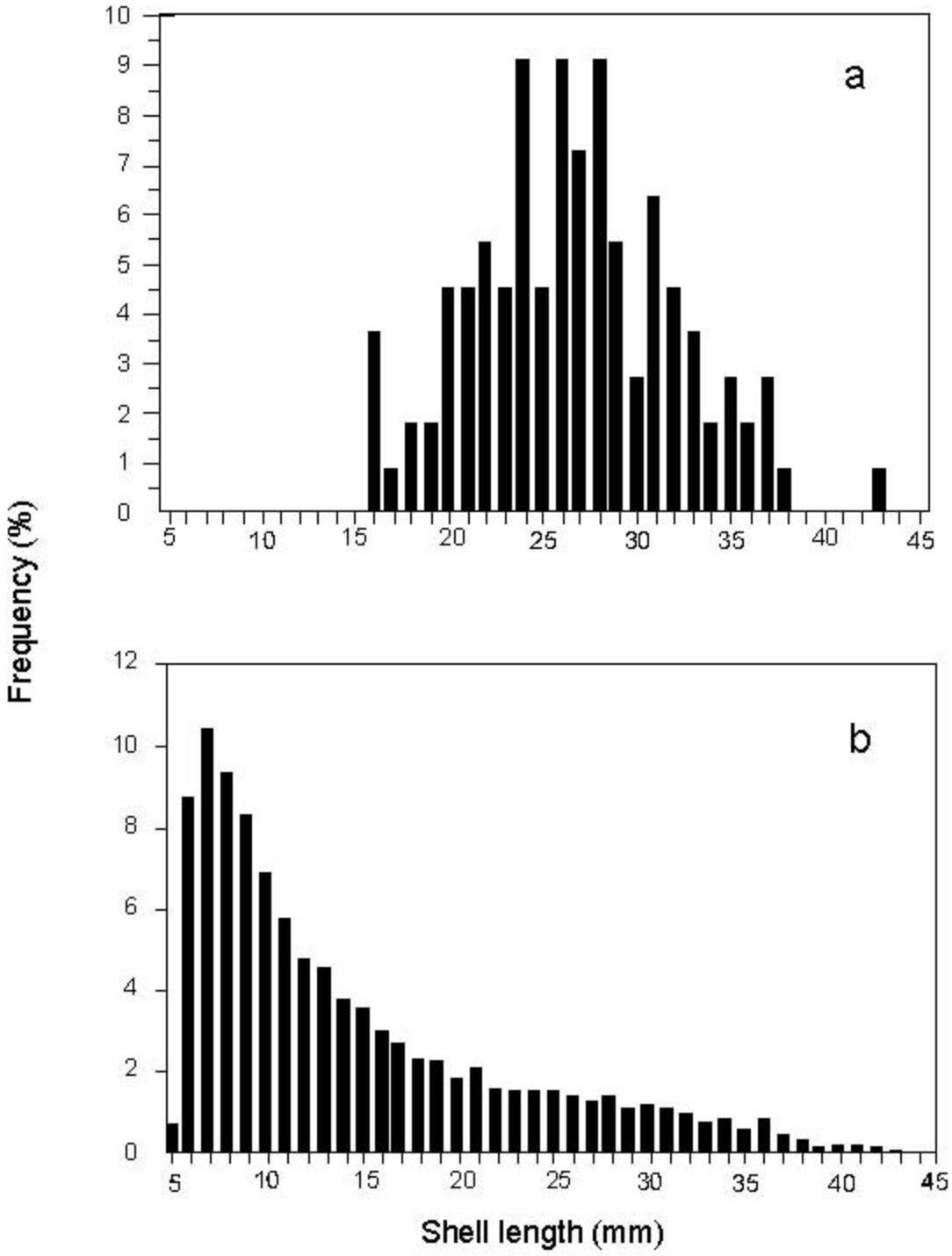

Figure 6. Length-frequency distribution of (a) tagged mussels, Mytilus trossulus, (n = 110) and (b) mussels (n = 18,196) randomly sampled between 0.6 and 1.2 m above mean lower low water at tagging sites on Montague Island and Knight Island in Prince William Sound, Alaska.

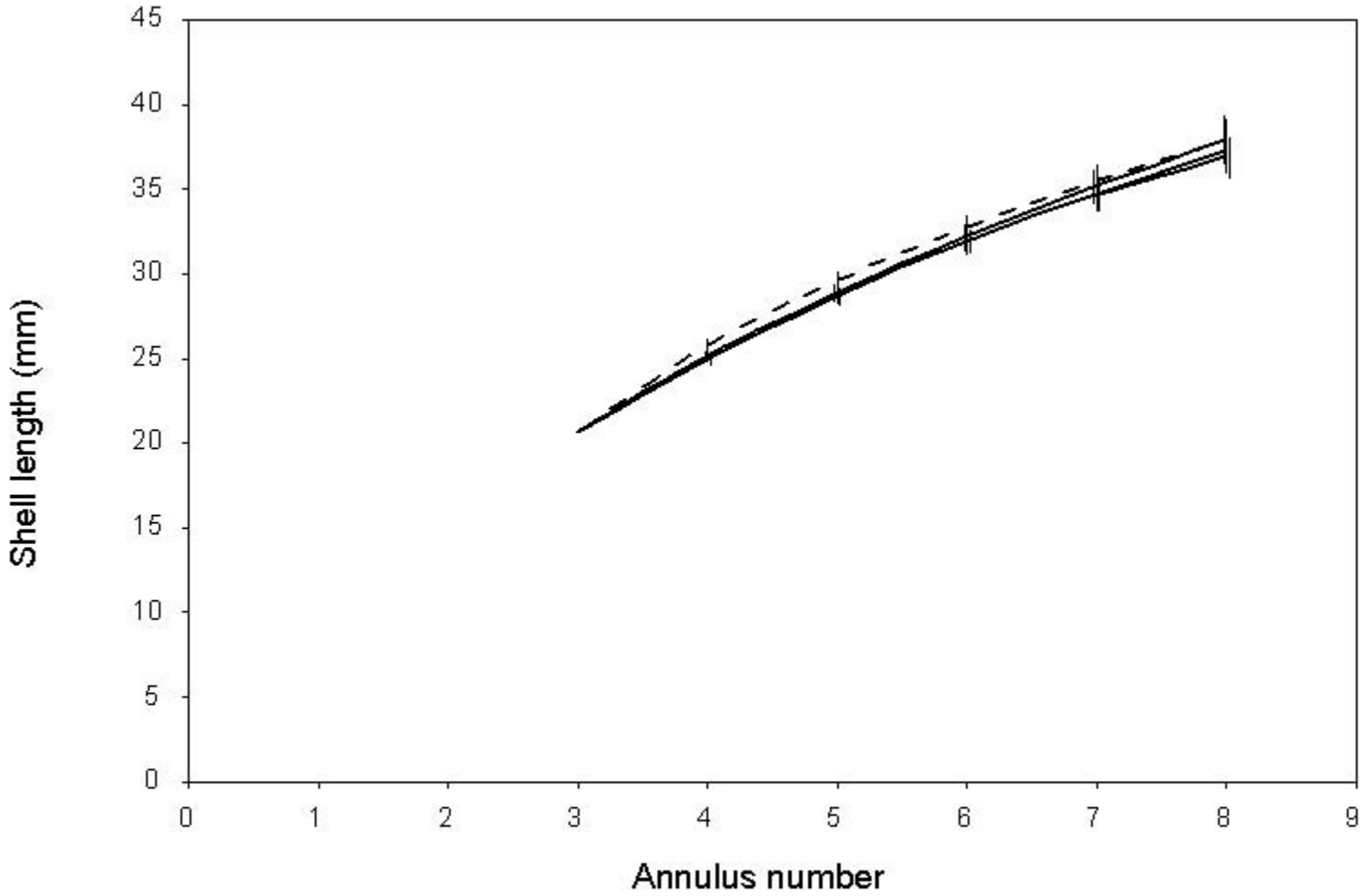

Figure 7. Curves of growth of 110 tagged Mytilus trossulus at Montague Island and Knight Island in Prince William Sound, Alaska depicting growth from annulus growth-increment data estimated by three observers (solid lines) and from tag growth-increment data (dashed line). Both sets of data were modeled by the Schnute analog growth equation for mark-recapture data. Vertical bars are bootstrap estimates of 95% confidence intervals.

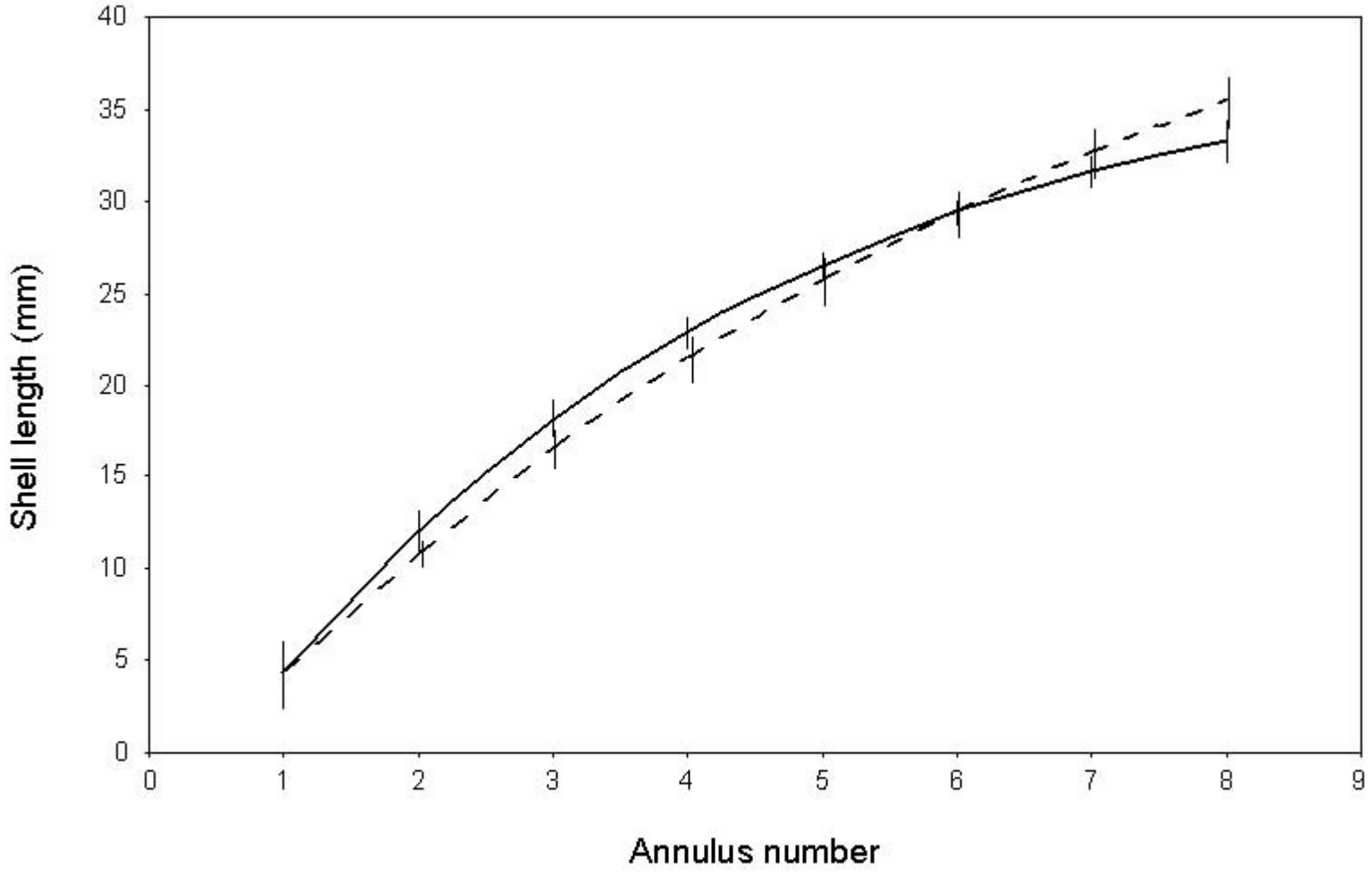

Figure 8. Curves of growth of 110 tagged Mytilus trossulus at Montague Island and Knight Island in Prince William Sound, Alaska depicting growth from age-length data modeled by the Schnute equation (solid line) and from annulus growth-increment data modeled by the Schnute analog growth equation for mark-recapture data (dashed line). Each curve depicts means of three observers. Vertical bars are bootstrap estimates of 95% confidence intervals (see Tables 4 and 7 for parameter values and confidence intervals). The curves include growth at the 1st and 2nd annuli estimated by substituting measurements to these annuli in a subset of the tagged mussels.

Appendix A

Submodels of the Schnute Growth Equation
(See Methods for definitions of terms)

Case 2: $a \neq 0$, $b = 0$

$$Y(t) = y_1 \exp\left[\log\left(\frac{y_2}{y_1}\right)\frac{1 - e^{-a(t-\tau_1)}}{1 - e^{-a(\tau_2-\tau_1)}}\right]$$

Case 3: $a = 0$, $b \neq 0$

$$Y(t) = \left[y_1^b + (y_2^b - y_1^b)\frac{t-\tau_1}{\tau_2-\tau_1}\right]^{1/b}$$

Case 3: $a = 0$, $b = 0$

$$Y(t) = y_1 \exp\left[\log\left(\frac{y_2}{y_1}\right)\frac{t-\tau_1}{\tau_2-\tau_1}\right]$$

Appendix B

Submodels of the Baker et al. (1991) Analog to the Schnute Growth Model for Mark-recapture Data
(See Methods for definitions of terms.)

Case 2: a ≠ 0, b = 0

$$Y_r = \exp\left[\ln Y_m e^{-a(t_r - t_m)} + (\ln y_2 - \ln y_1 e^{-a(\tau_2 - \tau_1)}) \frac{1 - e^{-a(t_r - t_m)}}{1 - e^{-a(\tau_2 - \tau_1)}}\right]$$

Case 3: a = 0, b ≠ 0

$$Y_r = \left[Y_m^b + (y_2^b - y_1^b) \frac{t_r - t_m}{\tau_2 - \tau_1}\right]^{1/b}$$

Case 4: a = 0, b = 0

$$Y_r = \exp\left[\ln Y_m + (\ln y_2 - \ln y_{1)} \frac{t_r - t_m}{\tau_2 - \tau_1}\right]$$

APPENDIX SO-05

Mesoscale Differences in Mussel, *Mytilus trossulus*, Population Structure in Prince William Sound, Alaska, in Relation to Oiling History and Predation Intensity[1]

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Running title: Predation and size structure of prey after disturbance

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Mesoscale Differences in Mussel, *Mytilus trossulus*, Population Structure in Prince William Sound, Alaska, in Relation to Oiling History and Predation Intensity

Charles E. O'Clair, Joshua Millstein and Mandy Lindeberg

ABSTRACT: To assess the relative importance of direct and indirect (sea otter, *Enhydra lutris*, and *Nucella* spp. predation) effects of the *Exxon Valdez* oil spill (EVOS) on mussel, *Mytilus trossulus*, population structure 7-8 yrs after the spill, we sampled mussel populations on 1,076 transects in May/June and July 1996 and 1997 on Knight Island (KI; oiled) where sea otter numbers were greatly reduced by the spill and on the northwestern shore of Montague Island (MI; unoiled) where sea otters were unaffected by the spill. The density of all mussels (≥ 5 mm in shell length) at KI exceeded that at MI in both years of our study, but the majority of mussels at KI were small, having recruited to the population in the previous 1-2 yrs. The length-frequency and biomass (ash-free dry mass [AFDW]) distributions of the mussels at KI were strongly skewed to the right in both years. The density and biomass of mussels ≥ 20 mm in shell length did not differ between study areas in 1996, but was higher at MI than at KI in 1997. The density of ≥ 40 mm mussels (the size range preferentially consumed by sea otters) was very low at both study areas. The density did not differ between study areas in 1996, but in 1997 was greater at MI than at KI. The mean biomass density of mussels ≥ 40 mm at KI slightly exceeded that at MI in 1996, but it was less than 1/6 that at MI in 1997. The results indicate that release from sea otter predation on Knight Island after the EVOS did not result in an increase in the abundance of large mussels there 7-8 yrs after the spill as would be predicted if sea otters controlled the size-structure of mussels in Prince William Sound. There is no evidence that the direct effects of oil on the mussel population of KI extended beyond 1995. The mean density of *Nucella lima* on KI exceeded that on MI by 2.4 x in 1996 and by 6.6 x in 1997, and the mean density of *N. lamellosa* on KI exceeded that on MI by 80 x in 1996 and by over 350 x in 1997. Estimates from laboratory feeding rate studies on *N. lima* and *N. lamellosa* of the total consumption of *Mytilus* by *Nucella* in our study areas were ≤0.3% and about 4% of the total mussel population in the study areas at MI and KI, respectively. The level of predation of *Nucella* on *Mytilus* at KI may have contributed to the creation of a bottleneck in the supply of large mussels in the population despite the release of the large mussels from sea otter predation at KI in the 7-8 yrs after the EVOS.

KEY WORDS: *Mytilus trossulus*, Sea otter, *Nucella*, Predation, *Exxon Valdez* oil spill, Population structure, Size structure

INTRODUCTION

The rate of recovery of populations of marine organisms after catastrophic disturbance, whether natural or anthropogenic in origin, depends on factors intrinsic to the organism such as reproductive rate and recruitment potential, but may also be strongly influenced by interspecific interactions or the level of chemical or physical stress in the environment unrelated to the disturbance (Loya 1976, Harris et al. 1984, Suchanek 1993, Davenport et al. 1995, Carroll & Highsmith 1996). Interspecific interactions may facilitate or inhibit population recovery after disturbance (Harris et al. 1984, Carroll & Highsmith 1996). Identifying important links in the interaction web of an intertidal community through small-scale experiments though useful to understanding community organization (Paine 1980, Menge 1995), may have limited utility for the prediction of recovery from broad-scale catastrophic disturbance (McCook & Chapman 1997).

The *Exxon Valdez* oil spill (EVOS) of 24 March 1989 and subsequent cleanup efforts represented a widespread, catastrophic disturbance to rocky intertidal communities in western Prince William Sound (PWS), Alaska (Highsmith et al. 1996, Hooten & Highsmith 1996, Houghton et al. 1996, Lees et al. 1996, Stekoll et al. 1996, van Tamelen & Stekoll 1996). The mussel, *Mytilus trossulus* Gould 1850, suffered reduced abundance and biomass to various degrees or not at all on oiled shores depending on habitat type and tidal level (Highsmith et al. 1996). On shores treated to remove oil, mussels generally suffered greater reductions in abundance than on oiled but untreated shores. The impact on the mussel population depended on the type of treatment; mussels exposed to high-pressure, hot-water treatment suffered up to a 96% reduction in percent cover after treatment (Houghton et al. 1996, Lees et al. 1996). By 1995, six years after the spill, mussel populations at oiled sites (including those on treated shores) showed fluctuations in abundance indistinguishable from those at reference sites (Houghton et al. 1997, Coats et al. 1999).

In addition to the direct effects of oil on mussels, *Mytilus* population size and structure might have been influenced indirectly by the effects of oil on mussel predators. Mussels of the genus *Mytilus* are preyed upon by a wide variety of mammalian, avian and marine invertebrate species. In addition to limiting mussel abundance and influencing local patterns of mussel distribution, predators such as the sea otter, *Enhydra lutris*, some shorebirds, crabs and gastropods prey on particular size ranges of *Mytilus*, thereby potentially influencing the size structure of mussel populations (see Seed & Suchanek 1992 for review). On the west coast of North America *Mytilus trossulus* ranges from Alaska to California (Seed 1992). It co-occurs and hybridizes with *M. galloprovincialis* in parts of Washington and California, but is the only bay mussel in Alaska (McDonald & Koehn 1988, Rawson & Hilbish 1995, Suchanek et al. 1997). *M. californianus* overlaps in distribution with *M. trossulus* in Alaska, but inhabits exposed outer coasts, appears unable to tolerate freezing conditions, and is not abundant north of Sitka, Alaska (Seed & Suchanek 1992). Populations of *M. trossulus* in PWS are subject to predation by sea otters, gulls (*Larus glaucescens* and *L. canus*), seaducks (*Bucephala islandica* and *Histrionicus histrionicus*), shorebirds (*Haematopus bachmani* and *Aphriza virgata*) dogwinkles (*Nucella lamellosa* and *N. lima*) and seastars (*Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata*, and *Leptasterias* sp.; Paul & Feder 1975, Garshelis 1983, O'Clair & Zimmerman 1986, Van Blaricom 1988, Bishop et al. 1998, Chapter 3 Part B,

Chapter 4). Of these predators, only sea otters have been shown to control mussel size-structure under some circumstances in PWS. Sea otter predation shifts the size-distribution toward smaller mussels as the larger ones are preferentially consumed (VanBlaricom 1987, 1988). Prior to the EVOS mussels represented up to 40% of the diet of sea otters at Green Island, PWS (Estes et al 1981, VanBlaricom 1987, 1988). Mussels were a major part of the diet of females with large dependent pups and independent pups there (Garshelis 1983, Van Blaricom 1988). At locations where female sea otters with dependent pups and independent pups were abundant, sea otter predation influenced the size distribution of *Mytilus*, shifting the distribution toward smaller individuals as the larger mussels (chiefly those ≥ 40 mm in shell length) were preferentially consumed (VanBlaricom 1987, 1988). Foraging observations of sea otters at northern Knight Island and northwestern Montague Island in 1996-97 revealed that clams were the most commonly observed group of prey organisms in sea otter diets, and mussels represented a relatively small percentage (10-13%) of the diet of sea otters (Chapter 3 Part B). However, Dean et al. (Chapter 3 Part B) did not break their foraging observations down by the sex, age or reproductive status of the sea otter, and no observations were made in winter when sea otters might forage nearer protected shores to avoid storms. Therefore, it is not clear what influence sea otters may have had on mussel population structure in western PWS a decade after the EVOS.

That segment of the sea otter population in the path of the oil spill in western PWS suffered heavy mortality. The northern Knight Island region was in the direct path of the EVOS and extensive stretches of the shoreline of the islands in the region were heavily oiled (Galt et al. 1991). Sea otter mortality in the region approximated 90% immediately after the spill (Bodkin & Udevitz 1994). Montague Island was not in the path of the EVOS and no oil came ashore within our study area there (Galt et al. 1991). The sea otter population at Montague Island was not reduced after the EVOS (Chapter 3 Part A). The number of sea otters in the northern Knight Island region remained low in the decade following the EVOS. Through 1998 sea otter numbers in the region did not exceed about half the pre-spill numbers whereas at Montague Island sea otter numbers remained high (Chapter 3 Part A, Chapter 3 Part B). Presumably, the mussel population in the northern Knight Island region was subject to a much reduced level of sea otter predation in the 7-8 yrs. after the EVOS compared to the population on Montague Island, and might be expected to respond with an increase in the abundance of large mussels relative to Montague Island.

Here we compare abundance and size-structure in mussel populations in an oiled and an unoiled area of PWS. We assess several factors that may have influenced mussel populations after the EVOS, and report the relative contribution of direct and indirect (principally sea otter predation) effects of the EVOS on the mussel populations.

METHODS

Study sites. The study was conducted on two islands in Prince William Sound (PWS), Alaska. The environment of one study area on the northwestern coast of Montague Island (MI) was not contaminated by oil from the EVOS and populations of vertebrate predators remained at pre-spill levels after the oil spill. The other study area included two locations on Knight Island (KI), Herring Bay and Bay of Isles, in the path of the oil spill, and where numbers of vertebrate

predators declined after the spill. Mussels were sampled within 200-m long segments of shore (sites) in the two study areas (Figure 1). The site was the sampling unit. The MI study area contained 250 contiguous sites; the KI area contained 155 contiguous sites in Bay of Isles and 187 contiguous sites in Herring Bay. The length of the shoreline including nearshore islands within each study area was measured on aerial photographs using a digital planimeter. The shoreline lengths were 51.5 km and 68.4 km at the MI and KI study areas, respectively. The dates of sampling were 29 May to 8 June and 2-12 and 19-30 July in 1996 and 18-28 May, 17-27 June, and 16-27 July in 1997.

Sampling design and mussel collection. Following cost and power analysis performed on preliminary mussel density data collected in 1995 a sample size of 60 sites per study area was chosen. Power analysis revealed that this sample size would allow the detection of a difference of 42 mussels 500 cm^{-2} (55% of the mean) at the $\alpha = 0.05$ significance level with power $1 - \beta = 0.8$. The actual number of sites sampled was 51 on MI and 57 on KI in 1996. In 1997 the number of sites sampled was 55 in each study area.

The sites were numbered sequentially throughout the study areas, beginning with the northern end of the MI. Each study area included the shorelines of islands adjacent to shore. The first site sampled in each study area was selected with a random number generator. The remaining sites were sampled systematically such that every 4th to 6th segment was sampled, depending on the study area.

Mussels were sampled in quadrats on ten vertical transects laid 20 m apart within each site. The first transect was placed a random distance between 0 and 20 m from the boundary of the site. The remaining nine transects were laid systematically at 20-m intervals starting from the first. Each transect was laid perpendicular to shore from the upper limit to the lower limit of the realized distribution of *Mytilus trossulus*, ie from the uppermost to the lowest mussel on the vertical transect. A 500 cm^2 quadrat was positioned a random distance along each transect. All mussels were removed from within the quadrat, placed in a plastic bag and frozen within 3-4 h. A total of 1,054 and 1,108 quadrats were sampled in 1996 and 1997, respectively.

Our density estimate for *M. trossulus* was restricted to the realized distribution of the mussel in the two study areas because it would have been unrealistic to attempt to estimate density over the potential distribution of the species based on habitat characteristics. *Mytilus trossulus*, like its congener *M. edulis*, is capable of inhabiting a broad range of intertidal habitats from protected habitat to that exposed to heavy wave action and including substrates such as bedrock or sediments ranging from boulder to mud (CEO, personal observations; Seed and Suchanek 1992). Moreover, the potential vertical distribution of mussels is modified by many physical and biological factors chief among which may be temperature extremes, desiccation and predators (Seed and Suchanek 1992). We were simply not able to adequately quantify the effect of site-specific differences in the magnitude of physical factors and biotic influences on mussel distribution to adjust the realized distribution of *M. trossulus* and thereby obtain an estimate of the potential distribution of the mussel at each site.

Several environmental variables were measured in conjunction with the mussel sampling. Invertebrate predators of mussels (asteroids and *Nucella* spp.) were counted 1 m either side of each transect. The width of the mussel zone was measured with a surveyor's tape. The slope of

the shore on each 200-m segment was measured with a clinometer. The substrate in each quadrat was classified according to the Wentworth grain-size scale (Holme & McIntyre 1984) . The segments were post-stratified into two strata based on substrate: 1) rocky (including bedrock and boulder areas) and 2) unconsolidated or mixed substrate (including various mixtures of silt, sand, granules, pebbles and cobble).

Mussel density, size and biomass. Mussel samples were sieved in the laboratory using 4 mm, 2 mm, 1 mm and 0.5 mm mesh sieves. Small mussels were counted in two size classes (0-2 mm and 2-5 mm) based on shell length. The shell length of all mussels ≥ 5 mm was measured to the nearest 0.1 mm with a digital caliper connected to a datalogger. A total of 58,432 mussels were measured for size-frequency analysis in 1996; 78,554 were measured in 1997.

A total of 280 mussels were selected from the main study locations (MI, Bay of Isles and Herring Bay) for the measurement of mussel mass. Mussels were selected from both mixed and rocky strata in as wide a range of shell lengths as possible. Each mussel was drip dried on a paper towel and then weighed. All measurements of mass were taken to the nearest mg on a precision balance. After the wet mass was obtained the mussels were placed in a drying oven at 60˚C, weighed at 24 h intervals until the weights stabilized, and then ashed in a muffle furnace for 5 hours at 550˚C. The ash-free dry mass (AFDM) was calculated by subtracting the mass of the ash from the dry mass of the mussel (Palmerini and Bianchi 1994).

***Nucella* laboratory feeding experiments**. Separate 60-day feeding experiments, run sequentially, were conducted in the laboratory in which *Nucella lima* or *N. lamellosa* were held together with *Mytilus trossulus* in perforated containers in a tank under flow-through conditions. The *N. lima* experiment was conducted from November to January; that with *N. lamellosa* from March to May. The mussels and dogwinkles were collected by hand at low tide or using SCUBA in or near Auke Bay, Alaska (lat 58˚22' N, long 134˚39'W) and transported to the Auke Bay Laboratory for the experiments. Three size classes of *Mytilus* were used in three treatments: 1) shell length (SL), 5-20 mm; 2) SL, 20.1 - 40 mm; SL, >40 mm. Ten mussels of the appropriate size class were haphazardly assigned to each container. Each treatment was run in triplicate. In the first experiment 10 *N. lima* were randomly assigned to each container (30 snails treatment^{-1}) using a computer random number generator. The snails averaged 21.3 mm in shell height (range, 14-29 mm). Because of the larger size of *N. lamellosa* (mean shell height, 37.4 mm; range, 24-45 mm), five snails were randomly assigned to each container in the second experiment. The experiments were monitored daily for drilled mussels, and a running mean predation rate expressed as no. of mussels eaten snail^{-1} d^{-1} was calculated. Mussels were replaced as they were consumed. Mean water temperature in the *N. lima* and *N. lamellosa* experiments was 7.8˚C (range, 4.8˚C - 8.9˚C) and 5.3˚C (range, 4.7˚C - 6.0˚C), respectively.

Quantitative analysis. Separate regressions of AFDM on shell length were calculated for each study location. The mass of each mussel ≥ 5 mm in shell length was calculated using the appropriate regression for the location where the mussel was collected. The density of mussels or mass M_l of mussel tissue per unit area in each study area was

$$M_l = \frac{\left(\sum_{j=1}^{s} \frac{\left(\sum_{i=1}^{q} \sum_{h=1}^{n} m_h \right)}{q} \right)}{s}$$

where m_h = the hth mussel or the mass of the hth mussel in the ith quadrat depending on whether density or biomass are being estimated, q = the number of quadrats of unit area in the jth shore segment, and s = the number of segments in the lth study area. The area of the mussel zone in each study area was the product of the length of the shoreline and the mean width of the mussel zone in the study area.

The number N_{kl} of mussels consumed by each species k of *Nucella* in each study area l was

$$N_{kl} = n_{kl} \times U_{kl}$$

where U_{kl} was the number of *Nucella* of species k in study area l, and the number n_{kl} of mussels eaten in one year by one *Nucella* of species k in study area l was

$$n_{kl} = \frac{1}{\sum_{a=1}^{3} \frac{p_a}{r_a}}$$

estimated from the proportion p_a of each of three size classes of mussels (shell lengths [SLs], 5-20 mm; SLs, 20.1-40 mm; SLs, >40 mm) and the rate r_a of consumption of mussels in each size class by each species of *Nucella* in one year, determined in the laboratory (see Results). The estimate of Carroll and Highsmith (1996) for the mid-intertidal feeding season in south central Alaska of 30 weeks was used to calculate r_a. Our estimate of n_{kl} rested on three simplifying assumptions: 1) that each species of *Nucella* preyed on *M. trossulus* in the study areas at the same rate as they did in the laboratory where alternative prey species were not available to them, 2) that the size classes of mussels were distributed randomly with respect to one another in the study areas, and 3) that *Nucella* exhibited no prey size selection of *Mytilus* at the study areas.

Analysis of variance was used to test for differences between study areas, years and strata for variables of mussel zone characteristics and mussel density and biomass. Homogeneity of variance was tested with Levene's test (Levene 1960). If necessary, transformations (log [y+1], $y^{-0.5}$, $y^{0.054}$) were used to stabilize variances. Transformations, except log (y+1), were derived according to the method of Taylor (1961). Planned comparisons were made with F-tests or Welch's approximate t-test with Satterthwaite's adjusted degrees of freedom for unequal variances (Day and Quinn 1989). Unplanned comparisons were made with the Kramer modification of Tukey's test for equal variances and unequal sample sizes (Day and Quinn 1989). Because the density and biomass data for mussels ≥ 40 mm in length and the density data for *Nucella lamellosa* on Montague Island contained many zeros we used the Mann-Whitney U-test to test for differences in density and biomass between study areas for these data. Analysis

of covariance was used to test for a difference in the slope of the relationship between log-transformed values of mussel shell length and AFDM between study locations. A two-tailed t-test was used to test the significance of the Pearson formulation of the product-moment correlation coefficient (r) describing the relation of *Nucella* spp. density with the modal length of the mussel length-biomass distribution at each site (Sokal & Rohlf 1995). The data were transformed (log [y+1]) for the test. Error values in the text and figures are one standard error of the mean.

RESULTS

Characteristics of mussel zone

The characteristics of the mussel zone and the substrate type available for mussel attachment differed between study areas. The mussel zone width averaged 4.3 x greater at Montague Island (MI; 37.2 ± 5.3 m) than at Knight Island (KI; 8.7 ± 0.6 m; data from 1996 and 1997 combined; Tables 1 and 2). The difference between study areas was not as pronounced in areas with rocky substrates as in areas with mixed substrates. As a result of the difference in mussel zone width between study areas the total area of the mussel zone at MI (1.53 km^2) exceeded that at KI (0.57 km^2) by 2.7 x. The mean slope of the shore within the mussel zone at MI (5.8° ± 0.4°) was one fourth as steep as that at KI (25.7° ± 1.7°; Tables 1 and 2). A greater proportion (63%) of the shore segments at MI were composed predominately of mixed substrate, whereas most (74%) of the shore segments at KI were predominantly rocky.

Mytilus density and biomass

Mytilus density differed between study areas depending on size-class, stratum and year. The mean density of all mussels ≥ 5 mm in shell length was higher at KI than at MI in 1996 and 1997 (Figure 2). Density of mussels ≥ 5 mm at KI (1,430 ± 245 m^{-2}) was 1.7 x that on MI (862 ± 191 m^{-2}) in 1996 (Tables 3 and 4; $F = 8.20$; $p < 0.01$). In 1997 the density at KI (2,020 ± 288 m^{-2}) exceeded that at MI (873 ± 125 m^{-2}) by 2.3 x ($F = 18.2$; $p < 0.01$). Significant study area by stratum and study area by stratum by year interactions for the ≥ 5 mm mussels indicated that pairwise comparisons of stratum means should be examined. When broken down to the stratum level only the rocky stratum at KI in 1997 significantly exceeded strata at MI (Tables 4 and 5).

The mean density of mussels ≥ 20 mm in shell length was higher at MI than at KI in 1997 but not 1996. Although no interaction terms were significant in the ANOVA, a two-tailed, planned comparison revealed that mean density of mussels ≥ 20 mm at MI (215 ± 36.1 m^{-2}) did not differ significantly from that at KI (134 ± 19.2 m^{-2}) in 1996 (Figure 2, Tables 3 and 4; $F = 2.53$; $p > 0.05$). In 1997 mean density at MI (280 ± 55.4 m^{-2}) exceeded that at KI (113 ± 24.2 m^{-2}) by 2.5 x ($F = 10.8$; $p < 0.01$). The ANOVA indicated a stratum effect. Within-year paired comparisons revealed that density was greater in mixed substrates (286 ± 60.1 m^{-2}) than on rocky substrates (127 ± 27.1 m^{-2}; Tukey-Kramer test; $p < 0.05$) in 1997 but not 1996 (mixed, 237 ± 31.6 m^{-2}; rocky, 122 ± 24.4 m^{-2}; Tukey-Kramer test; $p > 0.05$).

The density of large mussels (shell length ≥ 40 mm) was very low at both study areas (Figure 2). A large percentage of the quadrats at both areas contained no mussels in this size range (61% at MI, 63% at KI), therefore the data were not normally distributed. The Mann-Whitney U-test revealed no difference in mean density of large mussels between KI and MI in 1996 (test, $p = 0.07$; Figure 2). In 1997, the density at MI exceeded that at KI (Mann-Whitney U- test, $p = 0.004$; Figure 2). However, care must be taken in the interpretation of this result because of unequal variances (Levene's test, $p = 0.02$) in the large mussel data in 1997. When data from 1996 and 1997 were combined density did not differ between study areas (Mann-Whitney U-test, $p = 0.416$; Levene's test, $p = 0.027$).

The difference in mussel biomass between study areas depended on the size class of mussels and the year of sampling. The relationship of mussel mass (AFDM) to shell length did not differ between study areas (ANCOVA, study area x shell length interaction, $F = 0.132$, $p = 0.716$; Figure 3). The ANOVAS of biomass density of mussels in the size ranges ≥ 5 mm and ≥ 20 mm revealed in no significant effect of study area (Table 6). We then conducted paired *a priori* F- tests of biomass density of ≥ 5 mm and ≥ 20 mm mussels between study areas in 1996 and 1997. We observed no differences in biomass density of ≥ 5 mm between study areas in 1996 ($F = 0.27$, $p > 0.05$) or 1997 ($F = 2.91$, $p > 0.05$; Figure 4). Mean biomass density of ≥ 20 mm mussels at MI exceeded that at KI ($F = 10.7$, $p < 0.01$) in 1997, but not 1996 ($F = 1.06$, $p > 0.05$; Figure 4). Mean biomass density of ≥ 20 mm mussels also differed between strata (Table 6). *Post hoc* tests revealed greater biomass density in mixed sediment (29.0 ± 7.2 gm^{-2}) than on rocky substrate (10.3 ± 2.3 g m^{-2}; Tukey-Kramer test; $p < 0.01$) in 1997, but not in 1996 (mixed, 23.1 ± 3.2 g m^{-2}; rocky, 10.7 ± 1.9 g m^{-2}; Tukey-Kramer test; $p > 0.05$).

The effect of study area on the biomass density of large mussels (≥ 40 mm) varied between years. In 1996 mean biomass density of mussels ≥ 40 mm at KI exceeded that at MI by 43% (Mann-Whitney U-test, $p = 0.042$; Figure 4). In 1997 the relationship reversed and mean biomass density of large mussels at MI exceeded that at KI by 6.3 x (Mann-Whitney U-test, $p = 0.006$; Figure 4).

Mytilus length and biomass distribution

The length-frequency distribution of mussels ≥ 5 mm at both areas was skewed to the right in 1996 and 1997 (Figure 5; Table 7). Length-frequency modes occurred at shell lengths ranging from 6 to 8 mm at the two study areas over both years. The distribution at KI was more strongly skewed than that for MI indicating that mussel abundance was more strongly concentrated in the small size classes at KI (Table 7; Figure 5).

Differences between study areas in the distribution of biomass with mussel size were more pronounced than were differences between study areas in length frequency. At MI the distribution of mussel mass showed modes at shell lengths of 27 and 31 mm in 1996 and 1997, respectively (Figure 6). At KI modes occurred at shell lengths of 11 and 9 mm in 1996 and 1997, respectively (Figure 6). The MI distribution was skewed to the left in both years (Table 7). That at KI was skewed to the right in both years, and in 1996 was strongly platykurtic tending toward bimodal (Figure 6; Table 7).

Invertebrate predator density

A suite of invertebrate predators including two species of *Nucella* (*N. lima* and *N. lamellosa*) and five species of generalist seastar (*Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata*, and *Leptasterias* sp.) occur in the intertidal region in PWS and are known to prey on mussels or are potential mussel predators. The mean density of *Nucella lima* on KI exceeded that on MI in 1996 and 1997. In 1996 *N. lima* density on KI exceeded that on MI by 2.4 x (Figure 7, Table 8; Welch's t-test, $p<0.05$). In 1997, *N. lima* density on KI exceeded that on MI by 6.6 x (Figure 7, Table 8; Welch's t-test, $p<0.01$). Density of *N. lima* did not differ between years nor with substrate (Table 8).

Nucella lamellosa was rare at MI. It was observed on only nine (20%) shore segments there in 1996 and only 5 (9%) segments in 1997. The large number of zeros in the data set of *N. lamellosa* density precluded the use of parametric statistics. The mean density of *N. lamellosa* on KI exceeded that on MI by 80 x in 1996 (Figure 7; Mann-Whitney U-test, $p<0.001$). In 1997, *N. lamellosa* density on KI exceeded that on MI by over 350 x (Figure 7; Mann-Whitney U-test, $p<0.001$). The density of *N. lamellosa* on rocky substrate exceeded that on mixed substrate in 1996 by 476 x (rocky, 1.54 ± 0.48 m^{-2}; mixed, 0.003 ± 0.002 m^{-2}; Mann-Whitney U-test, $p<0.001$; study areas combined) and in 1997 by 701 x (rocky, 1.38 ± 0.36 m^{-2}; mixed, 0.002 ± 0.002 m^{-2}; Mann-Whitney U-test, $p<0.001$).

The density of *Nucella* spp. (both species combined) at the study sites was inversely correlated with the modal length of the mussel length-biomass distribution at the sites at KI in 1996 ($r = -0.633$, $p<0.01$) and in 1997 ($r = -0.463$, $p<0.01$; Figure 8). However, at MI these two variables were uncorrelated in both years (1996, $r = -0.068$, $p>0.05$; 1997, $r = -0.023$, $p>0.05$).

The density of large asteroids (*Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata*) on our transects did not differ significantly between study areas in 1996 or 1997 (Figure 7). Densities of the four species of seastars were lumped for the analysis. *Leptasterias* sp. was eliminated from the analysis because it is small and cryptic, and was difficult to count accurately. Abundance estimates of *Leptasterias* were made on the ordinal scale. Variances could not be stabilized with transformation of the seastar density data for the ANOVA (Table 8). Welch's approximate t-test was therefore used for within-year *post hoc* tests. The mean density of large asteroids was similar on KI (0.059 ± 0.019 m^{-2}) to that on MI (0.040 ± 0.013 m^{-2}) in 1996 (Welch's t-test, $p>0.05$) and 1997 (KI, 0.031 ± 0.006 m^{-2}; MI, 0.016 ± 0.007 m^{-2}; Welch's t-test, $p>0.05$; Figure 7). The density of large seastars on rocky substrate exceeded that on mixed substrate in 1996 (rocky, 0.076 ± 0.019 m^{-2}, mixed, 0.013 ± 0.005 m^{-2}; Welch's t-test, $p<0.01$) and 1997 (rocky, 0.037 ± 0.008 m^{-2}; mixed, 0.006 ± 0.002 m^{-2}; Welch's t-test, $p<0.001$; Table 8).

Nucella predation in the laboratory

Feeding rate was inversely related to mussel size in *Nucella lima*. On average, about one third of the *Nucella lima* fed at any particular time over the course of the experiment regardless of the size class of the mussels eaten (Table 9). However, feeding rate was highest for *N. lima* feeding on small (shell length, 5 - 20 mm) mussels. The mean feeding rate (averaged over 60 d) on small mussels was more than 4 x greater than that on medium (21.1 - 40 mm) mussels

(ANOVA, $F = 507$, $p < 0.001$; *a priori*, paired comparison, $F = 629$, $p < 0.001$; Table 9). The feeding rate on medium mussels was, in turn, more than twice that on large (> 40 mm) mussels (*a priori*, paired comparison, $F = 20$, $p < 0.01$; Table 9).

The relationship between feeding rate in *N. lamellosa* and mussel size class was similar to that for *N. lima*. The mean feeding rate on small mussels was 3x greater than that on medium mussels (ANOVA, $F = 43.9$, $p < 0.001$; *a priori*, paired comparison, $F = 40.1$, $p < 0.001$; Table 9). On average, a smaller percentage of the *Nucella lamellosa* fed at any given time than did *N. lima*. This was especially true of those feeding on large mussels (Table 9). The feeding rate of *N. lamellosa* on medium mussels was more than an order of magnitude greater than that on large (> 40 mm) mussels (*a priori*, paired comparison, $F = 7.9$, $p < 0.05$; Table 9).

Total annual consumption of *Mytilus* by *Nucella*

Estimates of total annual consumption of *M. trossulus* by *Nucella* spp. were markedly higher at KI than at MI in 1996 and 1997. The total number of mussels eaten by both species of *Nucella* combined at KI was about an order of magnitude greater than that at MI in both years (Table 10). The number of mussels consumed by *Nucella* spp. in 1996 represented 0.3% and 3.8% of the total number of mussels at the study areas on MI and KI, respectively. In 1997 *Nucella* spp. consumed 0.2% and 3.7% of the total number of mussels at MI and KI, respectively.

DISCUSSION

The direct effects of oil from the EVOS on mussel populations depended on habitat type and tidal level. Highsmith et al. (1996) estimated the abundance of *Mytilus* in western PWS in spring/summer 1990 and spring 1991. They found reduced mussel density throughout the vertical range studied at oiled sites compared to reference sites on coarse-textured substrate in spring 1990. In sheltered rocky and estuarine habitats only at lower tidal levels was mussel density reduced at oiled sites. In summer 1990 and spring 1991 mussel density was reduced at oiled sites in coarse-textured habitat at upper (summer 1990) and lower (spring 1991) tidal levels and in estuarine habitat at mid-tidal levels (summer 1990). The reduction in mean density at oiled sites relative to reference sites ranged from 20% to 90% depending on habitat, tidal level and year (Highsmith et al. 1996). Lees et al. (1996) estimated the abundance of *Mytilus* at 21 locations in western PWS in 1991-92 and found changes resulting from shoreline cleaning. They examined the effects of dispersants and beach cleaners, low-pressure warm water and high-pressure hot water (HP-HW) treatments on *Mytilus* abundance as well as that of other organisms on heavily oiled cobble beaches. Reductions in mussel percent cover after treatment ranged from 50% to 69% depending on the type of treatment used (Lees et al. 1996). The HP-HW treatment had the greatest impact on mussel cover. On a bedrock shore in a heavily oiled bay mussel percent cover was reduced 96% by HP-HW treatment (Houghton et al. 1996).

The direct effects of *Exxon Valdez* oil on mussel populations in PWS probably extended no more than a few years after the spill. The length-frequency distributions of *Mytilus trossulus* that Houghton et al. (1993) present for May, July and September 1991 in one heavily oiled bay (Herring Bay) show little evidence of recruitment there in 1991 (although the bimodal July distribution showed one mode at 5 mm). However, by 1993 mussels began recruiting to some

sites in Herring Bay. The length-frequency distributions of Highsmith et al. (1996) show evidence of good recruitment in June 1993, September 1994 and May 1995 at several sites in Herring Bay. In 1992 and 1993 Thomas et al. (1999) removed mussels from beds overlying sediments oiled by the EVOS in PWS. Total polynuclear aromatic hydrocarbon (TPAH) concentrations in the tissues of the mussels from the oiled beds ranged from about 0.2 to 6 $\mu g\ g^{-1}$, exceeding the tissue concentrations of TPAH in reference mussels by 20-95 x. Nevertheless, byssal thread production, condition index, clearance rate and glycogen content did not differ between mussels from the oiled beds and those from reference beds (Thomas et al. 1999). Highsmith et al. (1996) found reduced growth in mussels at oiled sites in parts of one heavily oiled bay (Herring Bay) that persisted until 1995. However by 1995, fluctuations in mussel abundance at oiled sites (including those treated to remove oil) were indistinguishable from those at reference sites (Houghton et al. 1997, Coats et al. 1999).

Among the indirect effects of the EVOS on mussel population structure, release from sea otter predation seemed a likely possibility to us. Before the EVOS VanBlaricom (1987, 1988) had shown that sea otter predation (especially that by female sea otters with dependent pups and independent pups) controlled the size distribution of *Mytilus* at Green Island near northwest Montague Island. Sea otter predation shifted the size distribution of *M. trossulus* toward smaller individuals as the large mussels (chiefly those ≥ 40 mm in shell length) were preferentially consumed. Immediately after the spill, sea otter mortality in the oiled northern Knight Island region approximated 90% (Bodkin & Udevitz 1994). Through 1998 sea otter numbers in the region did not exceed about half the pre-spill numbers (Chapter 3 Part A). At unoiled Montague Island the sea otter population was not reduced after the EVOS and numbers remained high in the decade after the spill (Chapter 3 Part A, Chapter 3 Part B). Presumably, the mussel population in the northern Knight Island region was subject to a much reduced level of sea otter predation in the 7-8 yrs. after the EVOS compared to the population on Montague Island, and might be expected to respond with an increase in the abundance of large mussels relative to Montague Island.

Our results did not support the hypothesis that the mussel population on northern Knight Island in 1996-97 responded in the predicted way to a 7-8 yr release from sea otter predation. Although the density of all mussels ≥ 5 mm in shell length at KI exceeded that at MI in both years of our study, the majority of the mussels at KI were small, having recruited to the population in the previous 1-2 yrs. As a result, the length-frequency distribution of mussels ≥ 5 mm was more strongly skewed to the right at KI than at MI in 1996 and 1997.

The density and biomass of large mussels either showed no differences between study areas or were greater at MI than at KI. The density and biomass of mussels ≥ 20 mm in shell length did not differ between study areas in 1996, but was higher at MI than at KI in 1997. Perhaps the most revealing comparison of mussel density and biomass between study areas is that of mussels ≥ 40 mm in shell length. In his pre-spill study in PWS VanBlaricom (1987, 1988) compared the length-frequency distribution of mussels in an area harboring female sea otters with dependent pups and independent pups with that in an area containing adult male otters only and found that where sea otter predation structured mussel populations, mussels ≥ 40 mm suffered the greatest mortality. The density of ≥ 40 mm mussels was very low at both of our study areas. The density of this size class did not differ between study areas in 1996. In 1997 the density of ≥ 40 mm mussels at MI actually exceeded that at KI. The mean biomass density of

mussels ≥ 40 mm at KI exceeded that at MI somewhat in 1996, but in 1997 the relationship reversed and mean biomass density of ≥ 40 mm mussels at MI exceeded that at KI by 6.3 x. Our results therefore indicate that release from sea otter predation on Knight Island after the EVOS did not promote an increase in the abundance of large mussels there.

Failure of the mussel population at KI to respond to release from sea otter predation in the predicted way was probably not a result of inadequate time for newly recruited mussels to grow into larger size classes at KI. If good recruitment of *Mytilus* to heavily oiled shores on KI after the EVOS were delayed until 1993 (see discussion of direct effects above), then these recruits would have had time to grow into the ≥ 20 mm size class at KI by 1996-97, according to the models of mussel growth for the region (Millstein & O'Clair 2001). Of course, juvenile and adult mussels that survived the oil spill would presumably continue to grow at some level and should further augment the large size class at KI by 1996-97.

Alternatively, *Nucella* predation may have structured the mussel population on KI. Carroll & Highsmith (1996) found that predation by *Nucella lima* prevented recovery of *Mytilus trossulus* at some sites in Kachemak Bay, Alaska where mussel abundance had been reduced by a severe winter freeze. They estimated that *N. lima* could eliminate 60-90% of mussels at a given site in one season. Suchanek (1978) reported similarly high levels of *Nucella* (= *Thais*) predation on *M. trossulus* (= *M. edulis* in Alaska) in southeastern Alaska where the average percentage of drilled mussel shells on the low shore ranged from 61% on protected shores to 95% on wave-exposed shores at sites with high numbers of *Nucella* spp. (*N. canaliculata*, *N. emarginata*, *N. lamellosa* and *N. lima*). In the present study, our estimate of the percentage of mussels at the study areas consumed by *Nucella* spp. in 30 weeks (the estimated mid-intertidal feeding season in south central Alaska determined by Carroll & Highsmith [1996]) was much lower than that of Carroll & Highsmith (1996), ranging from 0.3% to 3.8% (rather than 60-90%) depending on the study area. However, our estimate was based, in part, on an estimate of the mean density of *Nucella* spp. over lengths of shore of 51.5 km and 68.4 km at MI an KI, respectively, whereas the estimate of Carroll & Highsmith (1996) was at a site with a high density of *N. lima*. Nevertheless, we may have underestimated the number of mussels consumed in our study areas. Our estimate of the feeding rate of *Nucella* spp. on *M. trossulus* in the laboratory ranged from 0.0056 to 0.189 mussels d^{-1} depending on mussel size-class and the species of *Nucella*. This estimate was lower than those of Seed (1976; 0.31mussels d^{-1}) and Stickle et al. (1985; 0.1-0.6 mussels d^{-1} [see Seed and Suchanek 1992] depending on shell thickness and temperature) for *N. lapillus* preying on *M. edulis*. Our estimate was closer to that of Wickens and Griffiths (1985; 0.02-0.12 mussels d^{-1}) for *N. cingulata* feeding on *Aulacomya ater* and that of Hunt and Scheibling (1998; 0.1 mussels d^{-1}) for post-recruit *N. lapillus* feeding on *M. edulis/M. trossulus*. Hunt and Scheibling's (1998) estimate of the feeding rate of *N. lapillus* on *Mytilus* (0.1 mussels d^{-1} and 0.156 mussels d^{-1} for tidepools and emergent rock, respectively) in the field was similar to the estimate they obtained in the laboratory, indicating that the feeding rate of *Nucella* spp. at our study sites may not have been markedly different from the feeding rate estimate we obtained in the laboratory.

The negative correlation between the modal length of the mussel length-biomass distribution and the density of *Nucella* spp. at the study sites on KI is indicative of an effect of predation by *Nucella lima* and *N. lamellosa* on the population structure of *Mytilus trossulus* there. The lack of a correlation between these two variables at MI indicates that *Nucella* densities

on MI may have been too low to produce a detectable effect on mussel population structure. Although *Nucella lima* densities did not differ between substrate types, densities of *N. lamellosa* were found to be far greater in rocky areas (particularly on large immobile rock surfaces) than on mixed substrate, thus the increased *Nucella* spp. densities found at KI may be partially explained by the increased percentage of rocky shoreline there.

The strongly right-skewed length-frequency distribution of mussels resulting from a higher density of 5 - 20 mm mussels, in combination with the greater *Nucella* densities at KI (2.4 x - 6.6 x that at MI for *Nucella lima* and 80 x to over 350 x that at MI for *N. lamellosa*, depending on the year) was also consistent with, but not necessarily indicative of an effect of *Nucella* predation on mussel population structure at KI. Increased mussel recruitment alone at KI may have produced the strongly right-skewed length-frequency distribution of mussels there. However if mussel recruitment alone were responsible for the higher density of 5-20 mm mussels on KI one would not necessarily expect a relationship between the modal length of the mussel length-biomass distribution and the density of *Nucella* spp. at the study sites on KI. Alternatively, the differences in the mussel length-frequency distributions between study areas may have been the result of the interplay of two mechanisms: 1) an increase in the number of refuges for mussels of small size at KI and 2) prey size selection by *Nucella* spp.

The substrate at KI may have favored *M. trossulus* recruits. The shores of KI were predominantly rocky and included a large amount of bedrock with barnacle cover. This type of surface contained many small crevices and pits, which can provide refuge from *Nucella* for small mussels. As a mussel grows too large to obtain refuge in the pits and crevices in rock and between barnacles it can become prey to *Nucella*. By contrast, the predominantly mixed intertidal substrate at MI may not have provided a similar size refuge for mussels.

Nucella may prefer larger mussels thereby enhancing the size refuge provided by the habitat at KI. We did not examine size-selection of *M. trossulus* by *N. lima* or *N. lamellosa*. Experienced *Nucella lapillus*, a species comparable in adult size to *N. lima*, were found to prefer *Mytilus edulis* of 20-25 mm shell length (SL) in the laboratory (Hughes & Dunkin 1984). In Yorkshire, UK, Hughes & Burrows (1991) found that *N. lapillus* primarily preyed on mussels 10-20 mm SL. However, in Nova Scotia, predation by *N. lapillus* post-recruits ($\geq$5 mm SL) on mussels < 5 mm SL was not uncommon (Hunt & Scheibling 1998).

Nucella predation on KI may have contributed in large part to the failure of mussels of large size to increase in abundance at KI despite being released from sea otter predation during the 7-8 yr period after the EVOS. Mussel recruitment may have been enhanced at KI by habitat complexity. However, although *Nucella* spp. consumed < 4% of the estimated total population of *M. trossulus* in our study area at KI (compared to $\leq$ 0.3% at MI), that level of predation may have contributed to the creation of a bottleneck in the supply of large mussels by increasing mortality in mussels of intermediate size, thereby reducing the number of mussels growing to large size enough to compensate for the reduced mortality experienced by large mussels as a result of release from sea otter predation.

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Table 1. Mussel zone characteristics at study areas (Montague Island vs. Knight Island) in strata (rocky vs. mixed sediment substrates) in 1996 and 1997 in Prince William Sound.

			Montague Island			Knight Island		
Characteristic	Year	Stratum	Mean	SD	N	Mean	SD	N
Mussel zone width (m)	1996	Mixed	34.7	40.2	34	11.7	2.9	14
		Rocky	14.0	10.2	17	6.5	3.3	43
	1997	Mixed	64.6	82.2	35	19.2	10.6	14
		Rocky	16.7	14.6	21	6.3	3.3	41
Slope of Shore (˚)	1996	Mixed	5.3	2.8	34	12.7	6.3	14
		Rocky	7.4	5.4	17	21.4	12.6	43
	1997	Mixed	5.0	3.5	35	16.2	14.4	14
		Rocky	6.6	4.1	21	37.8	21.0	41
Lower Edge of Mussel	1996	Mixed	0.606	0.622	34	0.405	0.608	14
Zone (m)†		Rocky	1.369	0.544	17	0.938	0.491	43
	1997	Mixed	0.399	0.529	35	-0.021	0.491	15
		Rocky	1.215	0.531	21	0.739	0.634	41
Upper Edge of Mussel	1996	Mixed	2.560	0.563	34	2.497	0.257	14
Zone (m)†		Rocky	2.542	0.366	17	2.781	0.342	43
	1997	Mixed	2.643	0.651	35	2.516	0.731	15
		Rocky	2.635	0.260	21	2.917	0.601	41

Notes: SD, standard deviation; N, sample size.
† Height above mean lower low water.

Table 2. Analysis of variance of mussel zone characteristics in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in two years (1996 and 1997) in Prince William Sound.

Mussel Zone Parameter Source of Variation	df	MS	F	P
Mussel Zone Width				
Data transformed ($y^{-0.5}$); Levene's Test[a], P = 0.347				
Study area	1	0.406	61.4	<0.001
Year	1	0.041	6.23	0.013
Stratum	1	0.584	88.2	<0.001
Study area x Year	1	0.001	0.139	0.710
Study area x Stratum	1	0.034	5.12	0.025
Year x Stratum	1	0.008	1.26	0.263
Study area x Year x Stratum	1	0.002	0.348	0.556
Error	210	0.006		
Slope of Shore				
Data transformed (Log[y+1]); Levene's Test[a], P =0.002				
Study area	1	9.32	145.5	<0.001
Year	1	0.080	1.26	0.262
Stratum	1	2.07	32.3	<0.001
Study area x Year	1	0.324	5.05	0.026
Study area x Stratum	1	0.332	5.18	0.024
Year x Stratum	1	0.188	2.94	0.088
Study area x Year x Stratum	1	0.119	1.85	0.175
Error	211	0.064		
Lower Edge of Mussel Zone				
Untransformed data; Levene's Test[a], P = 0.486				
Study area	1	71.8	21.1	<0.001
Year	1	29.9	8.77	0.003
Stratum	1	253.9	74.5	<0.001
Study area x Year	1	2.14	0.630	0.428
Study area x Stratum	1	2.51	0.737	0.392
Year x Stratum	1	2.41	0.707	0.401
Study area x Year x Stratum	1	0.921	0.270	0.604
Error	212	3.41		

Table 2 (cont.)

Mussel Zone Parameter Source of Variation	df	MS	F	P
Upper Edge of Mussel Zone				
Untransformed data; Levene's Test[a], P = 0.002				
Study area	1	3.38	1.19	0.277
Year	1	3.37	1.18	0.278
Stratum	1	13.4	4.70	0.031
Study area x Year	1	0.012	0.004	0.947
Study area x Stratum	1	15.6	5.46	0.020
Year x Stratum	1	0.482	0.169	0.681
Study area x Year x Stratum	1	0.354	0.124	0.725
Error	212	2.85		

a. Test of homogeneity of variances.

Table 3. Mussel density (No./m^2) in three size classes in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in 1996 and 1997 in Prince William Sound.

			Montague Island			Knight Island		
Characteristic	Year	Stratum	Mean	SE	N	Mean	SE	N
Mussels ≥ 5 mm in shell	1996	Mixed	637	78.1	32	1098	148	15
length		Rocky	1243	491	19	1545	327	42
	1997	Mixed	974	184	35	693	120	14
		Rocky	705	129	21	2473	359	41
Mussels ≥ 20 mm in shell	1996	Mixed	231	43.0	32	250	38.6	15
length		Rocky	187	65.2	19	92.1	18.6	42
	1997	Mixed	340	81.7	35	149	32.0	14
		Rocky	178	52.8	21	101	30.6	41
Mussels ≥ 40 mm in shell	1996	Mixed	3.1	1.4	32	5.8	1.3	15
length		Rocky	0.43	0.34	19	1.8	0.65	42
	1997	Mixed	11.3	5.5	35	2.2	0.59	14
		Rocky	0.49	0.28	21	0.65	0.35	41

Notes: SE, standard error of the mean; N, sample size.
† Height above mean lower low water.

Table 4. Analysis of variance of mussel density (No./500 cm^2) in two size classes in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in two years (1996 and 1997) in Prince William Sound.

Mussel Size Class Source of Variation	df	MS	F	P
Mussels ≥ 5 mm in Shell Length				
Data transformed (Log[y+1]); Levene's Test[a], P =0.052				
Study area	1	3.07	15.9	<0.001
Year	1	0.017	0.089	0.766
Stratum	1	0.212	1.10	0.296
Study area x Year	1	0.005	0.028	0.868
Study area x Stratum	1	0.811	4.20	0.042
Year x Stratum	1	0.219	1.13	0.289
Study area x Year x Stratum	1	1.34	6.96	0.009
Error	211	0.193		
Mussels ≥ 20 mm in Shell Length				
Untransformed data; Levene's Test[a], P =0.117				
Study area	1	811.8	4.83	0.029
Year	1	0.444	0.003	0.959
Stratum	1	1152	6.86	0.009
Study area x Year	1	249.8	1.49	0.224
Study area x Stratum	1	0.0001	0.000	0.999
Year x Stratum	1	0.434	0.003	0.960
Study area x Year x Stratum	1	350.3	2.086	0.150
Error	211	167.9		

a. Test of homogeneity of variances.

Table 5. Difference between study areas (Knight Island - Montague Island) in density (No. x [500 cm^2]$^{-1}$) of mussels ≥ 5 mm in shell length within/between strata (rocky or mixed sediment substrates) in 1996 and 1997 in Prince William Sound and significance of Tukey-Kramer post hoc test. Data transformed (log [y+1]) for ANOVA. * = $p < 0.05$

			Montague Island			
			1996		1997	
Study Area	Year	Stratum	Mixed	Rocky	Mixed	Rocky
Knight Island	1996	Mixed	22.4	-7.0	6.0	19.1
		Rocky	44.1	14.7	27.8	40.8
	1997	Mixed	2.7	-26.7	-13.6	-0.6
		Rocky	89.1*	59.7*	72.8*	85.8*

Table 6. Analysis of variance of mussel biomass density (AFDW g/500 cm^2) in two size classes in strata (rocky vs. mixed sediment substrates) at study areas (Montague Island vs. Knight Island) in two years (1996 and 1997) in Prince William Sound.

Mussel Size Class Source of Variation	df	MS	F	P
Mussels ≥ 5 mm in Shell Length				
Untransformed data; Levene's Test[a], P =0.312				
Study area	1	2,417	0.788	0.376
Year	1	30.8	0.010	0.920
Stratum	1	8,242	2.68	0.103
Study area x Year	1	2,414	0.787	0.376
Study area x Stratum	1	345	0.113	0.738
Year x Stratum	1	206	0.067	0.795
Study area x Year x Stratum	1	11,993	3.91	0.049
Error	211	3,067		
Mussels ≥ 20 mm in Shell Length				
Untransformed data; Levene's Test[a], P =0.078				
Study area	1	5,640	2.93	0.088
Year	1	1.1	0.001	0.981
Stratum	1	17,393	9.04	0.003
Study area x Year	1	4,611	2.40	0.123
Study area x Stratum	1	117	0.061	0.805
Year x Stratum	1	26.2	0.014	0.907
Study area x Year x Stratum	1	3,914	2.04	0.155
Error	211	1,924		

a. Test of homogeneity of variances.

Table 7. Sample statistics for skewness (g_1) and kurtosis (g_2) of length-frequency and biomass distributions of mussels at Montague Island (MI) and Knight Island (KI) in 1996 and 1997, and results of the t-test of significance of the deviation of the statistics from their parametric values under a normal distribution. s_{g1} and s_{g2} are standard errors of g_1 and g_2, respectively; t_s is the estimated t statistic.

Area	Year	g_1	s_{g1}	t_s	P	g_2	s_{g2}	t_s	P
Length-frequency Distribution									
MI	1996	0.866	0.115	7.53	<0.001	0.074	0.229	0.32	n.s.
	1997	0.700	0.119	5.88	<0.001	-0.330	0.237	-1.39	n.s.
KI	1996	1.892	0.093	20.34	<0.001	3.933	0.186	21.14	<0.001
	1997	2.007	0.078	25.73	<0.001	4.802	0.156	30.78	<0.001
Biomass Distribution									
MI	1996	-0.130	0.022	-5.91	<0.001	-0.430	0.044	-9.77	<0.001
	1997	-0.178	0.019	-9.37	<0.001	-0.469	0.038	-12.34	<0.001
KI	1996	0.154	0.023	6.70	<0.001	-1.010	0.046	-21.96	<0.001
	1997	0.544	0.023	23.65	<0.001	-0.482	0.047	-10.26	<0.001

Table 8. Analysis of variance of *Nucella lima* and large seastar[a] densities (No. m^{-2}) in rocky and mixed sediment substrates at Montague Island and Knight Island in 1996 and 1997 in Prince William Sound.

Predator Group Source of Variation	df	MS	F	P
Nucella lima				
Data transformed (log[y+1]); Levene's Test[b], P = 0.05				
Study area	1	0.168	8.98	0.003
Year	1	0.034	1.81	0.18
Stratum	1	2.9×10^{-5}	0.002	0.97
Study area x Year	1	0.020	1.06	0.30
Study area x Stratum	1	4.1×10^{-4}	0.02	0.88
Year x Stratum	1	0.012	0.66	0.42
Study area x Year x Stratum	1	1.6×10^{-4}	0.008	0.93
Error	200	0.019		
Large seastars				
Data transformed ($y^{0.054}$); Levene's Test[b], P < 0.001				
Study area	1	0.164	1.18	0.28
Year	1	0.633	4.53	0.04
Stratum	1	3.63	26.0	<0.001
Study area x Year	1	0.057	0.41	0.52
Study area x Stratum	1	0.365	2.61	0.11
Year x Stratum	1	0.038	0.28	0.60
Study area x Year x Stratum	1	0.470	3.36	0.07
Error	200	0.140		

a. Includes *Pisaster ochraceus*, *Evasterias troschelii*, *Pycnopodia helianthoides*, *Dermasterias imbricata.*

b. Test of homogeneity of variances.

Table 9. Mean percent feeding and day 60 running mean feeding rate of *Nucella lima* and *N. lamellosa* on *Mytilus trossulus* in a 60-d laboratory experiment. N_D, number of days feeding observations were made. Means are presented with standard errors of the mean as $\bar{x} \pm SE$. Feeding rates are means of three replicates.

	N. lima			*N. lamellosa*		
Mussel length class (mm)	N_D	Feeding (%) $\bar{x} \pm SE$	Feeding rate (mussels snail^{-1} day^{-1}) $\bar{x} \pm SE$	N_D	Feeding (%) $\bar{x} \pm SE$	Feeding rate (mussels snail^{-1} day^{-1}) $\bar{x} \pm SE$
5 - 20	58	35.6 ± 1.7	0.189 ± 0.005	56	22.9 ± 1.8	0.171 ± 0.019
20.1 - 40	58	32.5 ± 1.7	0.045 ± 0.004	56	22.7 ± 1.2	0.056 ± 0.011
> 40	58	36.9 ± 1.5	0.019 ± 0.003	56	9.8 ± 1.1	0.004 ± 0.004

Table 10. Total annual consumption of *Mytilus trossulus* in three size classes by *Nucella lima* and *N. lamellosa* at Montague Island (MI) and Knight Island (KI) in 1996 and 1997.

			Individual annual consumption (No. snail^{-1} yr^{-1})				Total mussels consumed (No. yr^{-1})			
Year	Study area	Predator	MLC1[a]	MLC2	MLC3	Total	MLC1	MLC2	MLC3	Total
1996	MI	*N. lima*	16.5	5.4	0.05	22.0	3.5×10^6	1.1×10^6	1.1×10^4	4.7×10^6
		N. lamellosa	17.1	5.6	0.06	22.8	7.1×10^5	2.3×10^5	2.3×10^3	9.5×10^5
		Nucella spp.	-	-	-	-	4.2×10^6	1.4×10^6	1.4×10^4	5.6×10^6
	KI	*N. lima*	27.4	2.8	0.06	30.3	4.3×10^6	4.4×10^5	9.6×10^3	4.8×10^6
		N. lamellosa	26.1	2.6	0.06	28.8	2.5×10^7	2.5×10^6	5.5×10^4	2.8×10^7
		Nucella spp.	-	-	-	-	2.9×10^7	3.0×10^6	6.5×10^4	3.2×10^7
1997	MI	*N. lima*	13.3	6.0	0.16	19.2	2.7×10^6	1.2×10^6	3.3×10^4	4.0×10^6
		N. lamellosa	13.0	5.9	0.16	19.0	1.1×10^5	4.9×10^4	1.3×10^3	1.6×10^5
		Nucella spp.	-	-	-	-	2.8×10^6	1.3×10^6	3.5×10^4	4.2×10^6
	KI	*N. lima*	31.7	1.9	0.01	33.6	1.4×10^7	8.1×10^5	5.9×10^3	1.4×10^7
		N. lamellosa	30.1	1.8	0.01	31.9	2.8×10^7	1.6×10^6	1.2×10^4	2.9×10^7
		Nucella spp.	-	-	-	-	4.1×10^7	2.4×10^6	1.8×10^4	4.4×10^7

a. Mussel length classes (MLCs) were: MLC1, 5-20 mm; MLC2, 20.1-40 mm; MLC3, >40 mm.

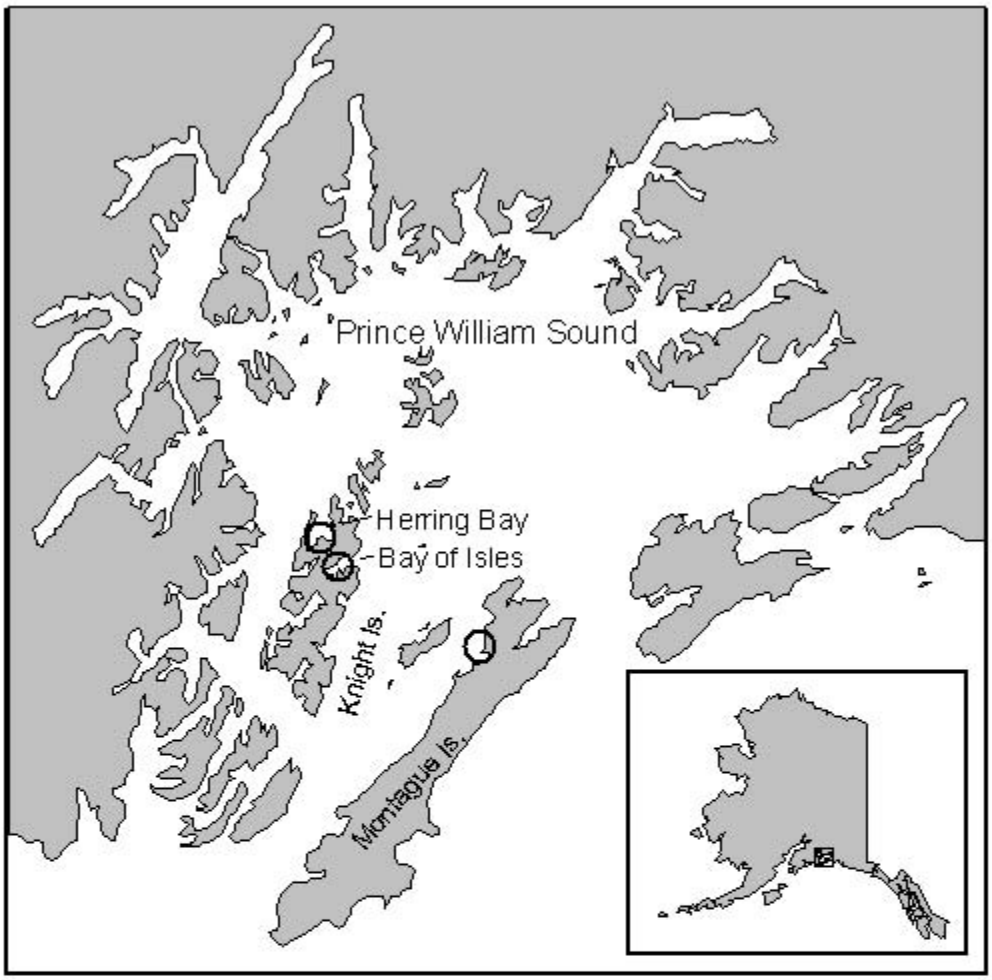

Figure 1. Location of study areas (circled) in Prince William Sound, Alaska.

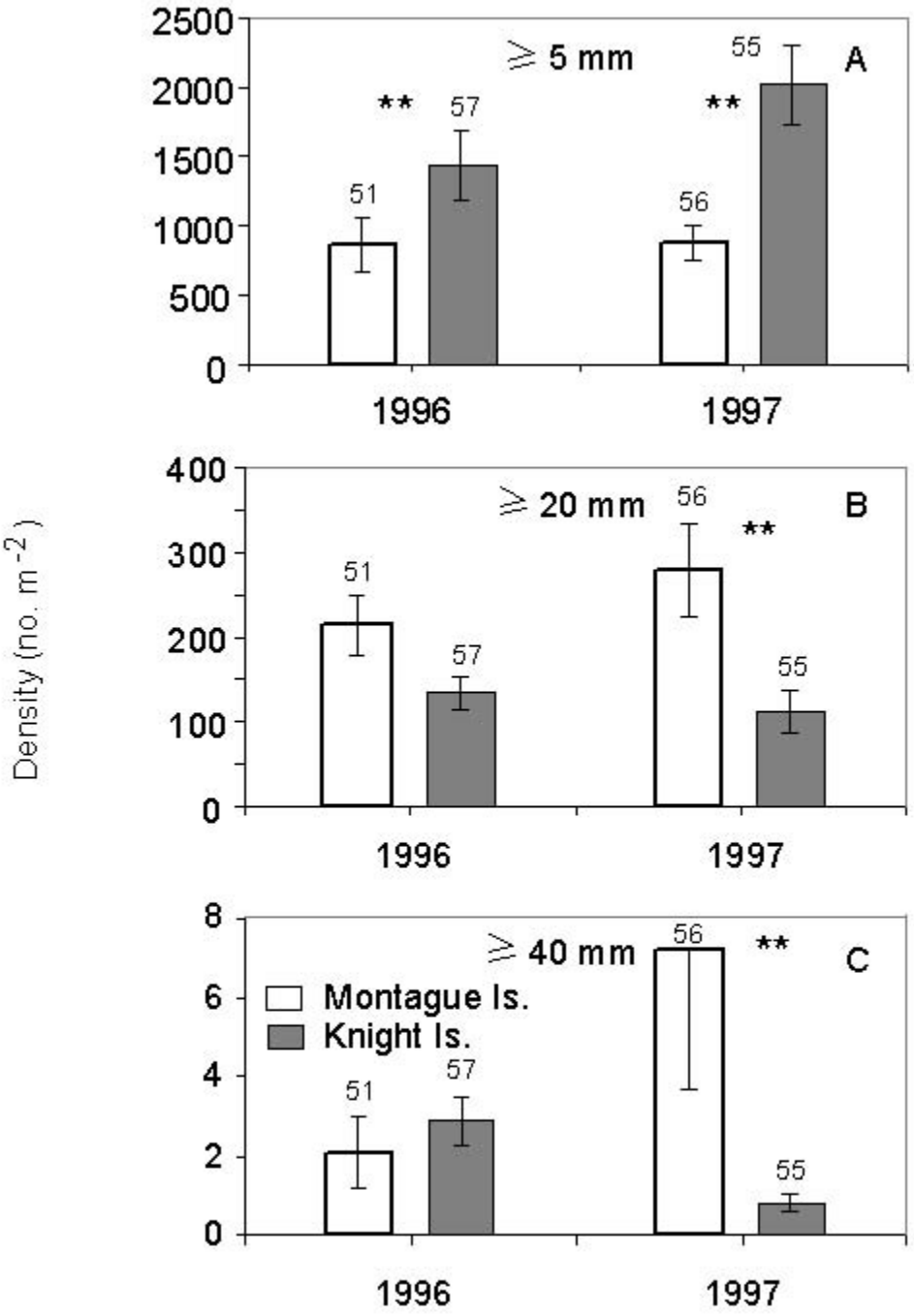

Figure 2. Mean density of mussels ≥ 5 mm (A), ≥ 20 mm (B) and ≥ 40 mm (C) in shell length at Montague Island and Knight Island in May-July 1996 and 1997. Error bars are one standard error of the mean. Numbers above the bars are sample sizes (no. of shore segments). **, $p<0.01$.

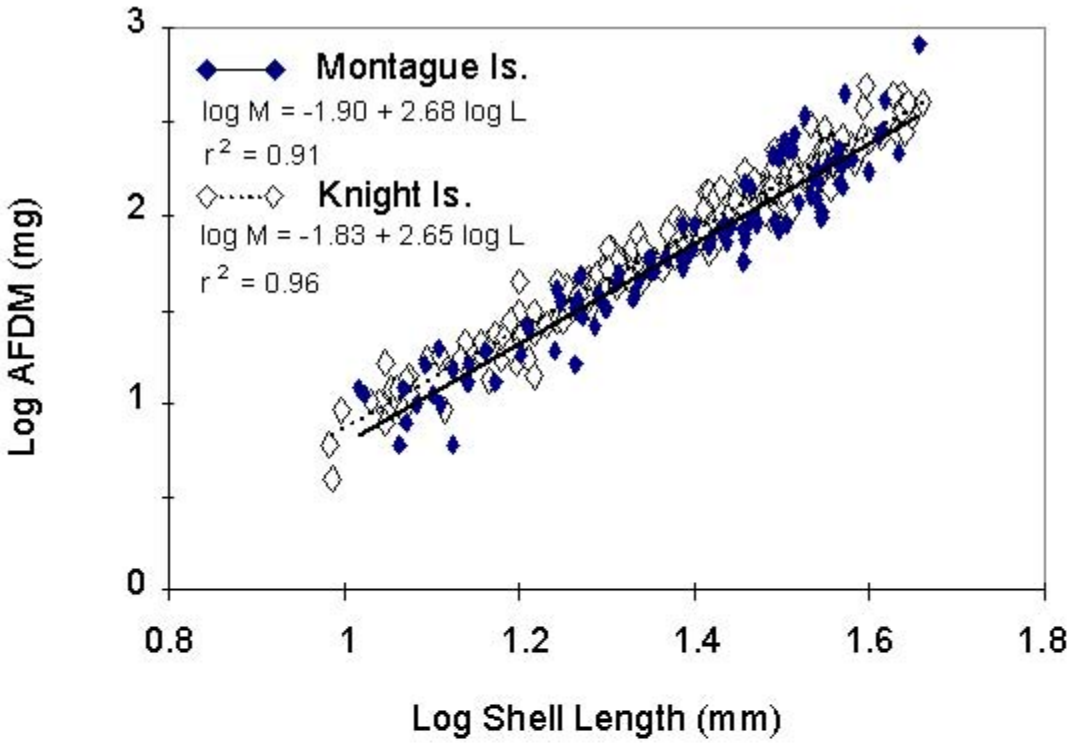

Figure 3. Regression of mussel ash-free dry mass (AFDM) with shell length (log scale) for Montague Island and Knight Island. Regression equation and the coefficient of determination (r^2) are shown for each location.

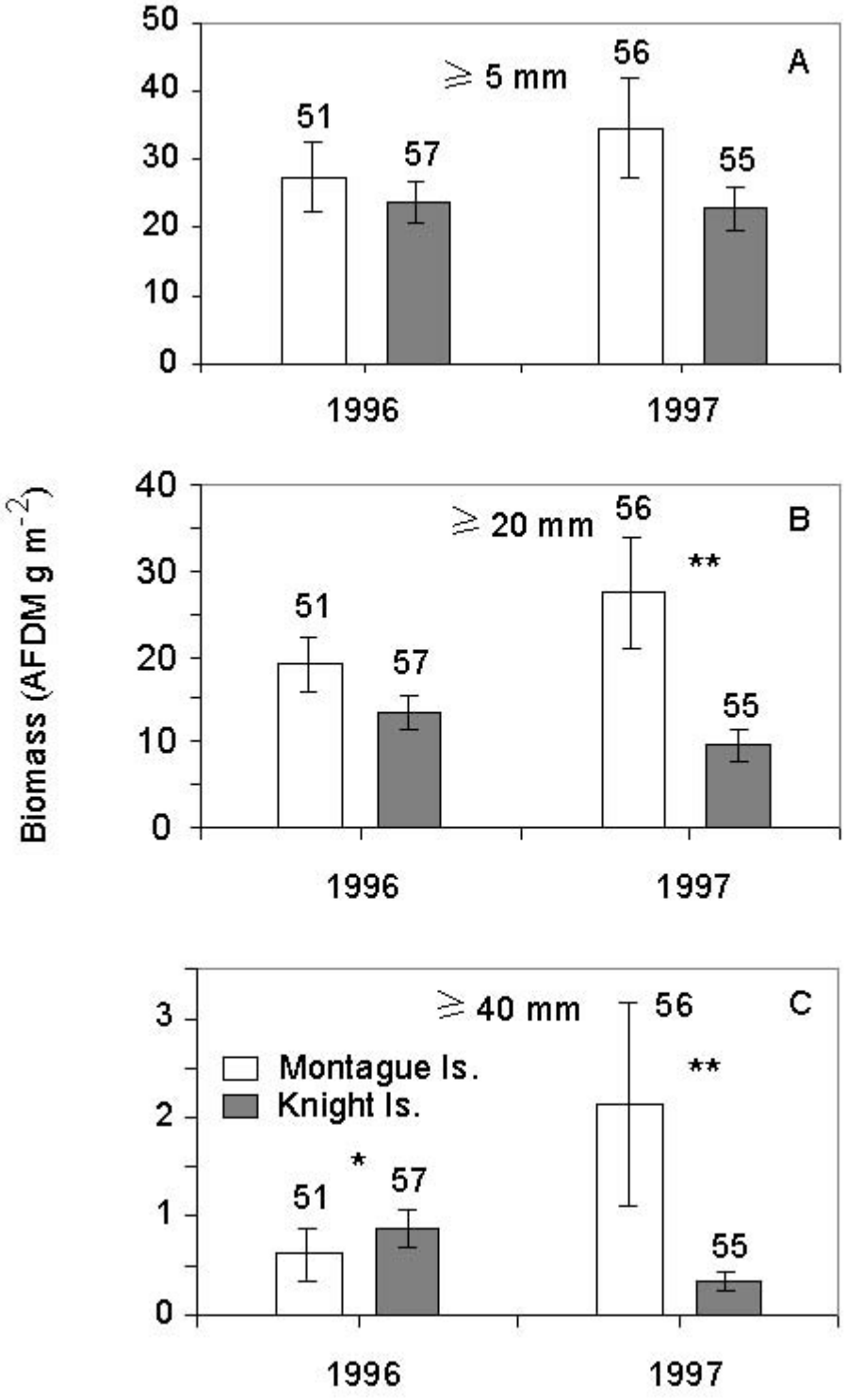

Figure 4. Mean biomass (ash-free dry mass) of mussels ≥ 5 mm (A), ≥ 20 mm (B) and ≥ 40 mm (C) in shell length at Montague Island and Knight Island in May-July 1996 and 1997. Error bars are one standard error of the mean. Numbers above the bars are sample sizes (no. of shore segments). *, $p<0.05$; **, $p<0.01$.

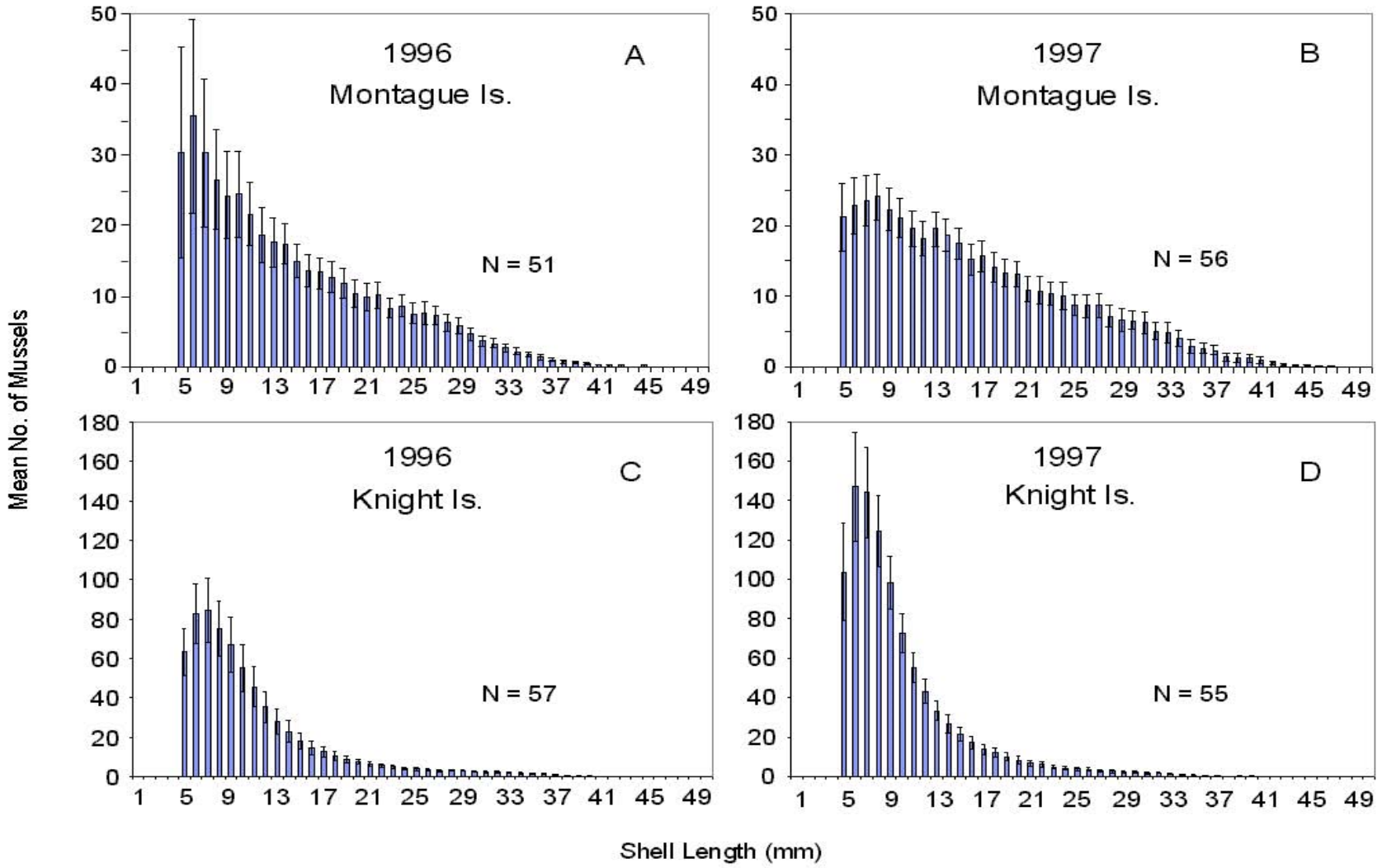

Figure 5. Length-frequency distribution of mussels ≥ 5 mm in shell length at Montague Island and Knight Island in 1996 and 1997. Error bars are one standard error of the mean. N = no. of shore segments sampled. Number of mussels measured was 20,722 (A), 24,693 (B), 37,710 (C) and 53,861 (D).

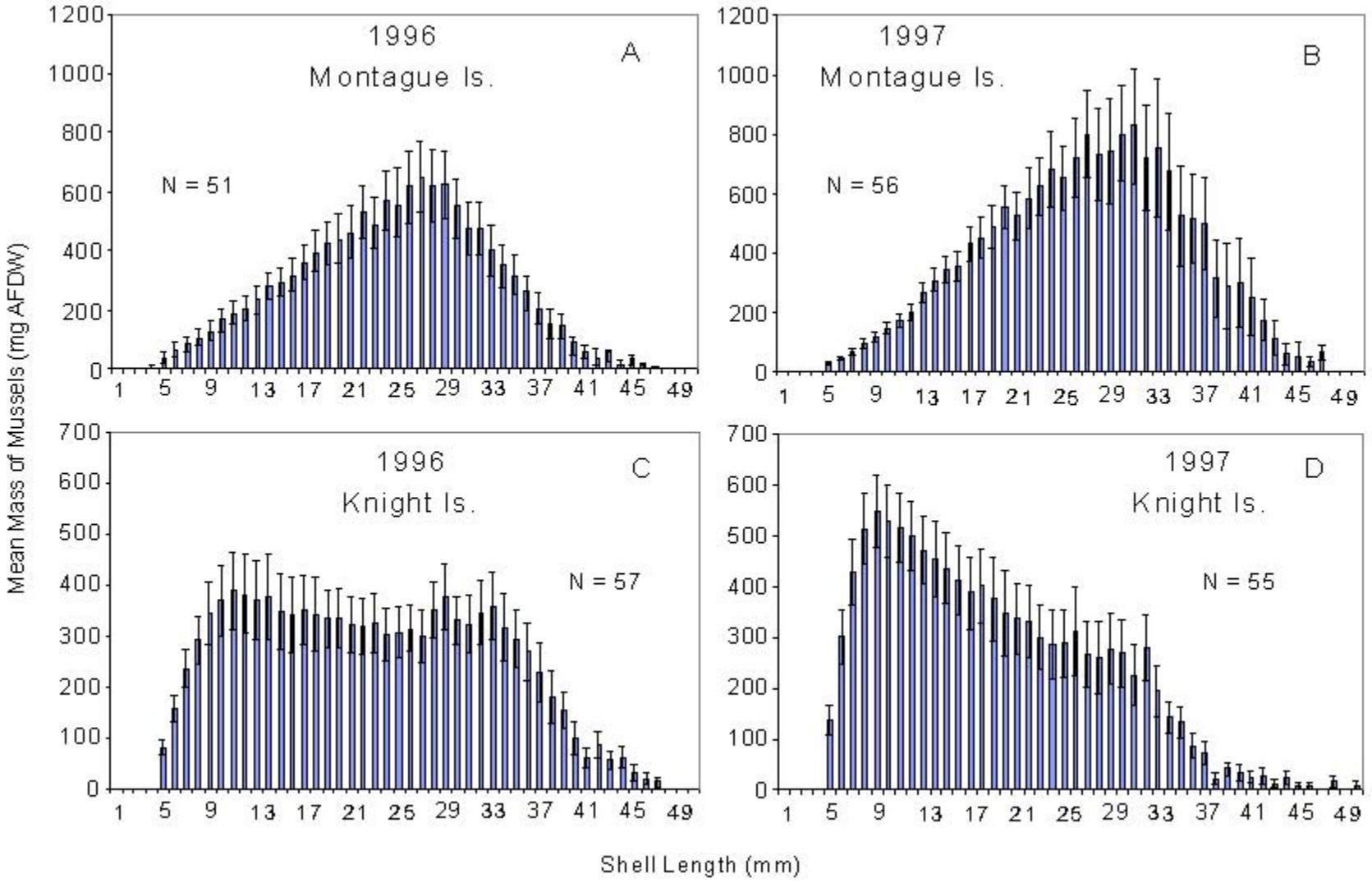

Figure 6. Distribution of mussel mass with shell length for mussels ≥ 5 mm in shell length at Montague Island and Knight Island in 1996 and 1997. Error bars are one standard error of the mean. N = no. of shore segments sampled. Number of mussels included was 20,722 (A), 24,693 (B), 37,710 (C) and 53,861 (D).

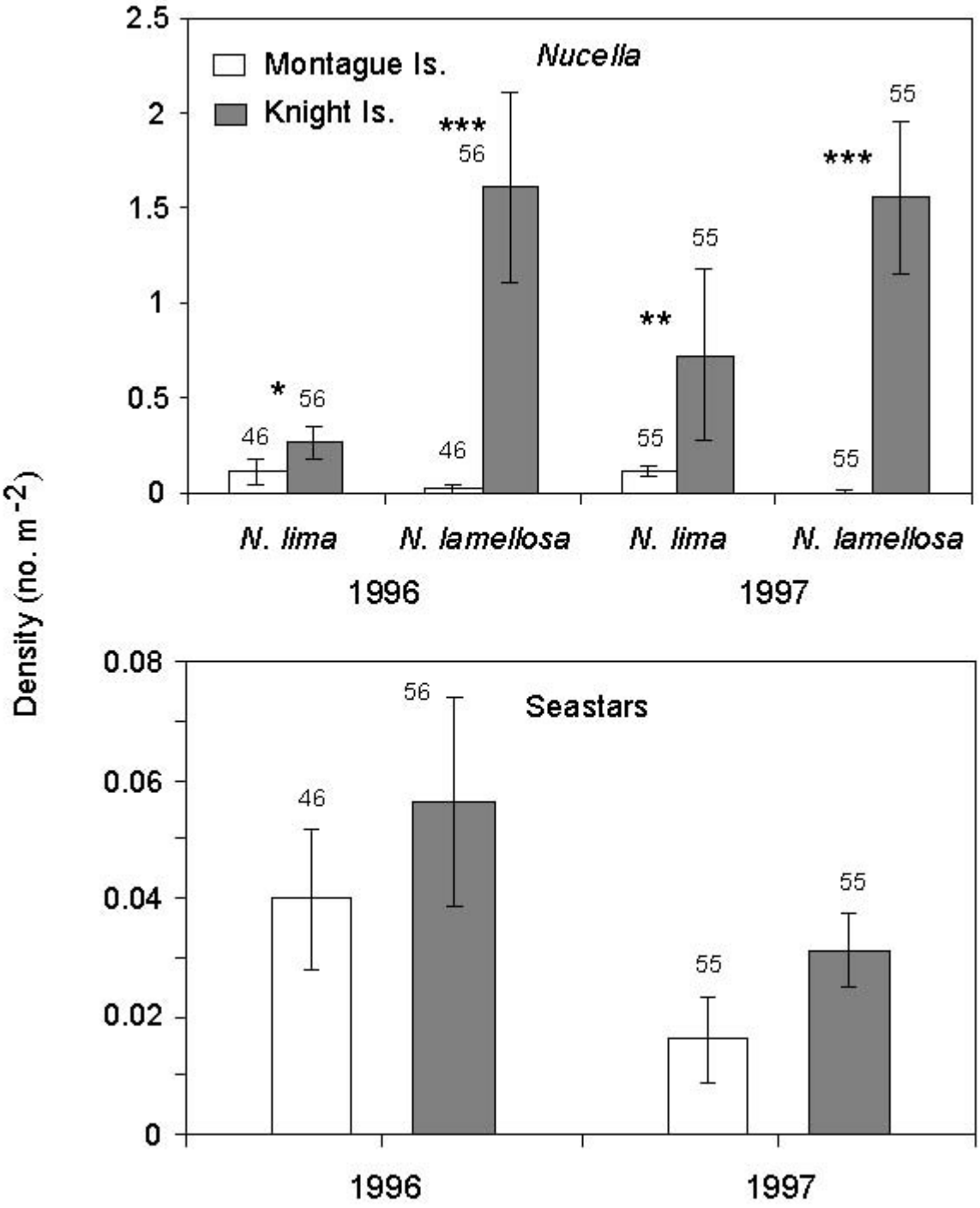

Figure 7. Mean density of *Nucella lamellosa* and *N. lima* and large seastars (*Evasterias troschelii*, *Pisaster ochraceus*, *Pycnopodia helianthoides*, and *Dermasterias imbricata*) at Montague Island and Knight Island in May-July 1996 and 1997. Error bars are one standard error of the mean. Numbers above the bars are sample sizes (no. of shore segments). *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

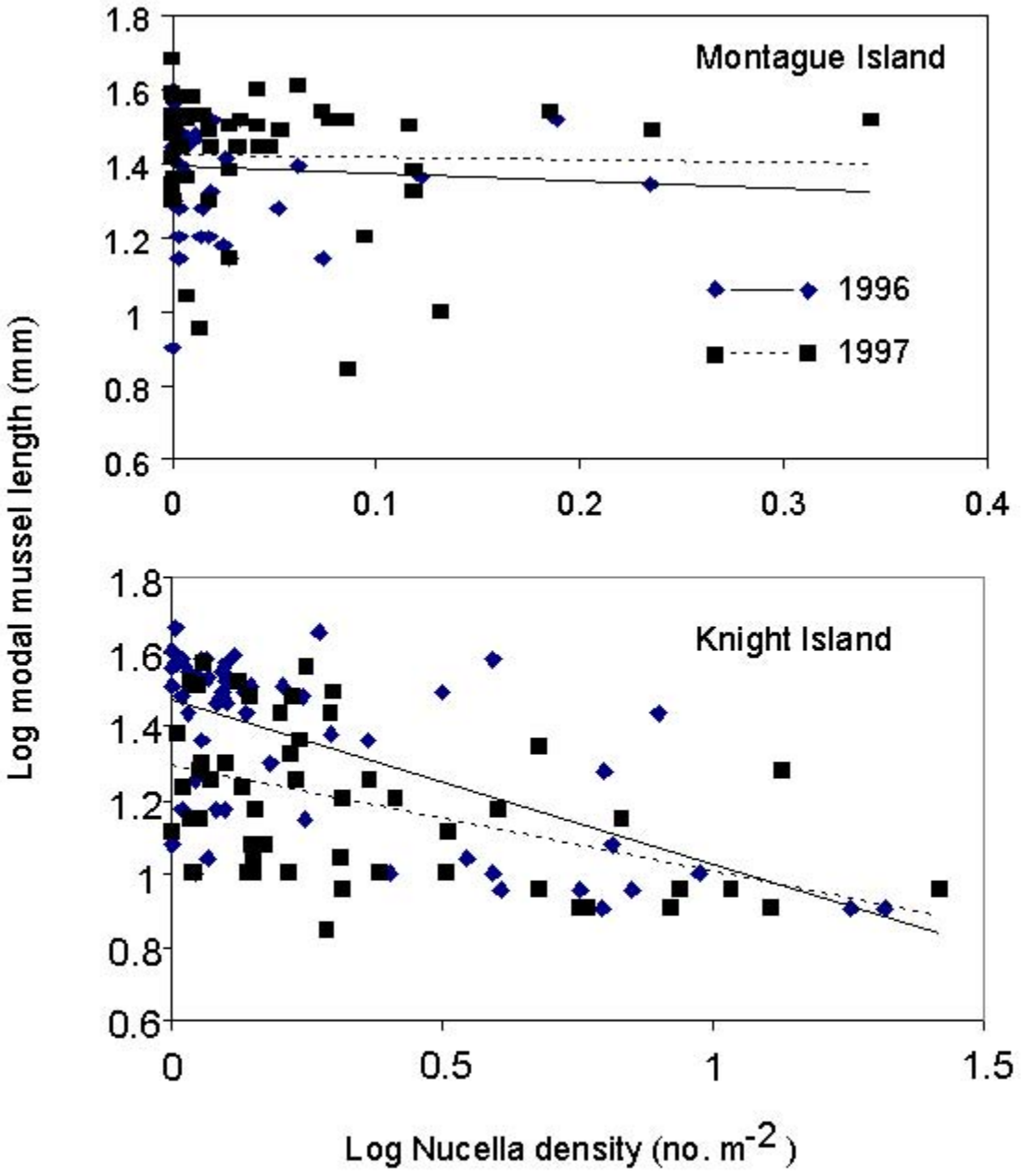

Figure 8. Correlation of the modal length of the mussel length-biomass distribution with the density of *Nucella lima* and *N. lamellosa* (grouped) at study sites on Montague Island and Knight Island in 1996 and 1997.

Appendix SO-06

Gage, T. K. 1998. Effects of invertebrate predators on clam populations in Prince William Sound, Alaska, with implications for the recovery of sea otters from the *Exxon Valdez* oil spill. M.S. Thesis, University of Washington, Seattle, Washington. (Copy available at Alaska Resources Library and Information Services, 3150 C Street, Suite 100, Anchorage, AK 99503 [907] 272-7547.)

Abstract: The abundance of sea otters (*Enhydra lutris*) in some areas of Prince William Sound, Alaska, has not yet recovered from the effects of the *Exxon Valdez* oil spill (EVOS). One possible explanation for the lack of sea otter recovery is the limited availability of food. I studied the effects of predatory benthic invertebrates (sea stars, snails, crabs) on the dynamics of clam populations, the primary prey of sea otters in Prince William Sound. I evaluated the hypothesis that high rates of clam consumption by predatory invertebrates are limiting the size of clam populations in oiled areas and, consequently, the local recovery of sea otters from EVOS. Field observations and laboratory studies were conducted to estimate the significance of clams in the diets of invertebrate predators.

I collected data on density, diet, and activity of predatory invertebrates intertidally and subtidally at 4–10 m depth in four bays. Two of the bays were oiled by EVOS and two were unoiled. I found the sea star *Pycnopodia helianthoides* to be the most abundant predatory benthic invertebrate in all four bays. Densities of *Pycnopodia* were not significantly different between oiled and unoiled areas. Published literature suggests broad overlap in diets of *Pycnopodia* and sea otters. My data, however, indicate that *Pycnopodia* in Prince William Sound have a diverse diet composed primarily of gastropods too small to be of significant nutritional value to sea otters. Clams were present in the diet of *Pycnopodia*, but at very low numbers in all areas. Clam species and size categories typically consumed by sea otters in Prince William Sound were poorly represented in the sampled *Pycnopodia* diet.

Laboratory studies were conducted to determine feeding times of *Pycnopodia* on the clam *Protothaca staminea*. *Protothaca* and other venerid bivalves are common in Prince William Sound and are preferred prey of sea otters. Separate studies were conducted with small (15–25 mm) and large (40–50 mm) clams. Studies showed that *Pycnopodia* less than 10 cm in radius were unable to consume large clams. Feeding times of larger *Pycnopodia* on large clams decreased as the size of *Pycnopodia* increased. Feeding times of *Pycnopodia* on small clams are variable. Density and dietary data collected in Prince William Sound were combined with laboratory feeding times to estimate feeding rates of *Pycnopodia* in Prince William Sound. *Pycnopodia* were estimated to consume less than 7.7% and 2% per year of the available large and small clams in Prince William Sound, respectively.

I conclude from the field and laboratory data that predatory invertebrates are not consuming clams at high rates in Prince William Sound. Therefore, invertebrate predators do not appear to be limiting the local recovery of sea otters from damage caused by EVOS by competing for the same resource.

Appendix SO-07

Dean, T. A., J. L. Bodkin, S. C. Jewett, D. H. Monson, and D. Jung. 2000. Changes in sea urchins and kelp following a reduction in sea otter density as a result of the *Exxon Valdez* oil spill. Marine Ecology Progress Series 199:281–291.

Abstract: Interactions between sea otters (*Enhydra lutris*), sea urchins (*Strongylocentrotus droebachiensis*), and kelp were investigated following the reduction in sea otter density in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill in 1989. At northern Knight Island, a heavily oiled portion of the Sound, sea otter abundance was reduced by a minimum of 50% by the oil spill, and from 1995 through 1998 remained at an estimated 66% lower than in 1973. Where sea otter densities were reduced, there were proportionally more large sea urchins. However, except in some widely scattered aggregations, both density and biomass of sea urchins were similar in an area of reduced sea otter density compared with an area where sea otters remained about 10 times more abundant. Furthermore, there was no change in kelp abundance in the area of reduced sea otter density. This is in contrast to greatly increased biomass of sea urchins and greatly reduced kelp density observed following an approximate 90% decline in sea otter abundance in the western Aleutian Islands. The variation in community response to a reduction in sea otters may be related to the magnitude of the reduction and the non-linear response by sea urchins to changes in predator abundance. The number of surviving sea otter may have been high enough to suppress sea urchin populations in Prince William Sound, but not in the Aleutians. Alternatively, differences in response may have been due to differences in the frequency or magnitude of sea urchin recruitment. Densities of small sea urchins were much higher in the Aleutian system even prior to the reduction in sea otters, suggesting a higher rate of recruitment.

Harlequin Duck (*Histrionicus histrionicus*) Appendices

(HD)

APPENDIX HD-01

CORRELATES OF HARLEQUIN DUCK DENSITIES DURING WINTER IN PRINCE WILLIAM SOUND, ALASKA: HABITAT ATTRIBUTES, HISTORY OF OIL CONTAMINATION, AND FOOD[1]

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ABSTRACT

We assessed sources of variation in Harlequin Duck (*Histrionicus histrionicus*) densities during winters 1995-1997 in areas of Prince William Sound, Alaska contaminated by the 1989 *Exxon Valdez* oil spill and in a nearby unoiled area. Habitat attributes that explained variation in duck densities included distance to streams and reefs, the degree of exposure to wind and wave action, and the dominant substrate type. We suggest that, on a broad scale, winter habitats of harlequin ducks are linked to their life history requirements for predictable environments to ensure high survival and adequate foods to meet high energetic demands. Finer scale habitat use presumably reflects an optimization process balancing costs and benefits of specific habitat features. After accounting for effects of habitat attributes, densities were consistently lower in oiled areas than unoiled, suggesting that population recovery from the oil spill was not complete, due either to lack of recovery from initial oil spill effects or continuing deleterious effects. Prey density and abundance were not strongly related to duck densities after accounting for habitat and area effects, although prey may influence harlequin duck densities as mediated through relationships of habitat attributes and prey density or abundance. Also, prey density and prey availability per duck were similar between oiled and unoiled areas, suggesting that food was not limiting harlequin duck population recovery from the oil spill, although we had low power to detect differences. Harlequin duck life history traits suggest that winter food availability is unlikely to limit populations under natural conditions. High levels of winter site fidelity, a reflection of the predictable environments of wintering harlequin ducks, likely affected our

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[1]Published: 2000. Condor 102:920–926.

results. High philopatry may be related to lower densities than expected in oiled areas due to chronic, residual oil spill effects on the same local aggregations of birds or due to low rates of immigration to enhance wintering groups with depressed numbers due to past oil spill effects.

Key Words: density, *Exxon Valdez* oil spill, food, habitat, Harlequin Duck, *Histrionicus histrionicus*, population recovery.

INTRODUCTION

Within a species, densities of birds vary among locations at every scale from biomes to microhabitats, in large part in response to variation in biotic and abiotic attributes of the environment. Use of habitats is thought to reflect an optimization process, in which birds select combinations of habitat attributes that lead to maximized fitness (MacArthur and Pianka 1966, Rosenzweig 1985). This process presumably seeks to balance benefits (e.g., energy intake) against risks (e.g., from predation or weather). Site fidelity also may influence bird distribution (Robertson and Cooke 1999) and individuals may return to a particular area despite changes in habitat quality (Hilden 1965, Cooch et al. 1993).

During winter, waterfowl must meet a variety of costs (e.g., maintenance, feather synthesis, courtship, mate defense, acquisition of nutrient reserves for reproduction), while balancing mortality risk. Most members of the seaducks tribe (Mergini) winter in the harsh, but relatively stable, nearshore marine environments of north temperate and subarctic latitudes. Seaducks typically exhibit life histories in which annual productivity is low and variable and reproductive life spans are long (Goudie et al. 1994). This strategy requires low rates of mortality during nonbreeding periods (Stearns 1992, Sæther et al. 1996) and, therefore, these species must select winter habitats that meet immediate demands and also confer a high likelihood of survival.

Within their holarctic distribution, Harlequin Ducks (*Histrionicus histrionicus*) are inextricably linked to nearshore marine environments during the nonbreeding portion of the annual cycle (Robertson and Goudie 1999). Adults leave coastal areas only for a few summer months, when they migrate to fast-moving streams to nest and raise broods (Robertson 1997; Cooke et al. 2000). Despite the importance of nearshore areas for Harlequin Duck populations, finer scale quantifications of winter habitat associations have rarely been conducted (Goudie and Ankney 1988).

In March 1989, the *Exxon Valdez* ran aground, spilling nearly 42 million L of oil into Prince William Sound, the wintering area for approximately 14,000 Harlequin Ducks (Lance et al. 1999). As much as 40% of the spilled oil was deposited in intertidal and subtidal zones of Prince William Sound (Galt et al. 1991, Wolfe et al. 1994), the areas used by Harlequin Ducks. Although much of the oil degraded and dissipated within a few years of the spill, some residual oil was still present in these areas during the course of our study (Hayes and Michel 1999). Immediate bird mortality from the *Exxon Valdez* oil spill was high (Piatt et al. 1990) and more than 1,000 Harlequin Ducks were estimated to have died as a direct result of the immediate effects of the spill (John Piatt, pers. comm.). Further, there have been concerns about continued effects of the *Exxon Valdez* oil spill on Harlequin Duck populations and lack of full population recovery (*Exxon Valdez* Oil Spill Trustee Council 1999).

We studied Harlequin Duck habitat associations in Prince William Sound during winter to: (1) identify environmental variables that correspond to high Harlequin Duck densities during winter, to better understand the habitat requirements of the species in light of life history requirements for high survival; and (2) to assess the status of Harlequin Duck population recovery from the *Exxon Valdez* oil spill. Evaluation of Harlequin Duck population recovery from the oil spill has been constrained by a paucity of prespill data from winter, the most important period for Harlequin Ducks in Prince William Sound and the "core" subpopulations

from a population structure perspective (Cooke et al. 2000). For this aspect of the study, we adopted a control-impact study design to assess potential oil spill effects, in which we compared densities of Harlequin Ducks between oiled and unoiled areas, recognizing the need to control for intrinsic area differences (Wiens and Parker 1995). Habitats within Prince William Sound are diverse, making it necessary to segregate effects of history of oil contamination from other environmental factors. Lower densities than expected on oiled areas (after accounting for other factors) could result from either failure to recover from immediate population impacts or from continuing deleterious effects of the spill; either case would lead to an interpretation of lack of full population recovery.

METHODS

Study Area

Located on the southcentral coast of Alaska, Prince William Sound encompasses about 39,000 km^2 including 4,800 km of shoreline. A temperate rainforest dominated by Sitka spruce (*Picea sitchensis*) and western hemlock (*Tsuga heterophylla*) covered most of the convoluted shoreline and islands in Prince William Sound. Temperatures seldom dropped below -20° C and, except for a few coves and lagoons, most areas remained ice-free during winter. Tidal amplitude averaged about 4 m. Predominant nearshore vegetation included rockweed (*Fucus distichus*), eelgrass (*Zostera marina*), and scattered offshore bull kelp (*Nereocyctis luetkeana*). All areas were undeveloped and human disturbance was low. Hunting was negligible for Harlequin Ducks in Prince William Sound.

Study locations were within oiled and unoiled areas of Prince William Sound (Figure 1). The oiled study area included 2 bays on Knight Island, Herring Bay and Bay of Isles, which were heavily oiled by the *Exxon Valdez* spill. The unoiled area was in the Stockdale Harbor and Port Chalmers region of northwestern Montague Island, which was selected because of the close proximity to the oil spill zone, thus minimizing area differences beyond habitat attributes (e.g., climate).

Harlequin Duck Surveys

We conducted surveys of Harlequin Duck numbers and distribution during 4-12 December 1995, 12-24 February 1996, 4-14 December 1996, and 14-23 February 1997, completing 5 replicates on Knight Island and 7 on Montague Island. Surveys involved a census of the area within 200 m from shore. Survey craft were staffed by a 2-3 person team consisting of a boat operator/observer and at least one observer/data recorder. We mapped locations and flock sizes of all Harlequin Ducks on mylar overlays of aerial photos. Locations were digitized using a geographic information system (GIS).

To generate average Harlequin Duck densities associated with each site at which habitat variables were measured (see below), we calculated the number of ducks detected during shoreline censuses within 200 m linear shoreline distance of the midpoint of each sampling site using GIS. Duck densities were expressed as the average number of birds within the designated shoreline segment over the replicate surveys.

Habitat Attributes

To select sites for sampling habitat attributes, the shoreline of each study area was divided into 200 m sampling units. From randomly selected start points, 216 sites (113 on Knight Island and 103 on Montague Island) were systematically selected for sampling during summers of 1996 and 1997.

For each site, a number of habitat variables were documented that we felt could be related to winter Harlequin Duck densities, including: *exposure* - a description of wind and wave action, categorized as full exposure, partial exposure, and not exposed; *dominant strata* - substrate type, categorized as rocky (bedrock and boulder areas) and mixed (unconsolidated or various mixtures of sand, pebbles, and cobble); *distance to stream mouth* - straight line distance from the midpoint of the sampling site to nearest stream mouth measured by GIS and categorized as <200 m, 200-500 m, 500-1000 m, and > 1000 m; *distance to reef* - straight line distance from the midpoint of the sampling site to the nearest offshore reef measured by GIS and categorized as 200-500 m, 500-1000 m, and > 1000 m; and *intertidal slope* - the average slope (in degrees) of the mussel zone. Observations with missing data for a habitat variable were excluded from habitat association models including that variable.

Habitat Association Models

We conducted general linear model analyses using SAS (SAS Institute Inc., Cary, North Carolina, USA) to assess relationships of habitat attributes (explanatory variables) to average Harlequin Duck densities (the response variable), using each sampling site as an observation. In examination of scatterplots of Harlequin Duck densities by habitat and food variables, we found that the distributions violated the assumption of linearity; by conducting a square root transformation of Harlequin Duck densities, the assumption of linearity was met, therefore we used the square root of average Harlequin Duck densities in all subsequent regression analyses. Categorical variables were included as a set of 1/0 indicator variables, with one level of each variable designated as the reference level and, thus, not included in model selection procedures (Ramsey and Schafer 1997).

We took two approaches to describing habitat relationships to duck densities and then assessing effects of oiling history after accounting for effects of habitat features. Under the first approach (Option A), we first analyzed data from each area independently to determine habitat attributes related to Harlequin Duck densities. For each area, we used Mallow's C_p values to direct model selection in data-based model selection context (Burnham and Anderson 1998). This method contrasts a number of models and uses the principle of parsimony to determine which model is best fit by the data (Hilborn and Mangel 1997), avoiding assumptions and biases of traditional stepping (i.e., forward, backward, and stepwise) model selection procedures (Anderson et al. 1994, Flack and Chang 1987). We then selected models that best explained variation in duck densities for both areas combined, using any variable that was included (i.e., explained significant variation) in the best-fitting model for either area along with interaction terms for each variable and area. Finally, an area (oiling history) term was added to each of the best-fitting models for both areas combined to determine whether oiling history explained additional variation in the data, i.e., variation beyond that already explained by the habitat

variables. We believe this approach was conservative with regard to assessing an effect of history of oiling because it accounted for any possible intrinsic area differences prior to assessment of oil history effects and, in fact, could have attributed some variation due to oiling history to other habitat attributes if these attributes differed by area.

In the second approach (Option B), areas were not analyzed separately and area (oiling history) was included during initial model selection procedures. Explanatory variables included in the analysis included all habitat parameters, their interactions with area, and an area (oiling history) term. This approach was simpler than the first analysis but allowed less control over confounding effects of area differences in Harlequin Duck densities related to habitat differences between areas and area differences due to history of oil contamination.

The Role of Food

Diets of Harlequin Ducks in marine areas consist primarily of intertidal and shallow subtidal benthic invertebrates, in particular amphipods, limpets, snails, chitons, and mussels (Vermeer 1983, Goudie and Ankney 1986, Gaines and Fitzner 1987, Goudie and Ryan 1991, Patten et al. 1998). Because prey abundance or density may influence seaduck distribution (e.g., Stott and Olson 1973, Guillemette et al. 1993), we estimated these parameters within each study area to assess relationships of prey availability to duck densities and compare measures of prey availability between areas.

To sample intertidal blue mussels (*Mytilus trossulus*), we established 10 transects perpendicular to the shoreline at 20 m intervals within each sampling site. We removed all mussels from within a 500 cm^2 quadrat placed at a randomly selected location along each transect and recorded the width of the mussel zone. Mussels were sorted by size class and counted. Lengths of mussels >5 mm were measured. Ash-free dry mass of mussels were determined using a muffle furnace.

Sampling for Harlequin Duck foods other than mussels was conducted at a subset of 15 of the systematically selected shoreline sites in each area. Because of generally low densities of Harlequin Ducks on Knight Island, 4 additional sites with relatively higher Harlequin Duck densities were selected to ensure that sampling represented the full range of Harlequin Duck densities. Similarly, 4 sites with moderate to low duck densities were added on Montague Island. Nonrandom sites were used in general linear model analyses, but not for characterization of the study areas. Samples were obtained at 3 locations at each of 2 depths (0.5 to -0.5, -0.5 to -1.5 mean lower low water [MLLW]) along each of the 200 m shoreline sites. At each location, divers collected and bagged all algae or eelgrass and scraped all visible epifauna from the substrate and airlifted them into a mesh bag. Epifauna were later scraped from algae and eelgrass, combined with epifauna from substrate, sorted, and identified to 7 prey types (limpets, chitons, lacunid snails, littorine snails, other snails, crustaceans, and amphipods). Samples from all locations within a site were pooled. Ash-free dry weights of prey were determined using a muffle furnace. Only ash-free dry weights of foods <25 mm were included in estimates of biomass, as these are the size-classes of prey likely taken by Harlequin Ducks.

For data analyses, prey data were included in 4 forms: *total food density* - the combined average densities (g/100 m^2) of mussels in the mussel zone and other prey items in the 0.5 to -1.5 m MLLW zone; *total food abundance* - an estimate of the biomass (kg ash-free dry mass) of

all food types within the 200 m sampling site, based on expansion of food densities to the areas of the mussel zone for mussels and the 0.5 to -1.5 m MLLW zone for other prey; *food density without mussels* - because biomass estimates of mussels were considerably higher (usually more than an order of magnitude) than other prey types, yet they constitute a relatively small part of the diet of Harlequin Ducks, we also used density estimates excluding mussels; *food abundance without mussels* - similarly, we used prey abundance estimates excluding mussels.

To examine effects of prey density and abundance on Harlequin Duck distributions, we assessed additional variation in duck densities related to food variables after accounting for habitat and area effects as determined by habitat association modeling (see above). We regressed residuals (observed Harlequin Duck densities - predicted densities) from the 5 best-fitting habitat association models of Options A and B against the 4 measures of prey abundance and density. This was not a powerful, direct test of the effects of food, as much of the variation related to food was likely accounted for by habitat attributes, as prey abundance and habitat were likely correlated. We took this approach, however, because sample sizes for sites with habitat measures only were several times higher than those for sites that included prey data and, thus, provided the best data set for examining correlates of Harlequin Duck density variation. Under this approach, relationships between food density or abundance and duck densities (after accounting for other effects) would suggest that food has an influence beyond that explained by correlations of prey abundance or density and other habitat attributes.

We also compared measures of prey density and abundance between areas to assess the potential role of food limitation to population recovery from the oil spill. Food limitation could constrain population recovery if the oil spill reduced prey availability, either through direct effects or indirectly through alterations of trophic web structure (Peterson 2001). Higher densities of prey or more prey (on a per duck basis; see below) on oiled areas would indicate that food limitation was unlikely. Conversely, higher densities or more prey on unoiled areas would indicate that food limitation might be involved in lack of population recovery. Similar prey density and abundance would be equivocal; however, these interpretations should be viewed with caution, as no studies have directly tested whether Harlequin Duck carrying capacity during winter is set by food availability.

Average food density per site was compared between areas using a t-test. Food abundance was compared in relation to duck abundance, under the premise that assessments of density dependent population limitation require per capita resource availability. We calculated food abundance per duck for each area as the average food abundance per site (for those sites where all prey were sampled) divided by the average duck abundance per site (calculated over all sites). Duck abundance for this calculation was the density over 400 m (as described above) divided by 2, so that both food and duck abundances were on the same scale. Variance was calculated for a ratio of 2 independent estimates (Seber 1973) and 2-tailed *Z* scores were calculated to compare areas (Snedecor and Cochran 1980).

RESULTS

Average Harlequin Duck numbers per site, excluding nonrandomly selected sites, were considerably higher at our unoiled study area than at the oiled area (Table 1) and our intent was to determine whether this was related to habitat differences, differences in history of oil

contamination, or some combination of differences in habitat attributes and oil spill effects. Some aspects of the habitat were distinctly different between areas (Table 1), particularly intertidal slope and dominant strata, with smaller differences apparent for exposure and distance to stream parameters. On both areas, Harlequin Ducks were almost always observed very close to shore, in intertidal and shallow subtidal habitats.

Habitat Association Models

On unoiled Montague Island, Harlequin Duck densities were related to a number of habitat attributes (Table 2). In all 5 of the best-fitting models, densities were positively related with having a reef within 500 m and a stream within 200 m; in 4 of the 5 models, positive associations were described between duck densities and occurrence of a stream within 200 - 500 m. In all models, densities on partially exposed sites were lower than those on unexposed or fully exposed sites and, in 2 of the 5 models, densities were higher on fully exposed sites than unexposed. Intertidal slope and dominant strata did not have consistent, strong effects.

Habitat association modeling using data from Knight Island only resulted in patterns similar to Montague Island (Table 2). For example, in all 5 best-fitting models, duck densities were positively related to having a reef in the 200 - 500 m interval. Also, on Knight Island, 4 of the 5 best models described positive associations between duck densities and occurrence of a stream within 200 m. Full exposure was associated with higher Harlequin Duck densities than for either partially exposed or nonexposed sites. Like on Montague Island, intertidal slope and dominant strata on Knight Island were not strongly or consistently related to Harlequin Duck densities. Similarities in patterns of results between Montague and Knight Islands, in the absence of consideration of area effects, suggest that similar habitat attributes are selected by Harlequin Ducks in both areas, despite overall area differences in the relative occurrence of the habitat attributes (Table 1) and differences in Harlequin Duck abundance (see intercepts; Table 2).

Under option A for both areas combined (Table 3), in which habitat parameters and area interactions were included in model selection prior to inclusion of an area term, there were unambiguous positive correlations in all 5 best-fitting models between Harlequin Duck densities and the closest stream and reef categories, consistent with results for each area independently. Full exposure was positively related to duck densities in all 5 models, although the negative interaction term indicated that the effect of full exposure was primarily expressed on Montague Island; further, negative interactions of area by partial exposure suggested that this habitat attribute was negatively related to duck densities on Knight Island. Mixed strata was positively associated with duck densities, but the stronger negative interaction terms suggested that the relationship was positive on Montague Island and negative on Knight Island. Results for habitat attributes within Option A should be viewed with some caution because, as mentioned in methods, this approach may have resulted in oiling history effects being attributed to habitat parameters due to the lack of an area term in the model selection process.

Relationships of habitat attributes and duck densities for both areas combined were more simply interpreted under Option B (Table 3), in which an area term was included during model selection. Again, the categories for closest reef and stream distances were consistently correlated

with higher Harlequin Duck densities. Full exposure also was related to higher duck densities for all 5 models and, under this Option, there were no associated negative interaction terms, suggesting that the positive relationship with full exposure was expressed on both areas. Negative effects of partial exposure were inconsistent (3 of 5 models) and relatively small. Positive parameter estimates for mixed strata in all models, in association with consistently stronger interaction terms, suggested positive associations of duck densities and mixed strata on Montague Island and negative associations on Knight Island.

Effects of History of Oil Contamination

Irrespective of the Option used for data analysis, area terms were consistently (and significantly under the Option A approach) negative (Table 3). In other words, duck densities were lower on Knight Island than Montague Island after accounting for effects of habitat attributes and differences in these attributes between areas, which we interpret as evidence that history of oil contamination was related to Harlequin Duck densities.

The Role of Food

Regressions of duck density residuals from habitat association models against 4 measures of food density and abundance gave exactly consistent results from all 5 best-fitting models of both Options A and B; therefore, we present results using residuals from Option A, Model 1 as representative of patterns in our data. Duck density residuals were not related to total food abundance (R^2 = 0.0011, F = 0.0338, df = 30, P = 0.8553), total food density (R^2 = 0.0004, F = 0.0114, df = 31, P = 0.9157), or food abundance without mussels (R^2 = 0.0450, F = 1.6945, df = 36, P = 0.2013; Figure 2). Food density without mussels was positively correlated with duck density residuals (R^2 = 0.1902, F = 8.6922, df = 37, P = 0.0055). However, this positive relationship was highly influenced by a single observation (Figure 3), a site on oiled Knight Island that was nonrandomly selected to represent high duck densities and which also had high densities of subtidal foods (especially snails and amphipods); without this observation, the relationship was nonsignificant (R^2 = 0.0734, F = 2.8513 df = 36, P = 0.0999). Taken together, these analyses suggest that variation in food data explained little variation in duck densities beyond that explained by habitat attributes. Food abundance or density may be important determinants of duck densities but, if this is the case, correlations between habitat attributes and food measures were sufficient to explain most of this variation.

Estimates of prey density and prey abundance per duck were similar between Montague and Knight Islands (Table 4); no statistical differences were detected between areas, although variation around these estimates was high and, thus, power to detect biologically meaningful differences was low. These data are somewhat equivocal with regard to assessing the role of food limitation on population recovery, particularly given the associated broad confidence intervals, although the similarities between areas for point estimates of all measures suggests that food availability on Knight Island was not dramatically lower as a result of the oil spill.

DISCUSSION

Habitat Relations to Harlequin Duck Densities

We found that winter Harlequin Duck densities were related to a number of the habitat attributes that we measured. We assume that habitat associations that we observed were related to habitat profitability and reflected, to some degree, solutions to the optimization process of balancing benefits of habitats against detrimental aspects (Abrahams and Dill 1989, Guillemette et al. 1993). This balance is influenced by structural and functional characteristics of the species (Hilden 1965), such as the life history requirement for high winter survival, as well as high levels of philopatry (see below), in the case of Harlequin Ducks.

Occurrence of a stream within 200 m was consistently and positively related to Harlequin Duck densities, both for each area independently and for both areas combined. Presence of a stream may influence prey distribution and, also, may provide freshwater to reduce osmotic stress for birds that ingest salts while feeding on marine invertebrates (Nyström and Pehrsson 1988). Similarly, a nearby reef was always positively associated with Harlequin Duck densities for both areas separately and when areas were combined. Reefs serve as safe haul-out sites and also offer intertidal foraging opportunities. Fully exposed sites tended to have higher Harlequin Duck densities than partially exposed or unexposed sites. Fully exposed sites may have higher productivity, and hence higher prey abundance, than less exposed sites, although birds at these sites also may be more vulnerable to deleterious weather effects. We were unable to conduct surveys during foul weather when birds may have moved to less exposed sites.

Relations of Harlequin Duck densities to strata type varied by area, with tendencies of positive associations of duck densities with mixed strata on Montague Island but negative associations on Knight Island, suggesting that substrate type is not an important aspect of harlequin duck winter habitat. Intertidal slope was not related to Harlequin Duck densities in most models; we had predicted negative associations resulting from duck responses to increased foraging areas in areas with shallower slopes. Lack of a relationship suggested that more foraging area does not correspond to higher numbers of ducks; in turn, this may indicate that food abundance does not limit harlequin duck populations (see below).

Few other studies have quantified winter Harlequin Duck habitat associations. Goudie and Ankney (1988) documented that Harlequin Ducks were closer to shore and used reefs more than other seaduck species in Newfoundland. Harlequin Duck winter habitats have been qualitatively characterized by a number of authors and have been consistently described as being very close to shore and in a varied mix of substrates (Fleishner 1983, Gaines and Fitzner 1983, Hirsch 1980, Vermeer 1983), in agreement with our findings.

Harlequin Duck habitat use and life history are inextricably linked. Among ducks, Harlequin Ducks are long-lived and have low and variable annual productivity (Goudie et al. 1994), a life history that requires high survival. High survival, in turn, depends on selection of stable and predictable habitats (Stearns 1992). On a broad scale, coastal habitats are thought to offer more stable wintering environments for waterfowl than inland sites (Diefenbach et al. 1988). Within coastal habitats, Harlequin Ducks occupy the narrow intertidal and shallow subtidal zone, a productive part of the marine environment. Goudie and Ankney (1986) described Harlequin Ducks as living near an energetic threshold as a result of their small body

size and relatively harsh wintering environments. As a result, Harlequin Ducks must forage nearly continuously during daylight hours of winter (Fischer 1998, Goudie and Ankney 1986) and, thus, require habitats with readily available and predictable food. Although benthic intertidal communities can vary in species composition over time, invertebrate biomass in intertidal and subtidal zones is generally high and stable. The generalist diet of Harlequin Ducks is thought to reflect their need for continuous foraging and, hence, reduced latitude for prey selectivity (Goudie and Ankney 1986). Harlequin Duck foraging strategy is reflected in their habitat use. Use of shallow water reduces dive and search times for more time efficient foraging (Guillemette et al. 1993). Some selected habitat attributes may correspond to higher prey density or quality (e.g., fully exposed sites). Use of areas near streams and reefs may reduce energetic costs and time of transit between foraging areas and other resources (e.g., fresher water, haul-out sites). In summary, Harlequin Ducks must use habitats that predictably allow them to meet daily energy costs within their time-limited foraging regime, while minimizing risk of mortality in concordance with their life history requirement for high survival probabilities.

Effects of History of Oil Contamination

We found that after accounting for effects of habitat attributes, history of oil contamination from the *Exxon Valdez* spill was related to duck densities, with densities lower on oiled Knight Island than would be predicted based on the habitat attributes that we measured. Our data were consistent with a hypothesis that Harlequin Duck populations were not fully recovered from the oil spill. We acknowledge that area differences also could be related to factors that we did not measure, although our intent at the onset of this study was to measure all intrinsic attributes that we suspected could be related to Harlequin Duck densities. We are unaware of other factors that we did not include that might be important and that are not closely correlated with the attributes that we measured.

Evidence from other studies is consistent with a hypothesis that Harlequin Duck populations were experiencing continued effects of the *Exxon Valdez* oil spill during the course of this study. Trust et al. (2000) concluded that Harlequin Ducks and the ecologically similar Barrow's goldeneye (*Bucephala islandica*) continued to be exposed to oil through 1998, based on higher induction of cytochrome P450 1A in oiled areas than unoiled. Also, Harlequin Duck adult female survival during winters 1995-1998 was lower on oiled areas than unoiled (Esler et al., unpubl. ms.) and lab studies support logical links between reduced survival rates and oil exposure (Holmes et al. 1978, 1979). Because population dynamics of birds with life histories like Harlequin Ducks are particularly sensitive to variation in adult female survival (Goudie et al. 1994, Schmutz et al. 1997), lower survival on oiled areas may have led to population declines (Rosenberg and Petrula 1998) and hence lower densities on oiled areas than predicted, as found in this study. Harlequin Duck populations likely have relatively low intrinsic growth rates, so full recovery (i.e., duck densities at levels predicted from intrinsic habitat attributes) likely will not occur until long after deleterious effects of the oil spill have ceased.

Day et al. (1997) conducted an avian habitat use study in Prince William Sound in the period immediately following the *Exxon Valdez* spill (1989 - 1991). They concluded that Harlequin Ducks showed negative relationships to oiling intensity during summer through 1990 but recovery by summer 1991. Of more relevance for comparison to this study, Day et al. (1997)

found no oil spill effects during winter. Why were our results different from those of Day et al. (1997)? First, because deleterious effects of the oil spill continued through the period of our study and until at least 1998 (Trust et al. 2000, Esler et al., unpubl. ms., Rosenberg and Petrula 1998), differences in Harlequin Duck abundance relative to history of oil contamination may have been more pronounced during our study than during the studies of Day et al. (1997). Also, Day et al. (1997), presumably by necessity due to their broader study question to look at all marine birds over a wider geographic area, used bays as sampling units and characterized habitats at the scale of the entire bay. Our study demonstrated that Harlequin Ducks respond to much smaller scale variations in habitat attributes. Also, Harlequin Ducks exhibit high fidelity to specific shoreline segments (Robertson et al. 1999, Cooke et al. 2000). Therefore, we were able to account for differences in environmental attributes at the scale that Harlequin Ducks select habitats before testing for relationships to history of oil contamination, allowing for a finer scale and presumably more powerful test.

The Role of Food

Food has been suggested to be related to distribution and abundance of some seaducks (e.g., Nilsson 1972, Stott and Olson 1973, Guillemette et al. 1993). In our study, food did not explain additional variation in duck densities beyond habitat attributes; however, because habitat and prey distribution were likely related, our approach limited our ability to directly assess effects of food on harlequin duck densities. Also, because of logistical difficulties associated with quantification of subtidal prey, the number of sites sampled was relatively small and, in association with high variability in food measures, resulted in low power for detecting effects of food.

Characteristics of Harlequin Ducks suggest that they may be more time-limited than food-limited. Energetic requirements of this small-bodied seaduck result in nearly continuous feeding during daylight hours of winter and a generalist diet that includes many common benthic invertebrates (Goudie and Ankney 1986). This foraging strategy, particularly in association with high levels of winter site fidelity (see below), suggests that food may be abundant overall, and the crux for Harlequin Ducks is to maximize energy intake during a short daily foraging period. Under this scenario, Goudie and Ankney (1986) predicted that Harlequin Ducks would preferentially take prey with high energy densities but would maximize intake by limiting search times and consuming any prey they encounter. We also would predict that Harlequin Ducks would forage in shallow areas with high prey densities, as these attributes would increase habitat profitability by reducing time spent searching during dives (Guillemette et al. 1993). Our data are somewhat consistent with this prediction, as we found that Harlequin Ducks foraged in very shallow water and food density was slightly, positively related to duck densities after accounting for habitat effects (Figure 2); again, habitat effects may have accounted for considerable variation in prey density prior to this analysis. Some authors (Nilsson 1972) have found that food exploitation by wintering diving ducks was small relative to standing crop; we suggest that this is likely the case for Harlequin Ducks, given their foraging requirements.

We found no strong evidence that food availability was limiting Harlequin Duck population recovery from the *Exxon Valdez* oil spill (Table 4). We recognize that we had low power to detect differences between areas. However, as described above, Harlequin Duck life

history and foraging strategy suggest that winter food likely is not limiting under natural conditions. In light of this, similar point estimates and variability in food abundance and density between oiled and unoiled areas supports our conclusion of lack of food limitation in oiled areas.

Significance of philopatry

Seaducks have a general pattern of high philopatry throughout their annual cycle (e.g., Savard and Eadie 1989) and a growing body of data suggests that this is true for Harlequin Ducks (Cooke et al. 2000). High philopatry can be an adaptive behavioral strategy in predictable environments (Robertson and Cooke 1999). Harlequin Duck winter habitat use is likely influenced by high levels of philopatry (Cooke et al. 2000), which reflects high stability of nearshore environments coupled with advantages of philopatry, including site familiarity and interannual pair reunion (Robertson and Cooke 1999). High philopatry, in association with life history requirements for high survival, also suggests that winter food is predictably and adequately abundant.

From the perspective of oil spill recovery, high levels of winter site philopatry suggest: (1) if residual oil spill damages exist, birds from oiled areas are vulnerable to spill effects as they return to those areas annually (i.e., these birds are affected disproportionately and are subject to cumulative effects), and (2) if dispersal and movements among areas are limited, recovery of groups of birds in oiled areas can occur only through demographic processes specific to that group (i.e., numbers are not bolstered through immigration from other areas). Lower densities than expected on oiled areas detected in this study may be a result of one or both of these processes.

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Table 1. Summary of winter harlequin duck densities and habitat attributes at sampling sites within Prince William Sound, Alaska, 1995-1997. Data for categorical variables are shown as number of sites and, parenthetically, percentage of sites within each category.

Parameter	Montague Island (Unoiled)	Knight Island (Oiled)
Average (± SE) Harlequin Ducks (ducks/400 m)	2.99 (±0.22); n=103	0.59 (±0.08); n=113
Average (± SE) Intertidal Slope (degrees)	5.80 (±0.38); n=103	25.46 (±1.69); n=112
Exposure		
Full	25 (24.3%)	15 (13.3%)
Partial	35 (34.0%)	49 (43.4%)
None	43 (41.7%)	49 (43.4%)
Dominant Strata		
Rocky	39 (37.9%)	83 (73.5%)
Mixed	64 (62.1%)	30 (26.5%)
Distance to Stream Mouth		
0-200 m	10 (9.7%)	12 (10.6%)
200-500 m	10 (9.7%)	19 (16.8%)
500-1000 m	14 (13.6%)	22 (19.5%)
> 1000 m	69 (67.0%)	60 (53.1%)
Distance to Reef		
200-500 m	10 (9.7%)	8 (7.1%)
500-1000 m	18 (17.5%)	22 (19.5%)
> 1000 m	75 (72.8%)	83 (73.5%)

Table 2. Top 5 models describing relationships of habitat attributes and winter (1996-1998) harlequin duck densities (square root transformed) on Knight Island, which was oiled during the *Exxon Valdez* spill, and unoiled Montague Island, Prince William Sound, Alaska.

		Habitat Model Parameter Estimates[a]									
	Mallow's		Intertidal	Exposure		Strata	Stream Distance (m)			Reef Distance (m)	
Model	C_p	Intercept	Slope	Full	Partial	Mixed	0-200	200-500	500-1000	200-500	500-1000
Montague Island (Unoiled)											
1	4.9062	1.5563	-------[b]	-------	-0.3827	-------	0.4254	0.3770	-------	0.5635	-------
2	5.5172	1.4174	0.0215	-------	-0.3890	-------	0.5220	0.4364	-------	0.5765	-------
3	5.6628	1.4853	-------	0.1930	-0.3144	-------	0.4745	0.3917	-------	0.5097	-------
4	6.0325	1.5891	-------	-------	-0.3659	-------	0.3817	-------	-------	0.6055	-------
5	6.1322	1.2824	-------	0.3749	-0.2320	0.2264	0.4191	0.3407	-------	0.4995	-------
Knight Island (Oiled)											
1	4.5194	0.5013	-------	0.3451	-------	-0.1772	0.3320	-------	-------	0.5022	-------
2	4.5479	0.4546	-------	0.3860	-------	-------	0.2806	-------	-------	0.4971	-------
3	4.7895	0.6406	-0.0044	0.2921	-------	-0.2458	0.2971	-------	-------	0.4614	-------
4	5.0939	0.4229	-------	0.3738	-------	-------	0.2730	-------	-------	0.5351	0.1618
5	5.3358	0.4879	-------	0.3669	-------	-------	-------	-------	-------	0.5035	-------

[a] Reference values for categorical model parameters were: exposure = none; strata = rocky; stream distance = >1000 m; and reef distance = >1000 m.

[b] ------- indicates that the parameter was not selected for inclusion in the model.

Table 3. Top 5 models describing variation in winter (1996-1998) harlequin duck densities (square root transformed) in relation to habitat attributes, interactions of habitat attributes and areas (Montague and Knight Islands, Prince William Sound, Alaska), and area, which was interpreted as an indication of the effect of oil contamination on Knight Island, after having accounted for intrinsic habitat differences between areas. Results of 2 analysis approaches are presented: one in which the area term was added after model selection of habitat terms and habitat by area interactions (Option A) and one in which the area term was included in model selection procedures (Option B).

		Habitat Model Parameter Estimates[a]										Interaction Terms (Area by Parameter)[b,c]					
	Mallow's		Intertidal	Exposure		Strata	Stream Distance (m)			Reef Distance (m)		Partial	Full	Mixed	Stream	Reef	
Model	C_p	Intercept	Slope	Full	Partial	Mixed	0-200	200-500	500-1000	200-500	500-1000	Exposure	Exposure	Strata	200-500 m	500-1000 m	Area[c,d]
Option A																	
1	6.1865	0.7451	-------[e]	0.8761	-------	0.7245	0.2951	-------	-------	0.5168	-------	-0.2431	-0.7677	-1.0521	-------	-------	-0.5652(0.1704);0.0011
2	6.4185	0.7663	-------	0.8422	-------	0.6654	0.2943	0.3084	-------	0.4930	-------	-0.2487	-0.7192	-0.9680	-0.4988	-------	-0.5387(0.1733);0.0021
3	6.6935	0.7713	-------	0.8534	-------	0.7032	0.2716	-------	-------	0.5123	-------	-0.2543	-0.7378	-1.0043	-0.1947	-------	-0.5442(0.1737);0.0020
4	6.8275	0.7255	-------	0.8469	-------	0.7265	0.2874	-------	-------	0.5500	0.1313	-0.2646	-0.7557	-1.0401	-------	-------	-0.5616(0.1703);0.0011
5	7.5336	0.7151	-------	0.9081	0.1001	0.7185	0.3001	-------	-------	0.5112	-------	-0.3171	-0.7697	-1.0262	-------	-------	-0.7320(0.2094);0.0006
Option B																	
1	5.9305	1.1663	-------	0.4459	-------	0.3159	0.3433	-------	-------	0.5095	-------	-------	-------	-0.4784	-------	-------	-0.6861
2	6.1194	1.2644	-------	0.3589	-0.1292	0.2672	0.3298	-------	-------	0.5210	-------	-------	-------	-0.4593	-------	-------	-0.7077
3	6.2173	1.2502	-------	0.3244	-0.1535	0.2693	0.3211	-------	-------	0.5608	0.1518	-------	-------	-0.4482	-------	-------	-0.7131
4	6.6900	1.1404	-------	0.4315	-------	0.3249	0.3385	-------	-------	0.5393	0.1206	-------	-------	-0.4724	-------	-------	-0.6871
5	6.8285	1.2766	-------	0.3443	-0.1459	0.2615	0.3195	-------	-------	0.5410	-------	-------	-------	-0.4340	-------	0.1671	-0.7487

[a] Reference values for categorical model parameters were: exposure = none; strata = rocky; stream distance = >1000 m; and reef distance = >1000 m.
[b] Interactions of area with other habitat attributes were not selected in any model.
[c] Reference value for area = unoiled Montague Island.
[d] Results of Area term for Option A are presented as the parameter estimate (SE); *P* value.
[e] ------- indicates that the parameter was not selected for inclusion in the model.

Table 4. Average (±SE) density and abundance per duck of harlequin duck prey (amphipods, chitons, limpets, snails, and mussels < 25 mm) at sampling sites within Prince William Sound, Alaska, 1997.

Parameter	Montague Island (Unoiled)	Knight Island (Oiled)	*P*
Density (g AFDW[a]/100 m^2)	2030.76 (± 2077.18)	1964.13 (± 2474.37)	0.94 (t = 0.08)
Abundance (kg AFDW/duck)	51.75 (± 61.43)	100.48 (± 194.71)	0.81 (Z = 0.24)
Density w/o mussels (g AFDW/100 m^2)	45.89 (± 39.14)	42.80 (± 29.22)	0.80 (t = 0.251)
Abundance w/o mussels (kg AFDW/duck)	3.84 (± 4.71)	3.23 (± 5.72)	0.94 (Z = 0.08)

[a]Ash free dry weight.

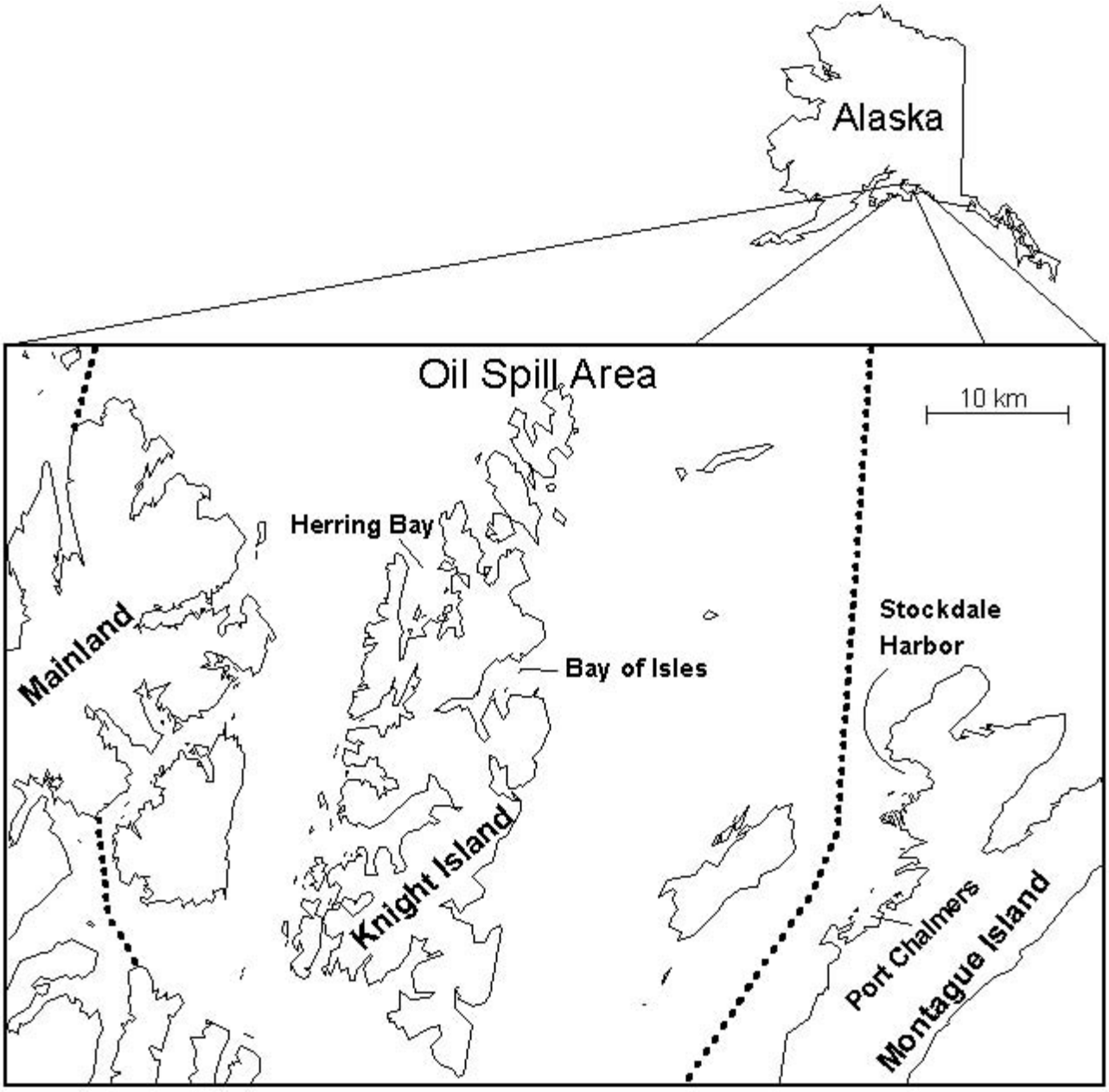

Figure 1. Study areas for assessing variation in winter harlequin duck densities, Prince William Sound, Alaska.

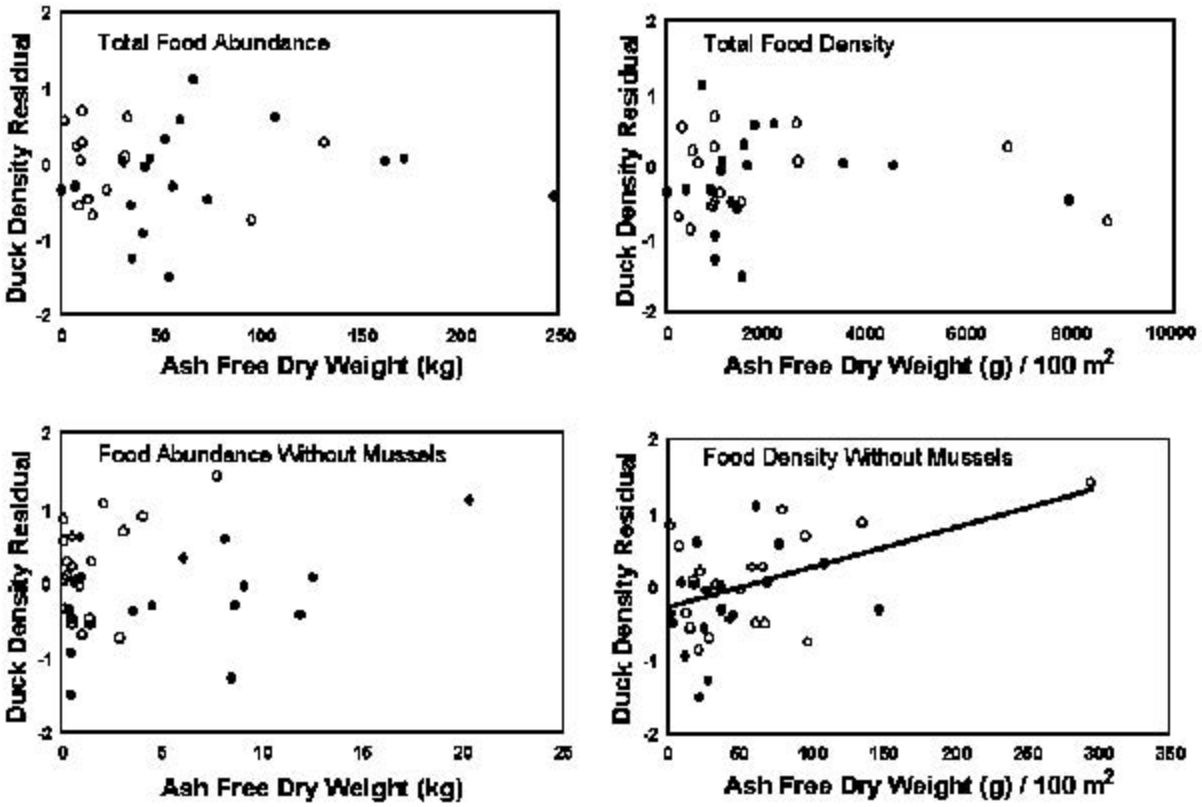

Figure 2. Linear relationships of residuals of harlequin duck densities (ducks/400 m shoreline; square root transformed) from a general linear model of habitat associations (Option A, Model 1, including area term) against measures of prey abundance and density. Open circles represent Knight Island (oiled) study sites and closed circles represent Montague Island (unoiled) sites.

APPENDIX HD-02

WINTER SURVIVAL OF ADULT FEMALE HARLEQUIN DUCKS IN RELATION TO HISTORY OF CONTAMINATION BY THE *EXXON VALDEZ* OIL SPILL[1]

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Abstract: Harlequin duck (*Histrionicus histrionicus*) life history characteristics make their populations particularly vulnerable to perturbations during nonbreeding periods. The 1989 *Exxon Valdez* oil spill was a significant perturbation to harlequin duck nonbreeding habitats in Prince William Sound, Alaska, which resulted in population injury. We used radiotelemetry to examine survival of adult female harlequin ducks during winters of 1995-1996, 1996-1997, and 1997-1998, to assess the progress of population recovery from the oil spill, and to evaluate potential continuing constraints to full recovery. We implanted 294 harlequin ducks (154 and 140 in oiled and unoiled areas, respectively) with transmitters and tracked their signals by airplane from October through March. We examined variation in survival rates relative to area and season (early, mid, and late winter). The 3 best models, as determined by comparisons of Akaike's information criterion (AIC_c) values, all indicated that survival of birds in oiled areas was lower than that in unoiled areas. Inclusion of body mass in the 3 best models did not improve their fit. In the model that best fit our data, survival was high in early winter for both areas, was lower during mid and late winter seasons, and was lowest in oiled areas during mid winter. Cumulative winter survival estimated from this model was 78.0% (SE = 3.3%) in oiled areas and 83.7% (SE = 2.9%) in unoiled areas. To assess whether area differences in survival were more likely related to oiling history versus geographic differences unrelated to oiling history, we contrasted a model with our original data set to a similarly structured model in which ducks (n = 75) from Green Island, an oiled site near our unoiled study area with similar intrinsic attributes, were recoded as being from the unoiled area. This alternate model fit less well (ΔAIC_c = 3.94), suggesting that oiling history was related to survival. Based on a demographic model, area differences in survival offer a likely mechanism for observed declines in populations on oiled areas. Other studies have found that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil as much as 9 years after the spill. We speculate that oil exposure, mortality, and population dynamics are linked and conclude that continuing effects of the oil spill restrict recovery of harlequin duck populations.

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Introduction

Harlequin ducks spend most of their annual cycle in nearshore marine environments, with breeding age birds leaving only for a few summer months to nest and raise broods on fast-moving streams (Robertson and Goudie 1999). Populations of harlequin ducks may be particularly sensitive to perturbations to their nonbreeding habitats. Harlequin ducks, like many seaducks, exhibit a life history in which variable and generally low annual reproductive effort is compensated by relatively high adult survival and, thus, long reproductive life spans (Goudie et al. 1994). This type of life history evolves under conditions of predictable and stable nonbreeding environments (Stearns 1992). Further, Goudie and Ankney (1986) described harlequin ducks, which are small-bodied relative to most other seaducks, as existing near an energetic threshold during winter, with little flexibility for increasing caloric intake or relying on stored reserves. While this strategy may be tenable under predictable and stable conditions, it does not accommodate perturbations that result in either decreases in energy acquisition or increases in metabolic costs.

The release of 11 million gallons of crude oil into the waters of Prince William Sound as a result of the March 1989 grounding of the *Exxon Valdez* was a significant perturbation to the nonbreeding habitat of harlequin ducks. As much as 40% of the spilled oil was deposited in intertidal and subtidal zones of Prince William Sound (Galt et al. 1991, Wolfe et al. 1994), the habitats used by harlequin ducks, and some residual oil was still present in these areas during the course of our study (Hayes and Michel 1999). Immediate bird mortality from the *Exxon Valdez* oil spill was high (Piatt et al. 1990) and more than 1,000 harlequin ducks were estimated to have died as a direct result of the immediate effects of the spill (John Piatt, U. S. Geological Survey, pers. comm.). Further, there are concerns that there may be continued, longer-term effects on harlequin duck populations in oil spill-affected areas (*Exxon Valdez* Oil Spill Trustee Council 1999).

This study was part of a program to assess population recovery of harlequin ducks from the *Exxon Valdez* oil spill in Prince William Sound. We focused on adult female survival during winter because (1) population dynamics of long-lived waterfowl species are particularly sensitive to changes in adult female survival (Goudie et al. 1994, Schmutz et al. 1997); (2) harlequin duck populations are likely sensitive to perturbations on wintering areas; and (3) Prince William Sound is primarily used by harlequin ducks during nonbreeding life stages. Paine et al. (1996), in a critique of studies immediately following the *Exxon Valdez* oil spill, recommended that demographic measures likely provide a better assessment of injury than species occurrence or abundance. We agree, and suggest that demographic studies not only serve to assess injury or recovery status, but also can lend insight into the processes and mechanisms underlying any constraints to full recovery.

Methods

As described by Paine et al. (1996), the *Exxon Valdez* oil spill was an imperfect experiment - a one-time perturbation without replication and, as in the case of wintering harlequin ducks, with little prespill data for comparison. Under these conditions, our approach was to compare oiled and unoiled areas, while attempting to minimize or account for differences

between areas that might confound interpretation of oil spill effects (Wiens and Parker 1995). We recognize that our statistical inference is to areas only, and that assessment of oil spill effects is subject to interpretation. We present ancillary data relevant for this interpretation.

Data Collection

This study was conducted in Prince William Sound (60°N, 148°W), the area most affected by the oil spill, during winters of 1995-1996, 1996-1997, and 1997-1998. We used radio telemetry to estimate survival of adult female harlequin ducks captured throughout the oil spill zone and on nearby unoiled Montague Island (Fig. 1).

Harlequin ducks, unlike most waterfowl, undergo wing molt on their marine wintering areas (Robertson and Goudie 1999). We herded flocks of flightless birds into funnel traps using sea kayaks during 20 August to 17 September, 1995-1997, the dates of peak wing molt by adult females. Captured harlequin ducks were removed from the trap, placed in holding pens, and transported by skiff to a larger vessel for processing. All birds were banded with unique U.S. Fish and Wildlife Service aluminum bands. We identified sex based on plumage characteristics and estimated age class by probing bursal depth (Mather and Esler 1999). Body mass ($\pm$ 1 g) was measured on an electronic balance.

Radio transmitters were surgically implanted into adult (after third year) female harlequin ducks using modifications (Mulcahy and Esler 1999) of the procedure described by Korschgen et al. (1996). Surgeries were conducted by veterinarians experienced in avian implant surgeries. Implanted transmitters have been successfully used in waterfowl studies (e.g., Olsen et al. 1992, Haramis et al. 1993), and an increasing body of literature suggests that radio transmitters implanted into wild waterfowl are less disruptive than external methods of attachment (see Esler et al., unpubl. ms.). Specifications of transmitters used in this study were described by Mulcahy and Esler (1999). Birds recovered from anesthesia for at least one hour before being released at the sites of their capture.

Radioed harlequin ducks were monitored approximately weekly from an airplane to determine mortality status and location. Monitoring flights began after the first birds were radioed and continued through the last week of March. Transmitters were equipped with mortality sensors that indicated death of a bird by a doubling of the transmitter pulse rate. Indicated mortalities were confirmed either by recovery of the radio or location of the radio signal in upland habitats, which harlequin ducks do not use during the nonbreeding season. Monitoring of radios for which signals were lost continued through the end of March.

Data Analysis

Unbiased survival estimation using telemetry requires meeting several critical assumptions (Pollock et al. 1989a), including: (1) radioed animals are representative of the population of interest; (2) survival is independent among individuals; (3) radio-marking does not affect survival during the study period; and (4) censoring of animals for which signals are lost is independent of the fate of those animals (i.e., missing animals are no more or less likely to be dead than animals for which fate is known). We felt that the first 2 assumptions were met based on our capture technique and marking regime. We perceived little chance of a systematically

biased sample based on susceptibility to capture, as we often were able to catch most birds within a given shoreline segment. Also, because we were marking only adult females, we felt that survival among individuals was independent beyond shared area effects, e.g., we were not marking both members of a pair or a mother and her offspring. We explicitly tested assumptions 3 and 4 (Esler et al., unpubl. ms.) and found that these were met for our sample.

For each week's sample of relocations, we counted mortalities and numbers of harlequin ducks at risk of mortality, following procedures outlined in Pollock et al. (1989a, 1989b) and Bunck et al. (1995). We used 1 October as the beginning of the data analysis period to ensure that all birds in the sample had survived a 14-day post-surgery censor period (Mulcahy and Esler 1999) and had completed wing molt. We made an *a priori* decision to combine data from all years to assure adequate power for detecting biologically meaningful differences between areas. A small number of birds (n = 6) moved between oiled and unoiled areas during winter; if a bird was detected in a different area for $\geq$ 2 consecutive observations, we included those observations in the at-risk data set for the newly occupied area.

We defined seasons as early winter, midwinter, and late winter, corresponding to the first 9 weeks of data collection, the middle 8 weeks, and the final 9 weeks. Our most general survival model contained 52 parameters, 26 for each area, and corresponded to the Kaplan-Meier method (Pollock et al. 1989a) of computing binomial estimates of survival. Variance estimates for this model were calculated using Greenwood's formula (Pollock 1989a). We examined the effects of season, area, and several season by area interactions on survival by comparing a series of reduced (fewer parameters) models. For all such model comparisons, we constrained survival to be equal among weeks within each season and area. The best model was that with the lowest Akaike's information criterion, adjusted for small sample size (AIC_c) (Burnham and Anderson 1998). The AIC_c balances the goodness-of-fit of the model (from the maximum likelihood) with the number of parameters to be estimated. Survival estimates and variances were calculated by iterative solution of the likelihood using program MARK (White and Burnham 1999).

We also assessed effects of body mass on survival by adding standardized body mass to the best-fitting models as determined above. A reduction in AIC_c value would indicate that the addition of the body mass term resulted in a more parsimonious model and thus that body mass was related to winter survival. Body mass was standardized to account for annual, geographic, and molt stage variation unrelated to our hypothesis of interest by using residuals around a general linear model (Esler et al., unpubl. ms.) as the body mass parameter. Body mass residuals could not be calculated for 12 of the radioed birds, thus model comparisons were conducted excluding these individuals.

Results

At 1 October, the beginning of the survival monitoring period, 294 radio-marked adult female harlequin ducks were included in the sample (154 from oiled areas and 140 from unoiled areas). Kaplan-Meier estimates of cumulative winter survival were 76.6% (SE = 4.0%) on oiled areas and 86.6% (SE = 3.2%) on unoiled (Fig. 2).

We contrasted 11 different models with various area and season combinations (Table 1). The best fitting model (Model 1) was one in which survival varied by season, with estimates higher in early winter than other seasons and lower in oiled than unoiled areas during midwinter

(Table 2). Cumulative winter survival estimated from this model was 78.0% (SE = 3.3%) in oiled areas and 83.7% (SE = 2.9%) in unoiled areas. Two other models had AIC_c values <2 units higher than Model 1. In Model 2, survival varied by season and was lower in oiled areas than unoiled during midwinter (Table 2). In Model 3, survival was high in the fall for both areas, lower and constant during mid and late winter on the unoiled area, and lower on oiled areas than unoiled during mid and late winter, particularly during midwinter (Table 2). These 3 best models all included an area effect, with survival on oiled areas lower than on unoiled areas (Fig. 2). The sum of AIC_c weights for models without an area effect was < 0.05, indicating that area effects were strongly supported by the data. Similarly, seasonal effects were well supported by the data, with survival during early winter consistently higher than in mid and late winter in the top 3 models. Inclusion of body mass increased AIC_c values of Models 1, 2, and 3 ($\Delta AIC_c \geq 0.69$), indicating that mass during wing molt was not strongly related to survival.

A difficulty of this study design was determining whether survival differences between oiled and unoiled areas were more likely related to intrinsic differences (such as habitat, disease, climate, social influences, or predator densities) rather than history of oil contamination. To address this, we looked more closely at data for birds (n = 75) from the Green Island area. Although Green Island was in the oil spill area, it was closer to unoiled Montague Island than to other oiled sites (Fig. 1). Also, habitats and duck densities (Esler, unpubl. data) were similar to the Montague Island study area. We found that the Kaplan-Meier estimate of cumulative survival of birds captured at Green Island (76.8%; SE = 5.7%) was more similar to that for all oiled areas combined than to unoiled Montague Island. We also contrasted a general season by area model (modified Model 8, Table 1; 3 areas = Green Island, other oiled areas, and unoiled Montague Island) to 2 models each with 2 areas (1 model with Green Island pooled with other oiled areas and 1 model with Green Island pooled with Montague Island). The AIC_c for the model with Green Island pooled with other oiled areas was $\geq$ 3.94 units lower than either of the other 2 models, suggesting that oiling history better explains differences in survival between areas than do intrinsic area differences.

Discussion

Winter survival of adult female harlequin ducks was lower on oiled areas than unoiled areas, primarily due to poorer survival during the midwinter period. In both areas, survival during early winter was higher than during mid or late winter. To understand how these estimates of survival might influence population dynamics, we incorporated the overall cumulative winter survival estimates for each area from Model 1 into a harlequin duck population model (Robertson 1997), holding all other parameters constant. The estimate of annual population change (λ) was 0.9464 for oiled areas (i.e., annual population declines of about 5.4%). For unoiled areas, λ = 1.0054, suggesting a relatively stable population. These estimates are consistent with trends estimated from population surveys conducted during fall 1995-1997 (Rosenberg and Petrula 1998). Differences in adult female survival offer a likely mechanism for differences in population trends between areas and, further, poor survival on oiled areas may be responsible for population declines.

Our data suggest that area differences in winter survival are more likely due to history of oil contamination than intrinsic area differences. For oiling history to affect survival

probabilities, and subsequent population trends, there must be some mechanism by which birds from oiled areas are compromised. One potential mechanism is that the immediate effects of the spill or subsequent effects of residual oil resulted in reductions of harlequin duck prey. However, during the period of this study, densities of harlequin duck prey were similar between oiled Knight Island and unoiled Montague Island and winter body mass of female harlequin ducks was similar between oiled and unoiled areas (Esler, unpubl. data), suggesting that differential food abundance did not explain differences in survival between areas.

Exposure to residual *Exxon Valdez* oil is another potential mechanism by which harlequin duck survival could be affected, as oil exposure is known to have deleterious toxic (Leighton 1993) and metabolic (Jenssen 1994) consequences. To determine if harlequin ducks in Prince William Sound were still being exposed to residual oil, Trust et al. (unpubl. ms.) measured induction of cytochrome P4501A (P450), which can indicate exposure to polycyclic aromatic hydrocarbon constituents of crude oil, in harlequin ducks captured during winter 1998 in both oiled and unoiled areas. P450 induction was much higher in harlequin ducks from oiled areas than those from unoiled areas, and Trust et al. (unpubl. ms.) concluded that this was almost certainly due to exposure to residual *Exxon Valdez* oil, as background hydrocarbon levels were negligible in intertidal areas of Prince William Sound prior to the oil spill (Short and Babcock 1996) and PCB levels were low and similar between areas (Trust et al., unpubl. ms.). Further, some residual oil was documented in nearshore habitats contemporary to our study (Hayes and Michel 1999). Finally, P450 results from harlequin ducks are consistent with those from several other nearshore vertebrates from oiled areas (Brenda Ballachey, U.S. Geological Survey, unpubl. data).

Could exposure to residual *Exxon Valdez* oil result in lower survival probabilities and concomitant population declines? Most lab studies have shown that mallards (*Anas platyrhynchos*) are tolerant of internal ingestion of oil, with toxic effects not evident until very high doses. These studies have been used to suggest that harlequin ducks should be unaffected by residual *Exxon Valdez* oil (Stubblefield et al. 1995, Boehm et al. 1996). However, other studies have found that the addition of other stressors such as cold temperatures caused oiled ducks in the lab to suffer considerably higher mortality than unoiled birds (Holmes et al. 1978, 1979). This compounding effect of environmental stress and oil exposure seems to be a more appropriate analog for wild harlequin ducks, which exist under relatively harsh winter conditions with little flexibility for accommodating additive stresses (Goudie and Ankney 1986). Our data indicate that mid and late winter may be stressful periods in the annual cycle of harlequin ducks even under unperturbed conditions, as survival on unoiled areas was lower during these seasons than during early winter.

The divergence of survival probabilities between oiled and unoiled areas during midwinter (Fig. 2) is consistent with a hypothesis of additive effects of oil in the presence of other stressors. Harlequin ducks are sight feeders and, during midwinter when day length is shortest, they spend most of their time foraging (Goudie and Ankney 1986, Fischer 1998). Prince William Sound is one of the farthest north wintering areas for harlequin ducks (Robertson and Goudie 1999), thus day light available for foraging is particularly limited. Because harlequin ducks have little flexibility for accommodating increased energy demands during winter (Goudie and Ankney 1986) that could result from either toxic insults or plumage oiling, they may not be able to handle additive effects of the oil spill, even if relatively small. We speculate that

differences in survival and populations trends may be related to documented differences (Trust et al., unpubl. ms.) in contaminant exposure.

Management Implications

Although populations of some animals may be unaffected or recover rapidly from oil spill effects (Wiens et al. 1996), others, such as harlequin ducks, have characteristics that make them vulnerable to population-level effects of oil spills for years following the event. For harlequin ducks, these characteristics include a life history requiring high adult survival, occurrence in habitats most affected by oil spills and which may hold residual oil for years, adaptation to stable and predictable marine environments, and high site fidelity. These traits also make harlequin ducks, and similar species, vulnerable to chronic, low-level oil pollution (Clark 1984). In the cases of either oil spills or chronic oil pollution, of course, the primary management recommendation is prevention; oil that does not go into the water does not threaten marine bird populations. Unfortunately, for harlequin ducks in the spill-affected area, there is little direct management action that now can improve winter survival. Hunter harvest of harlequin ducks is negligible in Prince William Sound and bag limits were already reduced following the oil spill. The extent of the *Exxon Valdez* oil spill zone is too large to recommend intensive habitat restoration; also, residual oil may be deeply buried in sediment (Hayes and Michel 1999) and oil removal efforts could result in significant disruption of intertidal habitats. Therefore, harlequin duck population recovery in Prince William Sound will depend largely on natural dispersal of residual oil and intrinsic population growth.

Factors that affect wintering aggregations likely are influencing subpopulations that are largely distinct demographic units (Cooke et al. 2000). Winter site fidelity of harlequin ducks is high (Robertson 1997, Cooke et al. 2000) and pair formation occurs on the wintering areas (Gowans et al. 1997, Robertson et al. 1998). Fortunately, levels of dispersal are high enough that subpopulations within the oil spill zone were not genetically distinct (Lanctot et al. 1999), i.e., the oil spill did not threaten a unique genetic resource. However, levels of dispersal are likely low and recovery of groups of birds in oiled areas must occur primarily through demographic processes specific to that group (i.e., numbers are not enhanced through immigration from other areas). Population recovery will require not only time for demographic processes to operate, but also elimination of continuing deleterious oil spill effects. Our data suggest that deleterious effects of the *Exxon Valdez* oil spill were evident as many as 9 years following the spill. Managers must recognize that, while oil spill effects may be short-lived for some species, full population recovery for species like harlequin ducks may require decades. In a broader context, the characteristics of harlequin ducks that make them vulnerable to oil spill effects also make them susceptible to population level consequences of other perturbations during nonbreeding periods, including human disturbance, habitat deterioration, and local overharvest.

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Table 1. Models used to estimate winter survival rates of adult female harlequin ducks in Prince William Sound, Alaska, using various combinations of season (early, mid, and late winter) and area (oiled and unoiled). The best model is that with the lowest Akaike information criterion, adjusted for small sample size (AIC_c; Burnham and Anderson 1998).

Models	Model description[a]	Number of parameters in model	AIC_c weight	AIC_c
1	EWO=EWU, MWO, MWU=LWO=LWU; survival varies between early winter and other seasons, areas differ during midwinter	3	0.314	480.7
2	EWO=EWU, MWO, MWU, LWO=LWU; survival varies among all seasons, areas differ during midwinter	4	0.199	481.7
3	EWO=EWU, MWO, MWU=LWU, LWO; survival varies between early winter and other seasons, areas differ during mid and late winter	4	0.144	482.3
4	EWO=EWU=MWU=LWU, MWO, LWO; survival does not vary seasonally in unoiled areas, areas differ during mid and late winter	3	0.100	483.0
5	EWO=EWU=MWU=LWO=LWU, MWO; survival varies between midwinter on oiled areas and all other season and area combinations	2	0.088	483.3
6	EW, MW, LW, O<>U; survival varies among seasons, with a constant area difference	4	0.074	483.6
7	EWO=EWU, MWO=MWU, LWO=LWU; survival varies by seasons	3	0.046	484.6
8	EWO, EWU, MWO, MWU, LWO, LWU; survival varies by all season and area combinations	6	0.028	485.6
9	EWO=MWO=LWO, EWU=MWU=LWU; survival varies by areas	2	0.004	489.3
10	EWO=EWU=MWO=MWU=LWO=LWU; survival does not vary by season or area	1	0.003	490.1
11	general model; estimates generated for each week and area	52	0.000	516.1

[a]EWO = early winter in oiled areas, EWU = early winter in unoiled areas, MWO = midwinter in oiled areas, MWU = midwinter in unoiled areas, LWO = late winter in oiled areas, and LWU = late winter in unoiled areas.

Table 2. Parameter estimates (SE) for the top 3 models describing adult female harlequin duck survival during winter in Prince William Sound, Alaska. See Table 1 for model descriptions.

Season[a]	Oiled Areas	Unoiled Areas
Model 1		
Early Winter	0.969 (0.012)	0.969 (0.012)
Mid Winter	0.870 (0.031)	0.934 (0.014)
Late Winter	0.925 (0.016)	0.925 (0.016)
Overall	0.780 (0.033)	0.837 (0.029)
Model 2		
Early Winter	0.969 (0.012)	0.969 (0.012)
Mid Winter	0.870 (0.031)	0.953 (0.020)
Late Winter	0.914 (0.021)	0.914 (0.021)
Overall	0.770 (0.034)	0.843 (0.029)
Model 3		
Early Winter	0.969 (0.012)	0.969 (0.012)
Mid Winter	0.870 (0.031)	0.940 (0.017)
Late Winter	0.910 (0.030)	0.933 (0.019)
Overall	0.767 (0.039)	0.850 (0.034)

[a]Seasons are of differing lengths (Early = 9 weeks, Mid = 8 weeks, and Late = 9 weeks).

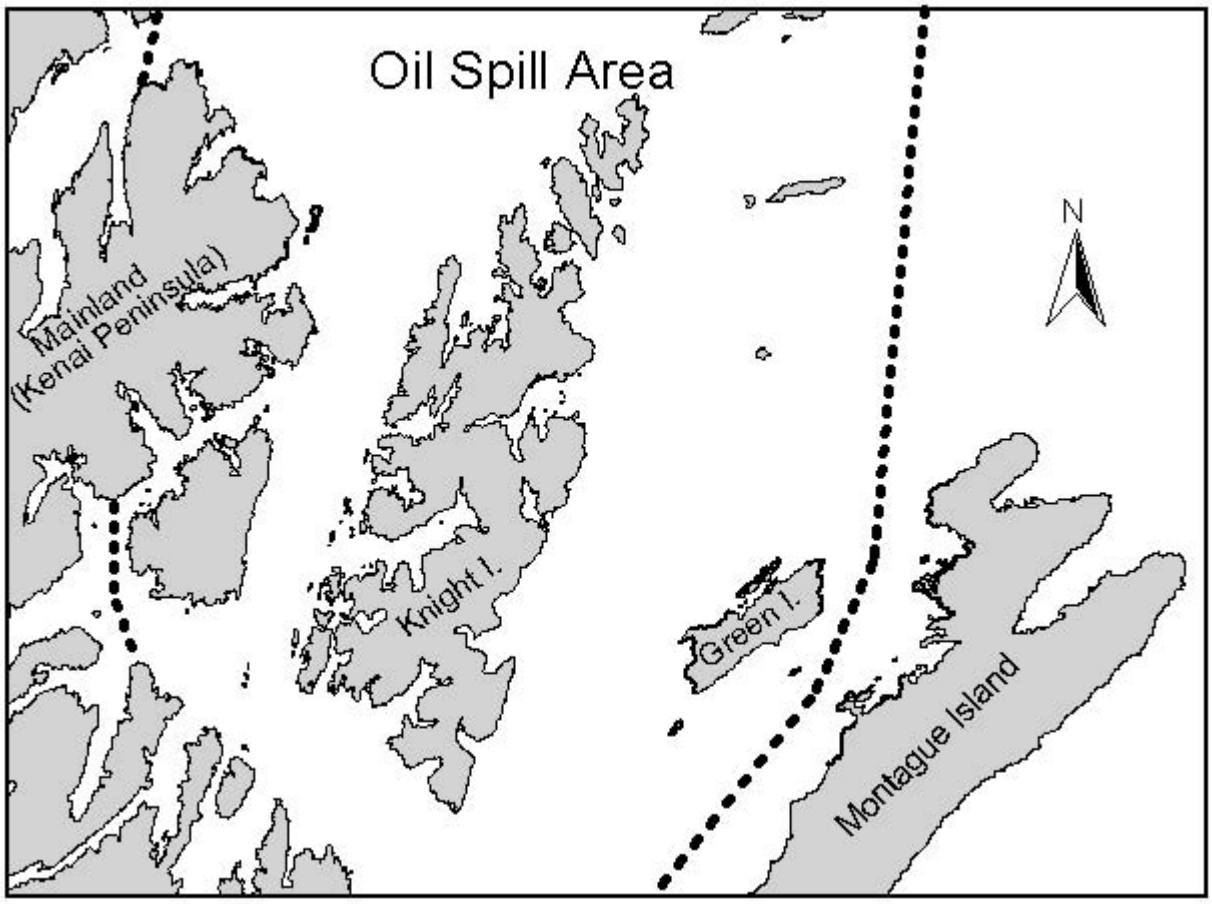

Figure 1. Study sites for estimating survival of adult female harlequin ducks in Prince William Sound, Alaska. Shorelines described by bold lines represent capture areas. The oil spill area is bounded by dashed lines.

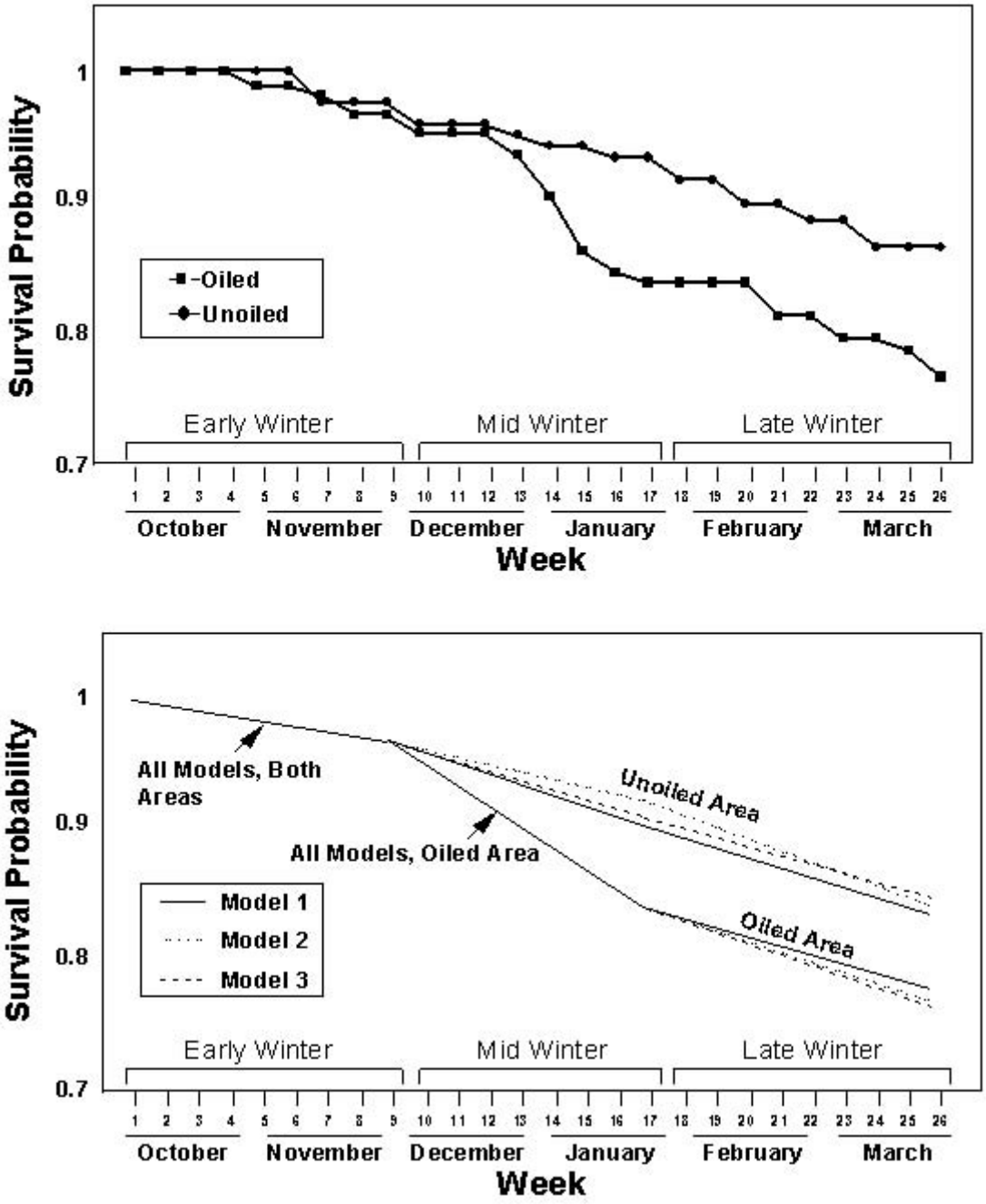

Figure 2. Winter survival probabilities for harlequin ducks in Prince William Sound, Alaska, based on Kaplan-Meier estimates (top) and the 3 best-fitting (see Table 1) reduced models (bottom).

APPENDIX HD-03

EVALUATION OF BURSAL DEPTH AS AN INDICATOR OF AGE CLASS OF HARLEQUIN DUCKS[1]

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Abstract

We contrasted the estimated age class of recaptured Harlequin Ducks (Histrionicus histrionicus) (n=255) based on bursal depth with expected age class based on bursal depth at first capture and time since first capture. Although neither estimated nor expected ages can be assumed to be correct, rates of discrepancies between the two for within-year recaptures indicate sampling error while between-year recaptures test assumptions about rates of bursal involution. Within-year, between-year, and overall discrepancy rates were 10%, 24%, and 18%, respectively. Most (86%) between-year discrepancies occurred for birds expected to be after third year (ATY) but estimated to be third year (TY). Of these ATY-TY discrepancies, 22 of 25 (88%) birds had bursal depths of 2 or 3 mm. Further, five of six between-year recaptures that were known to be ATY but estimated to be TY had 2 mm bursas. Reclassifying birds with 2 or 3 mm bursas as ATY resulted in reductions in between-year (24% to 10%) and overall (18% to 11%) discrepancy rates. We conclude that age determination of Harlequin Ducks based on bursal depth, particularly using our modified criteria, is a relatively consistent and reliable technique.

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Introduction

The bursa of Fabricius (hereafter bursa) has a long history of use for age assessment of wild waterfowl (e.g., Hochbaum 1942, Hanson 1949), although reliability estimates for the method rarely have been reported (Hohman and Cypher 1986, Esler and Grand 1994). The bursa is an immunosuppressive organ that forms as a sac on the dorsal side of the proctodeal region of the cloaca (Glick 1983). The bursa is present in juveniles, regresses as the bird matures, and eventually disappears in adults (Hochbaum 1942, Ward and Middleton 1971). Although the bursa has been used for age determination of ducks in spring (Anderson et al. 1969, LaGrange and Dinsmore 1988, Ankney and Alisauskas 1991,Young 1993), bursal involution may occur before or during an individual's first reproductive cycle, rendering it unreliable during that period (Hohman and Cypher 1986, Esler and Grand 1994). However, reliability may be higher during non-breeding seasons (Peterson and Ellarson 1978, Hohman and Cypher 1986). For birds that do not breed for two or more seasons following hatching (e.g., Canada Geese [Branta canadensis; Hanson 1949, Hochbaum 1942], Oldsquaws [Clangula hymelis; Peterson and Ellarson 1978], and other seaducks [Goudie et al. 1994]), the degree of bursal involution may be useful for differentiating age classes.

Our objective was to assess the utility of bursal characteristics for estimating age classes of Harlequin Ducks (Histrionicus histrionicus) by examining rates of discrepancies in age class designations of individuals over two or more capture events. In the absence of a known age sample, discrepancy rates provide a useful measure of reliability of bursal depth as an indicator of age class. Recaptures within-year provide an estimate of sampling error and recaptures between-year test assumptions about changes in bursal depth through time.

Methods

We captured flightless Harlequin Ducks during August and September of 1995-1997 in western Prince William Sound, AK using methods similar to Clarkson and Goudie (1994). We marked individuals with United States Fish and Wildlife Service leg bands. Age classes of all birds were estimated using internal bursal depth at each capture event. The bursa was exposed and a metal probe was inserted into the bursal sac to measure depth ($\pm$ 1mm). If the bursa was absent or $\leq$ 1 mm the birds were initially classified as after-third-year (ATY). Birds with bursal depths of >10 mm were classified as second-year (SY) and those with intermediate depths (2-10 mm) were classified as third-year (TY). Age classes are based on calendar year, thus, SY birds were approximately 14 months old, TY birds approximately 26 months, and ATY birds 38 months or older. Criteria for these initial classifications were based on those used in other studies of Harlequin Ducks (e.g., Goudie 1996) and the assumption that bursal involution should be complete after the third year when Harlequin Ducks reach breeding age (Hohman and Cypher 1986, Esler and Grand 1994, Goudie et al. 1994). Bursal depth for SY and younger birds is consistently >10 mm (Linduska 1945, Peterson and Ellarson 1978, Hohman and Cypher 1986, Henny et al. 1991). We assumed that bursal depth of TY birds would be intermediate as involution progressed (Ward and Middleton 1971). The age class criteria described above have been used to estimate age classes of harlequin ducks in other studies (e.g., Goudie 1996). Hatch year (HY) birds distinguished from older birds on the basis of size, presence of down, and

notched tail feathers; bursal depth of HY birds was not measured. These criteria were used to assign age classes throughout the course of the study. Bursal depths (mm), in contrast to age classification only, were recorded for birds estimated to be TY and ATY during the 1997 field season and late in 1996. Age class designations of recaptured birds were made without knowledge of age class estimates from previous captures.

Because of the lack of known-age birds, the accuracy of using bursal depth to determine age could not be tested directly. Instead, we used records from multiple captures to determine whether individuals could be consistently classified. Consistent classification (i.e., low discrepancy rates) for within-year capture events would suggest low measurement error. Consistent classification for between-year captures (i.e., an increase of one year in age class for every year between capture events) would support the original age class criteria and assumptions about the rates and timing of bursal involution.

To document discrepancy rates, we compared estimated to expected age classifications for each individual for each recapture event. Estimated age was the age classification based on bursal depth at the time of initial capture or recapture. An expected age class designation was generated at the time of recapture, based on previous age class designations and the time elapsed between capture events. Neither estimated nor expected ages were assumed to be correct; we simply contrasted the two to determine if there was a discrepancy or consistency. Discrepancies occurred when estimated age differed from expected age (i.e., within-year recaptures with different estimated age classes or between-year recaptures that differed from a pattern of one increase in age class estimate per year). We calculated frequency of discrepancies and identified classes of discrepancies that occurred. We compared frequencies of discrepancies among groups using chi-square goodness of fit tests.

Results

We recaptured 217 individuals one or more times for a total of 255 recaptures; 104 occurred within-year and 151 occurred between-years. Overall, estimated age classes of 82% (209) of recaptured ducks were consistent with expected age based on previous captures (Table 1). Of the recaptures, 176 were female, 79 were male. Proportions of consistencies and discrepancies did not differ between sexes (χ^2= 0.070, df = 1, P = 0.79).

Of within-year recaptures, 90% of estimated and expected ages were consistent (Table 1). This suggests that at least one of the age class estimates resulted from measurement error in 10% of cases, under the assumption that bursal depth would not change within a capture season.

Discrepancies between estimated and expected age classes occurred in 24% of between-year recaptures (Table 1), which is higher than would be expected if errors resulted only from measurement error (10%; see above). Most (86%) between-year discrepancies occurred when age class was expected to be ATY but estimated to be TY (Table 1). Bursal depths were recorded for 25 of 31 birds classified as between-year ATY-TY discrepancies. Of these ATY-TY birds, 22 (88%) had bursal depths of 2 or 3 mm. Five ATY-TY discrepancies were known to be ATY (see below) and had bursal depths of 2 mm. These results suggest that harlequin ducks with bursal depths of 2 or 3 mm should be classified as ATY. By reclassifying these birds as ATY, the overall proportion of discrepancies decreased from 18% to 11% (χ^2 = 5.77, df = 1, P = 0.02), the proportion of between-year discrepancies decreased from 24%

to 10% (χ^2 = 10.404, df = 1, $\underline{P} < 0.01$), and the proportion of within-year discrepancies did not change (χ^2 = 0.203, df = 1, $\underline{P}$ = 0.65). These results are consistent with the 10% measurement error predicted from within-year recaptures.

We had one instance in which a within-year discrepancy was associated with a between-year capture event. This individual was originally captured in 1995 and classified as an ATY; in 1997 the bird was captured twice and classified as an ATY once and a TY (2 mm bursa) once. As we were certain that the bird was an ATY in 1997 (see below), we classified the between-year recapture as a consistency and the within-year recapture as a discrepancy based on the original criteria. Using modified criteria, age class designations at all captures were consistent.

We had a subset of 45 individuals known to be ATY. Thirty-six birds were captured twice, first in 1995 and again in 1997, and nine were captured all three years. Because no HY birds were recaptured, all birds originally captured in 1995 and recaptured in 1997 definitely belonged in the ATY age class. Our between-year discrepancy rate for these known ATY birds was 13% using the original classification criteria. Five of the 6 discrepancies (83%) detected in this group were birds with bursa depths of 2 mm. By reclassifying these birds as ATY, our known ATY bird discrepancy rate dropped to 2% (χ^2 = 3.873, df = 1, $\underline{P}$ = 0.049).

Discussion

Based on discrepancy rates, we found that bursal depth enabled classification to relative age class, particularly after adoption of modified criteria for age class designation. Our estimate of measurement error rate (10% within-year discrepancy rate) for bursal age determination is comparable to error rates reported for some other age determination techniques. For example, age classes of 93% of female American Wigeon (Anas americana) (Wishart 1981) and 87.5% of Northern Pintails (Anas acuta) (Duncan 1985) were determined accurately using wing feather characteristics of known-age samples.

Measurement error can result from observer error; we attempted to minimize this source of error in our study by having only four trained observers measure bursas. We recommend similar cautions for other studies. Another potential source of measurement error may result from damage to the bursa while probing. Improper or prolonged probing may abrade the bursa and, as a result, bursal depth may be altered during the healing process, resulting in an inaccurate age class designation upon recapture. It may also be possible to puncture the bursa by probing too hard. Hanson (1949) found that with a small amount of added pressure a recently closed bursa may be pierced.

Our data strongly suggest that our original age class criteria, for TY and ATY birds, were inappropriate. Classifying birds with bursas $\leq$ 3 mm as ATY, 4-10 mm as TY, and > 10 mm as SY resulted in significantly lower between-year discrepancy rates than the original criteria. After reclassification, many of the remaining discrepancies likely were due to measurement error at one or all of the captures. The results from our known ATY sample corroborate our conclusions.

The ability to determine age classes of waterfowl accurately is essential for understanding the effect of age on many aspects of population ecology (e.g., Johnson et al. 1992). Adoption of age determination methods, without indications of their accuracy or reliability, could lead to erroneous conclusions about the ecological significance of age. While our data suggest that bursal age determination of harlequin ducks is relatively reliable, we stress that investigators be

aware that errors in age class designation are likely to occur when using this, or any other, technique.

Acknowledgments

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Table 1. Age classifications of recaptured harlequin ducks based on bursal characteristics.

Age-Class		Frequency	
Expected age	Estimated age	Between years	Within years
Discrepancies			
ATY	SY	1	1
TY	SY	0	4
ATY	TY	31	4
SY	TY	0	1
TY	ATY	4	0
SY	ATY	0	0
Consistencies			
SY	SY	0	16
TY	TY	4	47
ATY	ATY	111	31
Total recaptures		151	104

APPENDIX HD-04

SURGICAL AND IMMEDIATE POST-RELEASE MORTALITY OF HARLEQUIN DUCKS (HISTRIONICUS HISTRIONICUS) IMPLANTED WITH ABDOMINAL RADIO TRANSMITTERS WITH PERCUTANEOUS ANTENNAE[1]

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Abstract

Radiotelemetry is an essential tool in the study of free-ranging bird populations and a variety of transmitter attachment methods has been developed. A promising new method is abdominal implantation of a transmitter with a percutaneous antenna. Researchers using this technique should be concerned about and aware of mortality during surgery and the immediate post-release period (the 14 d period following surgery). Of 307 radio implant surgeries done in 1995 through 1997 in Harlequin Ducks (Histrionicus histrionicus), we documented 7 (2.3%) deaths during surgery and anesthetic recovery. Of 295 birds released with implanted radios, 10 (3.4%) died during the immediate post-release period. Modifications to anesthetic procedures used in the 204 surgeries done in 1996 and 1997 reduced mortality to 1.5% during surgery and 1.5% during the immediate post-release period. Anesthetic modifications included: intubation of all birds, placing birds on an elevated platform that allowed the bird's head to rest at a level lower than that of its body during surgery, use of a heated water blanket under the birds during surgery, monitoring of body temperature, and use of a heart monitor in addition to Doppler ultrasound to monitor heart rates and arrhythmias. Low levels of mortality associated with abdominal implantation of radio transmitters may be unavoidable, but mortality can be minimized with adaptive adjustments to anesthetic technique.

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Introduction

Radiotelemetry has become an essential tool for studying aspects of wild bird populations. Transmitters externally attached to birds, e.g., using backpack harnesses,[3] can cause adverse effects on behavior,[7,9] breeding and reproduction,[5,15,20] flight speed and metabolism,[6,16,17] and survival and return rates.[4,8,15,22,23] External transmitters attached by glue or subcutaneous anchors can be lost at a high rate.[9,21,24] In an effort to reduce adverse attachment effects and to increase transmitter retention rates, researchers have developed methods for abdominal implantation of radio transmitters.[11,10]

Recently, an abdominal implantation technique incorporating a percutaneous antenna has been successfully used for satellite transmitters.[18] Because such techniques are relatively new, there have been few reports of mortality experienced during surgery and in the period immediately following release, when mortality directly related to surgery and anesthesia could occur. Surgery-related causes of post-release mortality could include infection, dehiscence of the incision, hypothermia or other metabolic alteration, and an increased susceptibility to predation. In this paper, we describe the surgical and immediate post-release mortality experienced during a project involving the implantation into Harlequin Ducks (Histrionicus histrionicus) of a large number of conventional, VHF radio transmitters with percutaneous antennas.

Methods

From 1995 through 1997, we surgically implanted approximately 100 VHF radio transmitters each year into Harlequin Ducks in Prince William Sound, Alaska as part of a study to assess over-winter survival of these birds following the 1989 M/V Exxon Valdez oil spill. Only free-ranging female Harlequin Ducks greater than 3 yr of age, based upon cloacal examination of bursal involution,[12] were implanted with transmitters. Capture of ducks and surgeries were done at the same time each year (last week of August through the third week in September) during the annual wing molt. Ducks were captured while flightless by herding them into traps.[2] Each bird was banded with a unique U. S. Fish and Wildlife Service (USFWS) aluminum leg band.

A standard procedure was used to surgically implant transmitters.[10] Most of the surgeries were done by one of the authors (D.M.M.) with the remainder done by other veterinarians, all with previous experience implanting radio transmitters into birds. Briefly, anesthesia of the birds was induced with isoflurane (Aerrane, Ohmeda PPD, Inc., Liberty Corner, New Jersey 07938, USA) delivered by a cone with a vaporizer setting of 4.0 to 5.0% and was maintained at vaporizer settings ranging from 1.5 to 4.0%. Oxygen flow rate was maintained at 1 L/min. Following pre-surgical preparation, a midline incision was made into the abdomen and the right abdominal air sac. The antenna was passed through a trochar inserted from outside the bird as dorsally as possible at the intersection of the right pubic bone and the synsacrum. The transmitter was fitted into the right abdominal air sac and the incision was closed with 3-0 vicryl suture (Ethicon, Somerville, NJ 08876). The sole attachment of the transmitter to the body of the duck consisted of a single interrupted suture of 3-0 vicryl through the skin, body wall, and the catheter collar at the base of the antenna. Birds were allowed to recover from anesthesia for at least 1 hr before being released at their capture sites. Surgeries were done in a covered but

unheated workspace on the aft deck of a chartered motor vessel. Anesthesia was administered primarily by two biologists given training in the procedure or by veterinarians.

Birds were not intubated during the first half of the fieldwork in 1995. During the last half of 1995, and during both 1996 and 1997, the birds were intubated with uncuffed endotracheal tubes and were placed on foam pads with one end formed into a sloping ramp. The foam pads were designed so that the bird's head, when enclosed in the induction cone or when the bird was intubated, rested below the level of its body. Anesthetic monitoring in 1995 consisted of a Doppler ultrasound monitor (Parks Medical Electronics Inc., Aloha, Oregon 97007, USA) placed on the left cranial tibial artery. The anesthetist monitored anesthetic depth and respiration. In 1996 and 1997, body temperatures were monitored using a temperature sensor (Electro-Therm TM99A, Cooper Instrument, Middlefield, Connecticut 06455, USA) inserted into the cloaca. Also, in 1996 and 1997, we used a heart monitor with leads placed in standard positions and the birds were placed on a constant temperature water circulating pad (Gaymar T/Pump TP 400, Gaymar Industries Inc., Orchard Park, New York 14127, USA) to help maintain body temperature.

In 1995, transmitters (ATS, Isanti, Minnesota 55040, USA) weighed 15 g and were roughly spherical in shape (1.7-2.4 cm diameter), due to embedding in resin. In 1996 and 1997, transmitters (Holohil Corporation, Carp, Ontario K0A1L0, Canada) weighed 17.5 g, and were housed in brass cylinders measuring 4.0 cm by 1.5 cm, coated with a bio-compatible compound. A custom antenna collar (CBD-1, Vascath Corporation, Missisauga, Ontario L5A3V3, Canada) was added to the base of the antenna of each transmitter and was sealed with silicon adhesive. All transmitters had wire whip antennas. Rubber reinforcing was added to the basal 4 cm of the antennas in 1996 and 1997. The transmitters used in 1995 had a mortality switch activated by temperatures <27°C. In 1996 and 1997, transmitters were equipped with motion-sensitive mortality sensors activated by immobility of the transmitter for more than 12 hr. In all years, activation of the mortality switch doubled the transmitter pulse rate.

Radio tracking was conducted from fixed-wing aircraft. The first radio tracking flight occurred an average of 3.7 days (range: 3-5 days; $\underline{n}$=3) after the first radio was deployed each year. Intervals between flights during the period when any individual bird was within 14 days of surgery averaged 6.4 days (range: 3-12 days; $\underline{n}$=17). On each flight, mortality status and general location were noted for each bird. When a mortality signal was detected within 14 days of surgery, we tried to locate and recover the transmitter and carcass in order to examine the remains for the cause of death. In 1995 and 1996, gross necropsies were done on carcasses, but their use for unrelated analyses prevented histopathological examination. We used two-tailed Fischer's exact tests to assess difference ($\underline{P}$=0.05) in proportions of mortality between years. Proportions of interrupted surgeries and surgery or recovery deaths were calculated from total surgeries initiated, whereas proportions of post-release deaths were calculated from the numbers of birds released with transmitters and radio-tracked.

Results

A total of 307 surgeries were done on Harlequin Ducks during the 3 yr of the study, resulting in the release of 295 birds implanted with conventional radio transmitters equipped with percutaneous antennas (Table 1). Five surgeries were interrupted without implantation of a

transmitter, seven birds died during surgery or recovery, and 10 birds died within 14 days of release. Losses of birds during these periods decreased over the 3 yr course of the study (Table 1).

Seven birds died during the 307 surgeries and anesthetic recoveries, for an overall mortality rate of 2.3% (Table 1). Four of the deaths occurred in the first half of 1995, prior to the introduction of routine intubation of birds, the use of a foam ramp which placed the head of the bird lower than the body, and improved monitoring of body temperature and heart rate. In 1995, two birds died suddenly during surgery and could not be revived, and two birds experienced anesthetic difficulties after the abdominal incisions were made and the surgeries were continued but the birds died during recovery. Following the anesthesia modifications, the combined mortality rate for 1996 and 1997 was 1.5% (three surgery or recovery deaths of 204 total surgeries), compared to 3.9% (four deaths in 103 surgeries) in 1995 ($P=0.229$). In 1996, no surgery deaths occurred, but two birds died during anesthetic recovery. In 1997, one bird died during anesthetic recovery.

Mortality in the 14 day post-release period over all 3 yr was 3.4% (Table 1). Seven of the 10 post-release deaths occurred in 1995 during which the post-release mortality rate was 7.2% (Table 1). Four of the seven birds, and possibly a fifth, died within a few days of release; aspiration of fluids during surgery may have contributed directly or indirectly to death. Following introduction of the anesthesia modifications in 1995, there were only two post-release deaths of birds; both occurred in the second week after release. In 1996, post-release mortality was limited to three birds (3.1%), of which only one could have occurred within a few days of surgery. In 1997, no birds died in the immediate post-release period. When we pooled the 1996 and 1997 data (three post-release deaths of 198 radioed birds) to compare to the 1995 data, there was a decline in the post-release mortality rate to 1.5% ($P=0.017$).

Determination of an exact time of death in the post-release period was usually not possible because identifying death of a bird depended on detection of a mortality signal from the transmitter. Times of death were assigned to ranges of days due to intermittent scheduling of tracking flights. Of the 10 birds that died after release, all or parts of the carcasses were recovered from nine birds. Seven birds were subjected to predation or scavenging by bald eagles (Haliaeetus leucocephalus), or a mink (Mustela vison), or an unknown mammal, possibly a river otter (Lutra canadensis). The cause of death was not determined for one bird, despite recovery of an intact carcass, and one carcass was not recovered. In 1995, one bird that had died from an infection at the site of the transmitter implantation was recovered less than two days after surgery. This case of air sacculitis was the only known post-surgical infection in any of the implanted birds. Overall mortality rates (surgery, recovery, and post-release, combined) were significantly ($P<0.008$) lower in 1996 and 1997 (6 of 204; 2.9%) than in 1995 (11 of 103; 10.7%).

Surgeries were interrupted on five occasions over all 3 yr. For one bird in 1996 and one bird in 1997 anesthetic difficulties (severe, or repeated episodes of apnea) occurred before the abdominal incision was made (Table 1). Accidental incision of an abdominal organ (small intestine or ventriculus) occurred twice in 1995 and once in 1996. The incised organs were repaired and the birds were released without radios.

Discussion

Few reports have been published detailing mortality of wild waterfowl during surgery to implant transmitters or reporting the deaths of birds shortly after release. No deaths from surgeries occurred when 10 mallards (Anas platyrhynchos) were implanted with transmitters with percutaneous antennas and held for 28 days after surgery.[10] About 2% of 253 canvasbacks (Aythya valisneria) implanted with transmitters with internal antennas died during surgery and recovery.[14] An 8.2% mortality rate occurred when 49 mallards were implanted with transmitters with internal antennas.[4] No direct mortality occurred within 1-2 days of release of 12 spectacled eiders (Somateria fischeri) implanted with satellite transmitters with percutaneous antennas.[18] After adjustments to anesthetic technique, our surgical mortality (1.5%) was comparable or lower than the rates experienced in other studies. Mortality in 1996 and 1997 was reduced compared to 1995, which we attributed to improvements made to anesthetic technique. In 1996 and 1997, all birds were intubated and placed on foam ramps so that their heads were lower than their bodies which reduced the chance of aspirating fluids draining from the upper gastrointestinal tract and allowed for assisted breathing. Aspiration of fluids could have weakened the birds and made them more susceptible to predation. We had not previously used endotracheal tubes because of the short duration of the surgery (10 to 12 min) and of the total anesthetic period (18 to 24 min). Considering only 1996 and 1997, when anesthetic modifications were used for all surgeries, the surgery/recovery and immediate post-release mortality rates were each 1.5%. Although the effectiveness of heated water blankets for maintaining avian body temperature during extended surgery has recently been questioned,[19] we felt they were useful given the short duration of anesthesia and the low ambient temperatures (<10 °C).

Two syndromes causing mortality during surgery and anesthetic recovery were experienced: sudden, irreversible cardiopulmonary arrest that occurred during surgery and death of birds that failed to fully recover from anesthesia. The latter deaths typically occurred within about an hour of the completion of surgery. A similar syndrome of death during anesthetic recovery of rock doves (Columba livia) was attributed to putative hypothermia[1], but cloacal temperatures were normal in the Harlequin Ducks that died in 1996 and 1997 in our study. One or both of these syndromes have caused mortality during transmitter implantation surgery into spectacled eiders, common and thick-billed murres (Uria aalge and U. lomvia), and greater white-front geese (Anser albifrons) (D. M. Mulcahy, unpublished data).

The use of biologists to administer inhalant anesthesia to birds was a matter of practicality, but may have increased mortality. The use of technicians trained and experienced in anesthesia potentially could further reduce the mortality rate because of their ability to recognize anesthetic complications at an earlier stage.

Surgical sequelae must be differentiated from implantation effects. Complications (infection, dehiscence of the incision) directly related to the implantation surgery itself likely would occur within one week of the implantation; adverse effects of adjusting to an implanted radio (e.g., increased susceptibility to predation, effects on reproduction, adverse metabolic effects) might occur in the first week or later. Implantation of satellite transmitters altered nesting behavior of common and thick-billed murres.[13] We cannot assume deaths or disappearances of birds with transmitters within the first two weeks to be surgically related, as, without recovery and necropsy of the carcass, surgically related deaths cannot be distinguished

from birds killed by predators, for example. A 14 d period of data censoring following transmitter implantation has become standard.

The results of our study point out the importance of using the best possible anesthetic and surgical techniques. Although occasional deaths had occurred during transmitter implantation surgery of other species of birds, the rates were very low. At the beginning of our research on Harlequin Ducks research in 1995, mortality was high, until modifications in the anesthetic technique were introduced. This result suggests that species of birds may differ in the anesthetic and surgical obstacles they present when using an identical technique. Investigators using this technique of transmitter implantation must recognize that deaths are probably unavoidable, but can be minimized by careful attention and adaptive adjustment to anesthetic and surgical techniques.

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Table 1. Numbers of interrupted surgeries and deaths during surgery, the anesthetic recovery period, and the immediate post-release period (14 d) experienced during 3 yr of radio transmitter implants in Harlequin Ducks. Percentages are given in parentheses. The percentages of interrupted surgeries and surgery/recovery deaths are calculated on the number of total surgeries. The percentages of post-release deaths are calculated on the number of birds released with transmitters (1995, n=97; 1996, n=98; 1997, n=100; total for all years, n=295).

			Deaths (%)	
Year	Total Surgeries	Interrupted Surgeries	Surgery/Recovery	Post-Release
1995	103	2 (1.9)	4 (3.9)	7 (7.9)
1996	102	2 (2.0)	2 (2.0)	3 (3.1)
1997	102	1 (1.0)	1 (1.0)	0 (0.0)
Totals	307	5 (1.7)	7 (2.3)	10 (3.4)

APPENDIX HD-05

TESTING ASSUMPTIONS FOR UNBIASED ESTIMATION OF SURVIVAL OF RADIO-MARKED HARLEQUIN DUCKS[1]

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Abstract: Unbiased estimates of survival based on individuals outfitted with radio transmitters require meeting the assumptions that (1) radios do not affect survival and (2) animals for which the radio signal is lost have the same survival probability as those for which fate is known. In most survival studies, researchers have made these assumptions without being able to test their validity. We tested these assumptions by comparing interannual recapture rates (and, by inference, survival) between radioed and unradioed adult female harlequin ducks (*Histrionicus histrionicus*) and, for radioed females, between right-censored (i.e., missing) birds and birds with known fates. We found that recapture rates were not lower ($P = 0.585$) for birds equipped with implanted radio transmitters with external antennas (21.6 $\pm$ 3.0% [$\pm$ standard error]) than unradioed birds (21.7 $\pm$ 8.6%), evidence that radios did not affect survival. Recapture rates also were similar ($P = 0.486$) between right-censored (20.6 $\pm$ 5.1%) and known-fate individuals (22.1 $\pm$ 3.8%), suggesting that missing birds were not subject to differential mortality. We also determined that capture and handling resulted in short-term loss of body mass for both radioed and unradioed females ($P \leq 0.001$) and that this was more pronounced for radioed birds (15.4 $\pm$ 7.1 g; $P = 0.034$). However, after a year, body mass of recaptured females with radios was not different from expected ($P = 0.123$) under a hypothesis of no radio effect. Our study indicates that implanted radios are an unbiased method for estimating survival of harlequin ducks and likely other species under similar circumstances.

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Introduction

Radio telemetry has been used widely in studies of wildlife survival (White and Garrott 1990). Unbiased survival estimation using telemetry requires meeting several critical assumptions (Pollock et al. 1989), including: (1) radioed animals are representative of the population of interest; (2) survival is independent among individuals; (3) radio-marking does not affect survival during the study period; and (4) censoring of animals for which signals are lost is independent of the fate of those animals (i.e., missing animals are no more or less likely to be dead than animals for which fate is known). The first 2 assumptions often can be met through application of an appropriate experimental design, whereas the latter 2 are under less control by researchers and can not necessarily be assured by *a priori* planning. In most studies, investigators must make these latter 2 assumptions without being able to test their validity. In this study, we tested assumptions about radio effects and censored individuals for adult female harlequin ducks outfitted with implanted radio transmitters with external antennas.

A considerable body of literature exists describing effects of radio transmitters on wildlife species. In birds, deleterious effects of externally mounted transmitters (particularly those attached with backpack harnesses) have been documented in numerous studies, including changes in behavior (Massey et al. 1988, Pietz et al. 1993), reduced reproductive effort (Pietz et al. 1993, Rotella et al. 1993, Paquette et al. 1997, Garrettson and Rohwer 1998), and reductions in survival or return rates (Marks and Marks 1987, Burger et al. 1991, Cotter and Gratto 1995, Ward and Flint 1995, Dzus and Clark 1996). Although not all studies have shown negative effects of external transmitters (e.g., Hines and Zwickel 1985, Foster et al. 1992), the broad occurrence of documented deleterious effects clearly raises concern about generating unbiased survival estimates using externally mounted transmitters. Surgical implantation of transmitters into the abdominal cavity (Korschgen et al. 1984, 1996; Olsen et al. 1992) offers a promising alternative. In direct comparisons, implanted transmitters have consistently shown fewer deleterious effects than externally-attached radios (Rotella et al. 1993, Dzus and Clark 1996, Paquette et al. 1997), although no previous studies have contrasted long-term survival of birds with internal radios to unmarked individuals.

Survival estimates from radioed animals are generated based on the assumption that the probability of detecting animals is independent of their mortality status (Bunck et al. 1995), an assumption that is critically important for animals for which radio signals are lost and remain undetected through the rest of the telemetry monitoring period (i.e., right-censored). Recognizing potential violation of this assumption, some investigators (e.g., Conroy et al. 1989) have presented results that include maximum survival estimates, where all right-censored animals are assumed to have lived through the study period, and minimum estimates, where they are assumed to have died. Most investigators, however, produce survival estimates under the assumption that mortality rates of undetected animals are the same as detected animals. We are not aware of any studies that have directly addressed this assumption. Two studies (Miller et al. 1995, Cox et al. 1998) have reported returns of failed radios from hunter-killed northern pintails (*Anas acuta*), documenting that some right-censored birds were alive and in the study site during telemetry monitoring; however, the proportional frequencies of returns of known fate and right-censored birds were not compared.

Our study offered a unique opportunity to test assumptions of survival estimation of radio-marked animals. Harlequin ducks have high fidelity to molt sites (Robertson 1997), high annual survival (Goudie et al. 1994), and are susceptible to capture during wing molt. These traits, in conjunction with deployment of relatively large numbers of radios, allowed for good sample sizes to compare recapture rates and, by extension, survival differences among groups of birds. To test the assumption of a lack of radio effects on survival, we compared recapture rates of radioed and unradioed birds. We also compared recapture rates of radioed birds of known fate with those that were right-censored due to a lost radio signal to test the assumption of similar survival probabilities between these groups. Recapture probability of an individual is the product of between-year fidelity to the study site, capture probability if the bird is on the study site, and survival between capture events. Because site fidelity and capture probability of previously captured birds should not be related to radio status, we assumed that differences in recapture rates among groups of birds would reflect survival differences. We recognize that previously captured birds may exhibit trap shyness; however, because all birds included in this study, irrespective of radio status, were subjected to similar capture methods, handling, and holding time upon their original capture, we assume that the degree of trap shyness would not vary based on radio status. Also, we examined body mass changes of both radioed and unradioed individuals recaptured within- and between-years to assess potential short- and long-term effects of radio transmitters on body mass. Body mass has been positively related to survival probability for some waterfowl species (Conroy et al. 1989, Longcore et al. 1991, Bergan and Smith 1993) and, thus, is important to assess as a potential mechanism affecting survival of birds with radio transmitters.

Methods

Harlequin ducks were captured in Prince William Sound, Alaska as part of efforts to examine winter survival probabilities in relation to history of contamination by the *Exxon Valdez* oil spill. Captures occurred during 20 August to 17 September, 1995-97, the period of peak wing molt by adult females. Harlequin ducks were captured by using sea kayaks to herd molting, flightless birds into a funnel trap along shore. Once captured, birds were transported by boat to the main vessel for processing. Each bird was leg-banded with a unique U. S. Fish and Wildlife Service aluminum band, which was used to identify recaptured individuals. Sex was identified based on plumage characteristics and age class was estimated by probing bursal depth (Mather and Esler 1999). Body mass ($\pm$ 1 g) was measured on an electronic balance; estimated mass of radio transmitters was subtracted from measured masses of birds recaptured with implanted radios.

Radio transmitters were surgically implanted into adult (after-third-year) female harlequin ducks. In 1995, transmitters (ATS, Isanti, MN) weighed 15 g and were roughly spherical in shape (1.7-2.4 cm diameter), due to embedding in resin. In 1996, transmitters (Holohil, Carp, Ontario) weighed 17.5 g, and were formed as brass cylinders measuring 4.0 cm by 1.5 cm and were coated with a bio-compatible compound. All transmitters had wire whip antennas with a dacron-covered silastic sleeve glued to the base of the antenna. To deter birds from breaking antennas, a rubber reinforcement was added to the basal 4 cm of the antennas in

1996, which extended 3 cm outside of the duck's body when implanted. Expected battery life was $\geq$ 7 months for 1995 radios and $\geq$ 18 months for 1996 radios.

A modification of the procedure described by Korschgen et al. (1996) was used to surgically implant transmitters (Mulcahy and Esler 1999). Briefly, anesthesia of the birds was induced and maintained with isoflurane (Aerrane, Ohmeda, Liberty Corner, NJ). Following pre-surgical preparation, a midline incision was made into the abdomen and the right abdominal air sac was breached. The antenna was passed through a trochar inserted from outside the bird and placed as dorsally as possible at the intersection of the right pubic bone and the synsacrum. The transmitter was fitted into the right abdominal air sac and the incision was closed with absorbable sutures. The sole attachment of the transmitter to the body of the duck consisted of a single interrupted suture through the skin, body wall, and the collar at the base of the antenna. Birds recovered from anesthesia for at least 1 hour before being released at the sites of their capture.

Radioed harlequin ducks were monitored approximately weekly from an airplane to determine mortality status, location, and radio signal strength. Monitoring flights began after the first birds were radioed and continued until the last week of March. Transmitters were equipped with mortality sensors that indicated death of a bird by a doubling of the transmitter pulse rate. The mortality sensor was activated by temperatures < 27°C for 1995 radios and by immobility for > 12 hr for 1996 radios. Indicated mortalities were confirmed either by recovery of the radio or location of the radio signal in upland habitats, which harlequin ducks do not use during the nonbreeding season. Monitoring of radios for which signals were lost continued through the end of the monitoring period.

We used a one-tailed Fisher's Exact Test (Ramsey and Schafer 1997:548) to test the null hypothesis that recapture rates (proportions of birds recaptured) were not lower for radioed adult females than unradioed. Recaptures were defined as the capture of an individual in the year subsequent to previous marking or handling. We also estimated the proportional reduction in recapture rates of radioed to unradioed birds, and the associated variance, following methods for double ratio estimation (Cochran 1977:183); values near or above 1 would be consistent with no radio effect. No unradioed adult females were released in 1995, therefore we compared recapture rates of unradioed birds released in 1996 to both recapture rates of radioed birds from 1995 and 1996 combined, and 1996 only in case there were annual differences in recapture rates of radioed birds that might influence the results. Four birds were captured and radioed in 1995 and not recaptured again until 1997; these were not included in our analyses, as unradioed birds with comparable capture histories were not available. Animals captured in all three years were represented by 2 recapture events. The sample of radioed birds included only those known to have survived the 14-day period following radio implant surgery, a censor interval designed to eliminate effects of surgery or handling (Mulcahy and Esler 1999).

To test whether survival differed between birds with known fates (i.e., known to have survived or died during the monitoring period) and birds for which radio signals were lost during the monitoring period, we compared recapture rates of these groups following the methods described above for radioed to unradioed comparisons. Our null hypothesis for the one-tailed Fisher's Exact Test was that the recapture rate of right-censored birds was not lower than that of birds of known fate. Again, we calculated proportional differences in recapture rates with values near 1 indicating no differences between groups.

To examine differences in body mass between recaptured birds with radios and those without, we first standardized mass to account for seasonal, annual, geographic, and individual variation unrelated to our hypotheses of interest. We used residuals around a general linear model as our measure of standardized body mass. The model was generated from body mass data from molting females captured during our studies (n = 607), including all birds used in subsequent analyses. We used only data for first captures of females within a year to generate the model. The best-fitting model was determined by comparison of Mallow's C_p values of all possible combinations of main effects in a data-based model selection context (Burnham and Anderson 1998). Main effects included in the model selection process were: Area (an indicator variable in which unoiled Montague Island = 0 and capture sites in oiled areas = 1); Year (1/0 indicator variables for 1996 and 1997, with 1995 set as the reference value); Age (1/0 indicator variables for juvenile and subadult age classes, with the adult age class set as the reference value); and Ninth Primary Length (a continuous variable indexing the stage of wing molt). The model with the lowest C_p value was of the form:

Mass = 606.18 - 9.61*Area - 18.64*Year 1996 - 15.06*Juvenile Age Class -
0.19*Ninth Primary Length.

Because subadult and adult age classes did not differ in body mass variation during wing molt (i.e., the Subadult Age Class variable was not included in the best-fitting model), we used birds of both age classes for subsequent analyses of body mass changes. For an individual, the difference in body mass residuals between the original capture and subsequent recapture reflects the relative change in body mass after accounting for variation due to other factors. Differences in body mass residuals could not be calculated for a small number of birds that, at $\geq$ 1 of their captures, had not shed their old primaries and therefore molt stage (Ninth Primary Length) could not be determined and the general linear model could not be applied.

To examine whether body mass was affected by radio implantation after a full year, we compared the average between-year change in residuals between recaptured birds that were radioed and those that were unradioed using a t-test. We also compared the average change in residuals to zero, the expected result under a null hypothesis of no effect.

We assessed the effects of radio status and duration between captures on short-term changes in body mass using a general linear model. The dependent variable was the change in body mass residuals between within-year capture events of individuals and independent variables were radio status and the number of days between capture events.

Results

Twenty-three adult female harlequin ducks were captured, banded, and released without radio transmitters during 1996; of those, 5 (21.7 $\pm$ 8.6% [$\pm$ standard error]) were recaptured in 1997. Of 185 adult females implanted with radio transmitters in 1995 and 1996 that survived the 14-day postsurgery period, 40 were recaptured, a rate (21.6 $\pm$ 3.0%) not lower (P = 0.585) than that of unradioed birds. Similarly, when considering only 1996 radioed birds, 23 of 95 were recaptured, a rate (24.2 $\pm$ 4.3%) comparable to our unradioed sample (P = 0.691). The proportional difference in recapture rates (radioed recapture rate/unradioed recapture rate) was

0.995 (± 0.417) when including all radioed birds and was 1.114 (± 0.485) when considering only 1996 radioed birds; these results suggest no reduction from 1, i.e., no evidence for a radio effect on recapture rate.

Radio signals were permanently lost during the monitoring period (right-censored) for 63 birds transmittered during 1995 and 1996. Thirteen (20.6 ± 5.1%) of the right-censored birds were subsequently recaptured, which was similar ($P = 0.486$) to the recapture rate of birds with known fates during the monitoring period (27 of 122; 22.1 ± 3.8%). The proportional difference in recapture rates was 0.932 (± 0.280). Dates of right-censoring occurred throughout the monitoring period (Fig. 1). The number of undetected radios increased during the final 4 weeks of the monitoring period, probably due to battery exhaustion of 1995 transmitters. We compared recapture rates of right-censored birds and birds with known fates, excluding those with signals lost during the final 4 weeks, to determine whether mechanisms resulting in signal loss other than battery failure could be related to survival. We found that the recapture rate (9 of 40; 22.5 ± 6.6%) of birds right-censored during the first 5 months of the monitoring period was not lower ($P = 0.613$) than that for birds with known fates reported above. Also, the proportional difference between groups (1.017 ± 0.345) was near 1. Most lost signals occurred during the winter following 1995 captures (Fig. 1). Of 17 radioed birds recaptured in 1996, 13 had broken off their antenna at or near the skin surface (Mulcahy et al. 1999), likely explaining some signal loss; however, we also recaptured some individuals with intact antennas that were right-censored, perhaps as a result of other types of radio failure.

Body mass residuals of unradioed adult and subadult females ($n = 42$) averaged 5.0 (± .3) g higher in the year of recapture than the previous year, a result not different from zero ($t_{41} = 1.176$, $P = 0.246$). For radioed adult females ($n = 34$), body mass residuals averaged 7.4 (± .7) g lower upon their recapture than in the year of their first capture, not different ($t_{33} = 1.584$, $P = 0.123$) from the expected value of zero under a hypothesis of no radio effect. The 12.5 (± 6.4) g difference between groups was marginally significant ($t_{74} = 1.961$, $P = 0.054$). Taken together, these results do not suggest a strong radio effect on body mass after a year.

For within-year recaptures, the number of days between capture events did not explain variation in the change in body mass residuals between capture events ($t_{50} = 0.031$, $P = 0.975$) within a general linear model including a radio status term. Also, average number of days between capture events did not differ ($t_{51} = 0.368$, $P = 0.714$) between radioed (13.0 ± 0.9) and unradioed (13.3 ± 0.6) birds. Therefore, the analysis reduced to t-test comparisons. Body mass residuals of unradioed females ($n = 33$) declined an average of 15.0 (± 4.3) g between capture events, a result significantly lower than zero ($t_{32} = 3.480$, $P = 0.001$). Body mass residuals of radioed females ($n = 20$) declined 30.3 (± 5.7) g, also different from zero ($t_{19} = 5.349$, $P < 0.001$). The 15.4 (± 7.1) g difference in changes in body mass residuals between groups was marginally significant ($t_{51} = 2.178$, $P = 0.034$). These results suggest that capture and handling have short-term effects on body mass for both radioed and unradioed birds but that these effects were greater for those birds receiving radio transmitters.

Discussion

We found no evidence to suggest that adult female harlequin duck survival estimation was biased by either deleterious effects of implanted radio transmitters or differential survival

between known-fate and right-censored birds. Recapture rates invariably were quite similar between groups, building confidence for using these methods to test hypotheses related to survival.

This study is the first to compare interannual survival between birds with implanted radios and unradioed birds. Our finding that recapture rates were not reduced for harlequin ducks with implanted radios supports a growing body of evidence suggesting that implanted radios are less likely to result in biased survival estimates than externally attached radios. In comparisons of birds with implanted radio transmitters to others with externally attached transmitters, survival of birds with implanted transmitters was higher (Dzus and Clark 1996, Paquette et al. 1997). Other studies have documented lower survival or return rates for sharp-tailed grouse (*Tympanuchus phasianellus*; Marks and Marks 1987), black brant (*Branta bernicla nigricans*; Ward and Flint 1995), and rock ptarmigan (*Lagopus mutus*; Cotter and Gratto 1995) with external transmitters than for unradioed birds. However, no differences in survival were detected between externally-transmittered and unradioed spotted owls (*Strix occidentalis*; Foster et al. 1992) and blue grouse (*Dendragapus obscurus*; Hines and Zwickel 1985). We recommend that investigators be aware of potential bias using externally attached transmitters and consider the use of implanted transmitters as a potentially unbiased alternative.

Disadvantages of radio implantation include longer handling time and requirement of veterinary support for implant surgeries, although these are relatively minor compared to the desirability of obtaining unbiased estimates of survival and minimizing adverse effects on marked individuals. Schulz et al. (1998) reported elevated heterophil:lymphocyte ratios in captive mourning doves (*Zenaida macroura*) following abdominal implantation of radio transmitters, although postsurgery body mass and other blood chemistry parameters were not affected. Also, extrusion and loss of implanted radio transmitters with external antennas was documented (Mulcahy et al. 1999) for some of the harlequin ducks in this study. This could result in bias in survival estimation if extrusion and loss resulted in undetected mortality. However, recapture rates did not differ between a year without known extrusions and 1 with documented extrusions, the incidence of extrusion and loss was relatively low, recaptured birds that had lost their radios were apparently healthy, and radio loss occurred after the monitoring period (Mulcahy et al. 1999). Further, our results from this study show that recapture rates of radioed birds, including birds that lost radios, were similar to those of unradioed birds, corroborating the conclusion of Mulcahy et al. (1999) that extrusions did not affect health of birds. Radio extrusions can be avoided largely through attention to radio design and surgical technique (Mulcahy et al. 1999).

Short-term effects of transmitter implantation in birds have been detected, including reduced nesting effort (Meyers et al. 1998), surgical and postrelease mortality (Mulcahy and Esler 1999), and reductions in body mass documented in this study. However, biases to survival estimation can be avoided by censoring data during the period immediately following implantation when these effects occur. For our studies, 14 days was an appropriate censor interval. Ten mortalities of radioed harlequin ducks (out of 295 radioed and released during 1995 - 1997) were documented during the 14 days following radio implant surgery (Mulcahy and Esler 1999), compared to zero during the next 14 days. Also, the results from this study show no evidence of differential survival of radioed birds after the 14-day censor interval relative to unradioed birds.

One potential bias resulting from using radio telemetry to estimate survival is that deaths potentially related to the radio-tagging process (i.e., within the censor interval) may not be distributed at random within the sample of captured birds and, thus, the assumption that the radioed birds entering into the monitoring period are representative of the population of interest may be violated. In other words, the small number of deaths associated with radio-marking (Cox and Afton 1998, Mulcahy and Esler 1999) may occur in birds that had a different (presumably lower) survival probability had they not been captured than birds that survived the censor interval. In this case, one might predict higher recapture rates for radioed birds that survived the censor period than unradioed birds; we did not detect this, although we had little power to detect these presumably subtle effects. We believe that this potential bias had little effect on our survival estimates, as the incidence of deaths within the censor interval is relatively low (Cox and Afton 1998, Mulcahy and Esler 1999) and deaths were related more to procedural attributes than individual variation. We encourage investigators to minimize deaths due to radio-marking by adaptive modifications to capture and radio-marking techniques.

Loss of radio signals and the subsequent assumption that right-censored individuals have the same survival probability as individuals with known fates, is an issue that has been difficult to address in field studies. In many cases, undetected radios likely result from radio failure (Miller et al. 1995, Cox et al. 1998, this study), but other plausible scenarios of loss of a radio signal exist that are not independent of mortality status, e.g., a predator destroys the antenna or radio during the predation event. We demonstrated that this bias does not exist for our study of harlequin duck survival. Due to the paucity of data addressing this bias, however, we recommend other attempts to test this assumption.

Short-term body mass loss associated with radio-marking has been previously documented (Dugger et al. 1994) and we found short-term reductions in body mass, presumably related to capture and handling in both radioed and unradioed individuals. Body mass loss is a concern when estimating survival because of the documented relationship between body mass and subsequent mortality in some situations (Conroy et al. 1989, Longcore et al. 1991, Bergan and Smith 1993), although not others (Dugger et al. 1994, Migoya and Baldassarre 1995, Miller et al. 1995, Cox et al. 1998). However, because there were no strong radio effects on interannual body mass change and, particularly, because of our finding that interannual recapture rates did not differ between radioed and unradioed birds, we conclude that the short-term mass loss associated with radio transmitter implantation does not affect subsequent survival.

Management Implications

Survival is an important demographic parameter for understanding population status and predicting population trends, as well as for identifying environmental or anthropogenic factors that affect wildlife species. This is particularly true for species with life history traits similar to harlequin ducks, i.e., long-lived with relatively low investment in annual reproduction (Goudie et al. 1994, Schmutz et al. 1997). Thus, it is critical to use methods for measuring survival that result in unbiased estimates. Our results suggest that use of abdominally implanted radio transmitters for estimating harlequin duck survival does not violate assumptions of (1) no effect of radio transmitters and (2) no differential survival between right-censored and known-fate individuals. Based on our results, and those of studies contrasting external transmitters with

implanted transmitters, we suggest that implanted transmitters likely offer investigators a less biased method. Finally, we recommend that investigators attempt to quantitatively test assumptions of survival estimation for their particular species of interest and situation. Generation of survival rates in an unbiased manner is critically important for making subsequent management decisions for wildlife populations.

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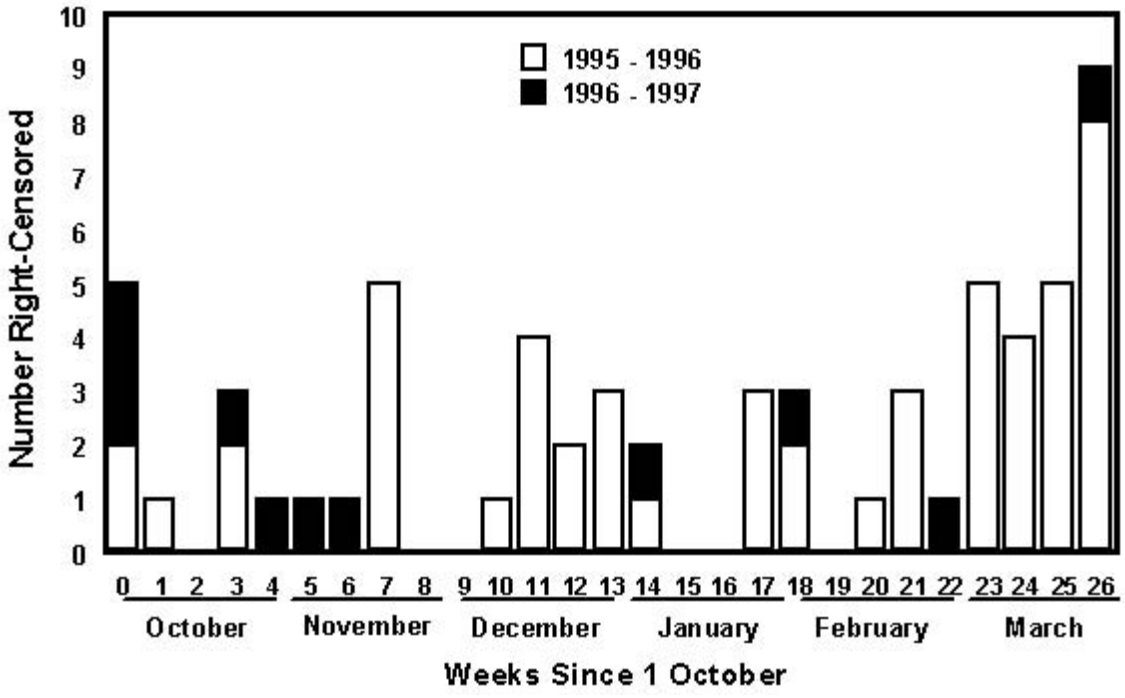

Figure 1. Distribution of dates of signal loss (right-censoring) of radio-marked adult female harlequin ducks in Prince William Sound, Alaska.

APPENDIX HD-06

LOSS FROM HARLEQUIN DUCKS OF ABDOMINALLY IMPLANTED RADIO TRANSMITTERS EQUIPPED WITH PERCUTANEOUS ANTENNAS[1]

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Abstract

We documented extrusion and loss of abdominally implanted radio transmitters with percutaneous antennas from adult female Harlequin Ducks (Histrionicus histrionicus). Birds were captured during wing molt (late August to mid-September) in 1995-1997. Of 44 Harlequin Ducks implanted with radios and recaptured, 7 (16%) had lost their transmitters and 5 (11%) had radios in the process of extruding. Most (11 of 12) extrusions and losses occurred in birds implanted with radios in 1996 and recaptured in 1997. We suggest that transmitter extrusions and losses were due largely to changes in transmitter design made between 1995 and 1996. Transmitters implanted in 1996 were cylindrical rather than spherical, had a flat end with an abrupt edge, and the lower portion of the antenna was reinforced. Radio losses occurred after the 7-mo monitoring period and caused no apparent harm to the birds. Investigators using implanted radios with percutaneous antennas for long-term projects should be aware of the potential for radio extrusion and should design minimize the problem by using transmitters that have no sharp edges and that are wide, rather than narrow.

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Introduction

Radio telemetry has been used widely in studies of wildlife survival, movements, habitat use, and breeding. An increasing body of literature suggests that radio transmitters surgically implanted into wild waterfowl are less disruptive than transmitters that are externally attached, based on differences in survival or return rates (Ward and Flint 1995, Dzus and Clark 1996), behavior (Pietz et al. 1993), and reproductive rates (Pietz et al. 1993, Rotella et al. 1993, Ward and Flint 1995, Paquette et al. 1997). The disadvantages of externally mounted transmitters stimulated the development of techniques for surgical implantation of transmitters (Korschgen et al. 1984, Olsen et al. 1992, Korschgen et al. 1996).

Waterfowl studies using implanted transmitters have reported high rates of success (e.g., Olsen et al. 1992, Haramis et al. 1993, Hohman et al. 1993, 1995). Loss of an internal transmitter has been documented only once, when a transmitter with an internal antenna was passed through the oviduct of a nesting female (Garrettson and Rohwer 1996). In this paper, we document the occurrence of extrusion and loss from Harlequin Ducks (Histrionicus histrionicus) of abdominally implanted radio transmitters with external antennas and we offer recommendations to minimize this problem.

Methods

We surgically implanted radio transmitters into adult female Harlequin Ducks from 1995-1997 as part of a study of their over-winter survival in Prince William Sound, Alaska. We captured birds and implanted transmitters each year during the last week of August through the third week of September during annual wing molt (when birds were flightless). Each bird was banded with a unique U. S. Fish and Wildlife Service (USFWS) aluminum leg band, which allowed identification of recaptured birds.

The procedure described by Korschgen et al. (1996) was used to surgically implant transmitters. Briefly, anesthesia was induced and maintained with isoflurane (Aerrane, Ohmeda, Liberty Corner, New Jersey). Following pre-surgical preparation, a midline incision was made into the abdomen and the right abdominal air sac was breached. The antenna was passed through a trochar inserted from outside the bird and placed as dorsally as possible at the intersection of the right pubic bone and the synsacrum. The transmitter was fitted into the right abdominal air sac and the incision was closed with absorbable sutures. The only attachment of the transmitter to the body of the duck consisted of a single interrupted suture through the skin, body wall and the collar at the base of the antenna. Birds recovered from anesthesia for at least 1 h before being released at the sites of their capture. Surgeries were done in a covered but unheated workspace on the aft deck of a chartered motor vessel.

The transmitters (ATS, Isanti, Minnesota) we used in 1995 weighed 15 g and were embedded in resin, which resulted in a roughly spherical shape (1.7-2.4 cm diameter). The transmitters (Holohil Systems, Carp, Ontario, Canada) we used in 1996 weighed 17.5 g and were enclosed in brass cylinders coated with a bio-compatible compound and measuring 4.0 cm by 1.5 cm. All transmitters had wire whip antennas with a dacron-covered sleeve glued to the base of the antenna. To deter birds from breaking antennas, rubber reinforcement was added to the basal 4 cm of the antennas in 1996, which extended 3 cm outside of the body.

In the second and third years of the study, we recaptured some birds that had been implanted with transmitters in a previous year. The presence of one of our leg bands and an external antenna or a visible antenna stump immediately identified a retained radio. We palpated the abdomens of all recaptured and transmitter-implanted birds to detect non-functioning radios that lacked a visible antenna stump and used a radio receiver, tuned to the proper frequency and placed immediately adjacent to the bird, to determine the presence of a functioning radio. In 1996 and 1997, most birds with non-functioning or missing radios were re-implanted with a new radio. After the abdomen was opened, a visual and tactile inspection was made of all accessible spaces in the abdomen and abdominal air sacs. We radiographed several birds in 1997 using a portable radiograph machine (Bowie Portable X-Ray Generator, Bowie Manufact., Lake City, Iowa) and instant film (Polaroid Transparent Radiographic Instant Film, Type TPX) to confirm that the transmitters had, indeed been lost, rather than migrated into the anterior thorax of the birds.

Results

We recaptured 44 ducks in 1996 and 1997 that had been implanted with radios in 1995 or 1996 (Table 1). Of the 40 ducks that were recaptured one year after radio implantation, 6 (15%) had lost their transmitters and 5 (13%) had radios that were in the process of extruding (Table 1). Of the 17 ducks implanted with spherical transmitters in 1995 and recaptured in 1996, 13 had broken off the antenna where it exited the skin, leaving either no stump or only 1-2 mm of antenna extending from the skin. None of the birds implanted in 1995 had lost the transmitter when recaptured in 1996. The transmitter was missing from one of the four birds implanted in 1995 and recaptured in 1997; the antennas were broken off of the transmitters in the other three birds (Table 1). We could palpate the transmitters in the caudal right quadrant of recaptured ducks as a firm mass of appropriate size and shape.

In 1997, we recaptured 23 ducks implanted with cylindrical transmitters in 1996. Of these, 12 (52%) had radios present, with no sign of extrusion; 2 of the 12 ducks had broken off the antennas at the end of the reinforced base (Table 1). Three birds (13%) had radios present internally, but with the dacron antenna collar pulled out through the body wall and skin, two birds (9%) had pulled both the dacron antenna collar and part of the transmitter out through the skin, and six birds (26%) had lost their radios entirely. In 1997, we confirmed by radiography for three ducks that the transmitters had been entirely lost from the body instead of having migrated to another location within the body.

We replaced 20 radios in recaptured birds 1-2 yr after the first implantation. During these surgeries, extensive adhesions were found involving intestines, air sac membranes, liver, and the ventral body wall. A thick (1-2 mm) fibrous sheath completely surrounded the entire transmitter body and had to be cut to remove the enclosed transmitter. The adhesion between the antenna collar and the body wall was broken by gently pulling on the transmitter body. Hemorrhage was minimal from the incised connective tissue sheath and the disrupted antenna collar attachment site.

We captured two birds in 1997 with the transmitters partially protruding through the body wall. In these birds, the arc of the caudal end of the transmitter body closest to the antenna attachment had been pulled first and farthest through the body wall, suggesting that a lever action

had been applied to the reinforced base of the antenna. The fit between the skin and the transmitter body was tight, preventing both the immediate loss of the transmitter and the leakage of water into the abdomen, which might have caused infectious peritonitis or air sacculitis. We removed both transmitters, after aseptic preparation of the site, by applying traction to the antenna base and gentle clearing of inflammatory debris from around the skin-transmitter interface, using the blunt end of a scalpel handle. We surgically reduced and closed the resulting fistulae.

Discussion

All attachments to wild animals, such as tags, bands, marks, and instrument packages, suffer a rate of loss specific to the species of animal, environmental conditions, type of attachment, and mechanism of attachment. We chose abdominal implantation of radio transmitters for this project because of the potential deleterious effects of externally mounted transmitters (Pietz et al. 1993, Rotella et al. 1993, Ward and Flint 1995, Dzus and Clark 1996, Paquette et al. 1997). Loss rates of surgically implanted transmitters are often assumed to be low (Zimmer 1997) compared to externally attached transmitters with only one documented loss of an implanted transmitter reported (Garrettson and Rohwer 1996). Because abdominally implanted transmitters equipped with internal coiled antennas suffer reduced signal strength due to the body mass of the animal, we used percutaneous antennas. The technique developed by Korschgen et al. (1996), utilizing a percutaneous antenna was used in one study without report of transmitter loss (Petersen et al. 1995).

We believe that the increased rate of loss of transmitters implanted in 1996 compared to those implanted in 1995 resulted from changes in dimensions and configurations of the transmitters. In 1995 the transmitters were rounder and wider than the cylindrical transmitters used in 1996. The 1995 transmitters were built by constructing the electronics around the partial circumference of a cylindrical battery and then embedding both in epoxy resin, resulting in a spherical shape. Only one bird that had been implanted in 1995 and recaptured in the 2 years following implantation had lost its transmitter. The 1996 transmitters and batteries were enclosed in a cylindrical brass case, lightly coated with a biocompatible material. The flat posterior end of the brass case met the curved side wall in an abrupt, 90 degree angle, which was inadequately blunted by the biocompatible coating. The antenna exited the side of the brass case adjacent to this sharp angle. A final change in design was the addition of rubber reinforcement around the initial 4 cm of antenna, in an effort to reduce the bird's ability to break the antenna at the level of the skin.

We speculate that the birds groom and manipulate the solid wire antenna, causing metal fatigue and failure where the antenna exits the skin, as documented with the 1995 transmitters. The additional reinforcement material added to the base of the antennas in 1996 reduced the antenna failure rate, although some birds still managed to break the antenna at the distal end of the reinforcement. The birds likely continued to groom and manipulate the remaining, reinforced antenna base until they pulled the antenna collar out through the body wall. Adhesions, contractures, and proliferative connective tissue prevented the transmitter from then falling back into the air sac and drawing the antenna back into the bird. The placement of the antenna on the side of the cylindrical transmitter body next to the flat end caused a lever action when the bird

pulled on the antenna base. This placed the abrupt edge of the transmitter's flat end adjacent to the body wall, which helped to enlarge the hole where the antenna exited. Eventually the entire transmitter was pulled through the fistula.

The smaller cross-section of the cylindrical transmitter used in 1996 was able to pass through a smaller fistula. Also, the narrower transmitter probably could pass more easily through the angle formed by the pubic bone and the synsacrum. To reduce this effect, the surgeon must assure that the antenna is passed through the body wall as dorsally as possible at the origin of the pubic bone on the synsacrum. The angle between these two anatomical structures narrows dorsally, and the intersecting soft tissues are thicker and stronger dorsally.

There have been few reports of long-term retention rates because abdominal implantation of transmitters in birds, especially those using percutaneous antennas, is a relatively new technique. The transmitter with a percutaneous antenna that we used is anchored in the short term by a suture through the antenna collar and body wall and in the long term by the body wall attachment to the antenna collar that occurs during healing. Therefore, there is little chance for the transintestinal expulsion of transmitters experienced in ictalurid and salmonid fishes (Summerfelt and Mosier 1984, Chisholm and Hubert 1985, Marty and Summerfelt 1986).

All of the ducks that had lost transmitters and were recaptured in fall 1997 had been located regularly from August-September 1996 through April 1997. Therefore, transmitters must have been lost between the end of April and the time the birds were recaptured in late August and September 1997. Loss of transmitters did not appear to affect the health of implanted ducks. Recaptured birds with lost or extruding transmitters appeared healthy. The fibrous sheath that developed around the transmitter body may have sealed the transmitter from the air sac and peritoneal cavity during extrusion of the transmitter through the skin. Recapture rates in the year following implantation were higher for birds implanted in 1996 (24%) than in 1995 (19%) suggesting that survival was not compromised by loss of the transmitter.

We believe that the loss of abdominally implanted transmitters can be reduced by designing wider transmitter bodies with no abrupt edges, careful placement of the percutaneous antenna as dorsally as possible and elimination of the reinforcement at the base of the antenna. We recommend that investigators using implanted transmitters with external antennas work closely with manufacturers to design a transmitter that is appropriate for their work and which minimizes risk of extrusion and loss. Loss of radios could result in erroneous conclusions in studies relying on radio telemetry.

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These data were collected under studies supported by the *Exxon Valdez* Oil Spill Trustee Council. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council. Data were collected with the assistance of Bryan Baetsle, Rick Ballas, Brad Benter, Tim Bowman, Kathy Burek, Jennifer DeGroot, Bob Jarvis, Danielle Mather, Dan Monson, Julie Morse, Dan Ruthrauff, Dorcas Schaeffer, Kim Trust, and the crews of the motor vessels *Auklet*, *Julia Breeze*, *Kittiwake II*, and *Waters*. D.V. Derksen, J. B. Grand and T. Van Pelt reviewed the manuscript. Use of trade names does not imply product endorsement.

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TABLE 1. Fate of radio transmitters implanted into female Harlequin Ducks during wing molt, Prince William Sound, Alaska, 1995-1997.

Year Implanted/Recaptured	n	Radios lost	Radios retained		
			Undamaged	Antenna Broken	Radio Extruding
1995/1996	17	0	4	13	0
1995/1997	4	1	0	3	0
1996/1997	23	6	10	2	5
TOTALS	44	7	14	18	5

APPENDIX HD-07

HEMATOLOGY AND SERUM CHEMISTRY OF FREE RANGING, MOLTING, FEMALE HARLEQUIN DUCKS AND A COMPARISON OF VALUES BETWEEN DUCKS FROM THE OILED AND UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA[1]

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Abstract

Hematology and serum chemistry reference ranges were established for molting female harlequin ducks (Histrionicus histrionicus) sampled from unoiled areas of Prince William Sound, Alaska. Blood values of harlequin ducks from the oiled areas of Prince William Sound sampled at the same time significantly differed only in having lower red blood cell counts and higher sodium and glucose levels.

[1]In preparation for submission to Marine Pollution Bulletin.

Introduction

Ten years after the event, harlequin ducks in Prince William Sound, Alaska, have not recovered from the effects of the Exxon Valdez oil spill. Harlequin ducks are true seaducks, spending all but the nesting portion of their life cycle in the marine environment. This ethology makes them very sensitive to the long-term effects of an oil spill that occurs in coastal waters.

Hematology and serum chemistries are standard tests used in the diagnosis of disease in animals. Considerable efforts are made to establish reference intervals for a variety of birds, however, the emphasis has been to define such values for species that are held in captivity, especially psittacines, raptors, and poultry (Cambell 1994, 1995, Fudge 1997, Heidenreich 1997, Hochleitner 1994). It is a challenge to define reference intervals and to use clinical pathology as a tool to study free-ranging birds in environments far from the laboratory. Besides the difficulties sometimes encountered in obtaining a sufficient number of samples of free-ranging birds, handling, storage, and transportation of blood samples collected during field studies is less than optimal. In addition, samples are rarely available from birds of both genders, from all ages, and at all stages of their life cycle, important variables in clinical pathology.

We present hematology and serum chemistry reference intervals and intervals for free-ranging female harlequin ducks captured during wing molt in an unoiled area of Prince William Sound, Alaska. We then compare these intervals to similar data obtained from birds at the same stage of their life cycle, but captured from an area that had suffered contamination from the oil spill in 1989.

Methods

Birds were captured in 1995 and 1996 from areas in Prince William Sound, Alaska, designated as oiled and unoiled study sites following the M/V Exxon Valdez oil spill. Only free-ranging female Harlequin Ducks greater than 3 yr of age, based upon cloacal examination of bursal involution (Mather and Esler 1999), were sampled. Capture of ducks was done at the same time each year (last week of August through the third week in September) during the annual wing molt. Ducks were captured while flightless by herding them into traps (Clarkson and Goudie 1994). All sampling and preliminary sample processing (to the point of separating serum from whole blood) was done onboard a chartered boat.

Two ml of blood was taken from the jugular vein using a 3-ml syringe and 21 gauge 25-mm needles. Duplicate blood smears were made on microscope slides, 1 ml was placed into a plastic conical tube and the remainder of the blood was placed into glass tubes. Blood was allowed to clot at ambient temperature for 1-12 hr before being centrifuged to separate serum that was then frozen at -20 C. Samples were held in the boat's freezer for up to three days until they could be shipped via floatplane to Cordova, Alaska, from which site they were transported by commercial courier service to an avian clinical pathology laboratory in California. A portion of the collected serum was sent for determination of haptoglobin levels.

In the laboratory, differential blood cell counts were made from the prepared blood smears. The presence of hemoparasites was noted. Hematology variables measured or calculated were: white blood cell count ($10^3/\mu L$), red blood cell count ($10^6/\mu L$), packed cell volume (%), mean corpuscular volume (fL), hemoglobin (g/dL), mean corpuscular hemoglobin

concentration (g/dL), azurophils (%), bands (%), heterophils (%), lymphocytes (%), monocytes (%), eosinophils (%), and basophils (%). The presence of a buffy coat, thrombocytes, reactive lymphocytes, and polychromasia and anisocytosis of erythrocytes was noted. Serum chemistry variables measured were: sodium (mEq/L), potassium (mEq/L), gamma glutamyltransferase (IU/L), alkaline phosphatase (IU/L), calcium (mg/dL), creatine phosphokinase (U/L), glucose (mg/dL), lactic dehydogenase (IU/L), phosphorus (mg/dL), aspartate aminotransferase (IU/L), total protein (g/dL), and uric acid (mg/dL). Sodium, potassium, gamma glutamyltransferase, and alkaline phosphatase were measured only in samples taken in 1996. Samples reported by the laboratory as hemolyzed were not included in any analysis. Within a year, some parameters could not be measured in some samples.

Reference intervals for blood parameters for molting female harlequin ducks were determined following recommendations of the National Committee for Clinical Laboratory Standards (1995). Only blood samples from ducks captured on the unoiled side of Prince William Sound were used to calculate reference intervals. In order to minimize the influence of potential mishandling, we eliminated samples for which there was an indication of suboptimal processing. We did not use samples with values of less than 2.0 mEq/mL for potassium or less than 155 mg/dL for glucose. We identified and eliminated outliers by calculating the ratio D/R where D was the absolute difference between an extreme observation (large or small) and the next largest or smallest observation, and R was the interval of all observations (Dixon 1953). Calculations of $D/R \geq 1/3$ were used as cut-off values (Reed 1971). Normality testing indicated that the distributions of most of the blood variables violated assumptions of normality or equal variance. Common transformations converted some but not all of the variables to normal distributions. A nonparametric procedure on the data without transformation was used to determine the reference intervals (National Committee for Clinical Laboratory Standards 1995). The lower reference limit, $\underline{r}_1$ (the 2.5^{th} percentile) was the observation corresponding to $\underline{r} = 0.025 (\underline{n} + 1)$ and the upper reference limit, $\underline{r}_2$ (the 97.5^{th} percentile), as the observation corresponding to $\underline{r} = 0.975 (\underline{n} + 1)$. We used the term reference interval rather than reference range (Dybkær and Solberg 1987)

We used the nonparametric, Mann-Whitney rank sum test to compare hematology and serum chemistry values obtained from female molting harlequin ducks captured from unoiled areas to values obtained from ducks captured from oiled areas of Prince William Sound using an experiment-wide error rate of 0.05. We used a sequential Bonferroni adjustment (Rice 1989) for $\alpha = 0.002$ for $\underline{n} = 27$, the total number of hematology and serum chemistry parameters compared. Statistical tests were done using commercial software (Sigmastat, Jandel Scientific Software, San Rafael, California, USA).

Results

The analytical laboratory reported erythrocyte polychromasia and anisocytosis and slightly degranulated heterocytes in all samples. Hemoparasites were observed in three samples: two leucocytozoon and one plasmodium; data from these samples were not used in determining reference intervals. Descriptions of blood cell types were not available from the laboratory.

Table 1 summarizes the hematological and biochemical values for the molting female harlequin ducks sampled from the unoiled area of Prince William Sound and includes the median

value, the 2.75 and 97.5 percentiles and the extreme values. Table 2 compares the hematological and biochemical values between blood from molting females in the unoiled areas to blood from female ducks in the oiled areas of Prince William Sound. Significant differences were found between the two groups of ducks for total red blood cell count, sodium, and glucose. Using the derived reference intervals, there were 43 (23 low, 20 high) individual values outside of the reference interval from birds in the unoiled areas and 130 (64 low, 66 high) individual values outside the reference interval from birds in the oiled areas of Prince William Sound.

Discussion

Tests for normality and equal variance showed that many of the blood and serum chemistry data that we collected were not normally distributed. Efforts to transform non-normally distributed data improved some but not all variables. Therefore, we chose to use nonparametric statistical methods to describe the reference intervals and to make our comparisons. When data are not normally distributed, nonparametric estimates of reference intervals, including rank tests such as the one we used, are more accurate than parametric methods (Potvin and Roff 1993, Reed et al. 1971). Although the majority of papers dealing with blood and serum chemistry data use parametric statistics and present reference intervals as means ±2 SD, many authors fail to test for normality and to adjust the use of parametric or nonparametric statistics accordingly. Others transform their data for the statistical comparisons, but present means ±2 SD without making clear whether these values were calculated from transformed or nontransformed data.

The large number of parameters that can be measured from a single blood sample also requires caution for analysis. Rice (1989) showed that there was a 95% chance of finding a significant difference between one of 12 pairs in a table of statistical parameters using a 0.05 level of significance. This led Work (1996), in his study of free-ranging seabirds, to use Rice's suggestion for a sequential Bonferroni adjustment for an experiment-wide error of 0.05. In comparing 26 pairs of blood and serum variables from ducks taken from unoiled area to ducks taken from oiled areas, our use of the same method reduced the number of significant differences from 10 to 3 (red blood cell count, sodium, and glucose). Although the red blood cell count was significantly lower in birds in the oiled areas of Prince William Sound, there was no report of Heinz-body anemia, as frequently occurs during acute exposure to oil (Leighton 1983, Yamato 1996). Also, the packed cell volume and red blood cell indices (mean corpuscular volume, hemoglobin, and mean corpuscular hemoglobin concentration) did not differ significantly. Sodium and glucose concentrations were significantly but moderately higher in birds from the oiled areas. Sodium and glucose levels could reflect a higher level of stress in birds living in oiled areas, or could be a result of different durations of capture chases, handling times, or effects of recent adverse weather prior to capture.

There was considerable variation in blood and serum chemistry variables in the ducks sampled from both the oiled and the unoiled areas. All the blood samples were analyzed at the same laboratory. However, there was no way to control for variations in the individual histories of each of the birds. Similarly, the facts that our study was done in a remote area and the samples had to be picked up and flown to a different state for analysis presented special problems. Also, our collection and preliminary processing of blood samples was done as an

adjunct to the major effort being made to surgically implant radio transmitters into harlequin ducks. Thus, there were inevitable variations in sample handling and processing and serum storage times. We feel that these problems are inherent in studies on free-ranging wildlife that are done in remote areas, with minimal equipment and facilities and with competing demands for space, time, and personnel.

Harlequin ducks are one of several species whose populations in Prince William Sound have not recovered from the effects of the 1989 Exxon Valdez oil spill. We used two approaches to determine if harlequin ducks living in areas of Prince William Sound that had been contaminated with oil in 1989 showed alterations in hematology or serum chemistry. First, we used blood samples from harlequin ducks living in an area of Prince William Sound that had never been oiled to establish normal reference intervals, and then compared values from samples taken from ducks in areas that had been oiled to the reference values. Second, we directly compared blood values from ducks taken from oiled areas to ducks from unoiled areas. The former approach represented a classical evaluation of blood samples from an experimental population. The latter approach helped control for variations, such as chase and holding times, and individual variations in short-term history because all samples from both areas were included.

Although oil spills in the marine environment occur relatively frequently and often involve considerable numbers of birds, most investigations of oiling in seabirds has focused on the acute effects and on the techniques for rehabilitation of oiled seabirds (Degernes 1995, Gibson and White 1990, Holcomb and White 1990, Leighton 1985, Leighton 1986, Leighton 1993, Tseng 1999, Tully et al. 1996, Yamato et al. 1996). Rehabilitation of acutely oiled seabirds is popular, but the results of cleansing and releasing oiled seabirds is not certain (Sharp 1996, Clark 1978). Because of the complexity of the question, relatively little research has been done on the long-term sequelae of acute exposure to oil or to chronic exposure to low levels of petroleum hydrocarbons. The primary targets of oil toxicity in birds, including seaducks, are the peripheral erythrocytes, with a resulting Heinz-body hemolytic anemia (Leighton 1983, Yamato 1996). The effect of low level, chronic exposure to hydrocarbons is undoubtedly complex, and may involve secondary effects, such as additive stress, suppressed immunity, and decreased reproduction (Leighton 1993). Rocke et al. (1984) found that ingestion of crude oil caused decreased resistance to infection by Pasteurella multocida caused by an impaired cellular response. A higher than expected prevalence of Plasmodium infection was found in oil-contaminated common murres (Uria aalgae); the involvement of the oil in the expression of the infection was not certain (Roertgen 1990).

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Table 1. Normal reference intervals and extreme observations for hematology and serum chemistry values of free ranging, molting female harlequin ducks taken from unoiled areas of eastern Prince William Sound, Alaska.

				Fractiles			
Parameter	Units	n	Median	0.275	0.975	Extremes	
Hematology							
White blood cell count	$10^3/\mu L$	72	14.0	6.60	30.0	6.60	32.0
Red blood cell count	$10^6/\mu L$	45	3.17	2.15	4.74	2.15	4.74
Packed cell volume	%	71	54	44	64	42	65
Mean corpuscular volume	fL	45	175	105	223	105	223
Hemoglobin	g/dL	45	15.7	8.6	20.1	8.6	20.1
Mean corpuscular hemoglobin concentration	g/dL	45	28.0	17.0	38.0	17.0	38
Azurophils	%	72	0	0	0	0	0
Bands	%	72	0	0	0	0	0
Heterophils	%	72	77	54	90	51	91
Lymphocytes	%	72	21	10	45	6	47
Monocytes	%	72	9	0	0	0	9
Eosinophils	%	71	1	0	7	0	9
Basophils	%	72	1	0	5	0	5
Serum Chemistry							
Sodium	mEq/L	30	155	138	202	138	202
Potassium	mEq/L	31	2.8	2.0	5.0	2.0	5.0
Gamma glutamyltransferase	IU/L	31	9	0	18	0	18
Alkaline phosphatase	IU/L	31	372	88	1020	88	1020
Calcium	mg/dL	72	10.7	8.50	14.2	8	14.6
Creatine phosphokinase	U/L	72	1121	366	4252	136	4968
Glucose	mg/dL	72	339	267	486	263	497
Lactic dehydogenase	IU/L	71	359	146	902	123	1000
Phosphorus	mg/dL	30	4.8	2.0	8.4	2.0	8.4
Aspartate aminotransferase	IU/L	71	64	16	163	12	195
Total protein	g/dL	72	3.65	2.60	5.40	2.50	6.40
Uric acid	mg/dL	72	9.7	4.0	16.8	4.0	16.9
Haptoglobin		68	90.5	34.9	235	24.4	257

Table 2. Comparison of hematology and serum chemistry variables of harlequin ducks captured from the unoiled and oiled areas of Prince William Sound, Alaska.

	Unoiled Area				Oiled Area			
Parameter	n	Median	Extremes		n	Median	Extremes	
Hematology								
Total white blood cell count ($10^3/\mu L$)	80	13.7	6.60	32.0	87	12	5	32
Total red blood cell count ($10^6/\mu L$)	48	3.17	2.15	4.74	51	2.85[a]	2.01	3.68
Packed cell volume (%)	79	54	42	64	86	55	5.0	69
Mean corpuscular volume (fL)	48	172.5	105	223	51	177	149	233
Hemoglobin (g/dL)	48	15.80	8.60	19.9	50	14.20	7.5	20.3
Mean corpuscular hemoglobin concentration (g/dL)	48	28.50	17	38	50	26.50	15	48
Azurophils (%)	80	0	0	0	88	0	0	0
Bands (%)	80	0	0	0	88	0	0	0
Heterophils (%)	80	76.5	51	91	87	77	47	93
Lymphocytes (%)	80	22	6.0	47	87	17	4	52
Monocytes (%)	80	0	0	9	87	0	0	4
Eosinophils (%)	79	1	0	14	87	2	0	24
Basophils (%)	79	1	0	5	87	1	0	6
Serum Chemistry								
Sodium (mEq/L)	37	155	135	202	42	161.5[a]	146	388
Potassium (mEq/L)	39	2.70	1.10	5	44	2.45	1.10	7.90
Gamma glutamyltransferase (IU/L)	39	9.0	0	18	44	11	3.00	19
Alkaline phosphatase (IU/L)	39	316	88	1200	44	305.5	16	1592
Calcium (mg/dL)	80	10.85	8	14.80	88	10.55	7.60	14.20
Creatine phosphokinase (U/L)	80	1074	136	4968	88	925.5	235	5556
Glucose (mg/dL)	80	336.5	153	497	88	376.5[a]	162	496
Lactic dehydogenase (IU/L)	80	363.5	123	1550	88	349	126	1916
Phosphorus (mg/dL)	39	4.80	1	15.60	44	4.80	2.4	11.6
Aspartate aminotransferase (IU/L)	80	65.5	12	379	88	58	17	522
Total protein (g/dL)	80	3.60	2.30	6.40	88	3.8	2.4	13.3
Uric acid (mg/dL)	80	9.75	4.0	16.9	88	10.5	0.9	21.2
Haptoglobin	76	97	24.4	257	83	102.8	21.6	236

[a] Value significantly different than value for same parameter among harlequin ducks from the unoiled areas ($P < 0.002$).

APPENDIX HD-08

CYTOCHROME P450 1A INDUCTION IN SeaduCKS INHABITING NEARSHORE AREAS OF PRINCE WILLIAM SOUND, ALASKA[1]

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Abstract

Following the *Exxon Valdez* oil spill, hepatic rates of EROD activity and thus, P450 1A expression were significantly higher in harlequin ducks (*Histrionicus histrionicus*) and Barrow's goldeneyes (*Bucephala islandica*) from oiled areas of Prince William Sound, Alaska, when compared to birds from unoiled sites. Polychlorinated biphenyl exposure did not account for area differences in P450 1A induction in harlequin ducks. Background hydrocarbon levels in Prince William Sound were negligible prior to the 1989 oil spill, but remnant *Exxon Valdez* oil was still present in nearshore habitats of the spill zone coincident with our study. We conclude that P450 1A induction in seaducks from areas oiled during the *Exxon Valdez* oil spill was likely due to exposure to residual oil. We speculate that biochemical and physiological changes in individuals chronically exposed to oil may be constraining population recovery of some seaduck species affected by the spill.

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Introduction

In 1989, the *Exxon Valdez* ran aground on Bligh Reef, spilling 11,000,000 gallons of crude oil into Prince William Sound (PWS), Alaska. Subsequent wind and ocean currents spread the oil southwest through western PWS and along the Kenai and Alaska peninsulas and the Kodiak Archipelago. As much as 40% of the spilled oil was deposited in intertidal and subtidal habitats of PWS (Galt et al., 1991; Wolfe et al., 1994), and some residual oil was still present in these habitats during the course of our study (Hayes and Michel, 1999). These nearshore environments are important for large numbers of vertebrates including molting and wintering waterfowl (Lance et al., 1999). Immediate postspill oil contamination caused acute mortalities of thousands of birds (Piatt et al., 1990), and concerns that continuing long-term oil exposure could be affecting avian populations remain. Exposure to oil through contaminated sediments or prey items could potentially elicit adverse physiological changes in birds (Leighton, 1993; Jenssen, 1994), which in turn, could have demographic consequences (e.g., Holmes et al., 1978, 1979) for the population. Populations of some species of birds, including harlequin ducks (*Histrionicus histrionicus*), have not fully recovered in areas of PWS affected by the oil spill (*Exxon Valdez* Oil Spill Trustee Council, 1999). Individuals may continue to be exposed to residual oil, and making that determination is important in understanding mechanisms constraining full recovery of bird populations.

Directly measuring oil constituents in bird tissues does not accurately reflect exposure to xenobiotic parent compounds (Lee et al., 1985). Polycyclic aromatic hydrocarbons (PAHs) are constituents of oil that, upon ingestion, are rapidly metabolized, thereby, making it difficult to determine the chemical structure of the original compound. One of the most sensitive and specific biochemical measurements for assessing exposure to PAHs is the induction of cytochrome P450 (P450), mixed-function oxygenase (MFO) systems (Woodin et al., 1997). Certain PAHs induce P450 responses, therefore measuring resultant enzyme production or activity can indirectly indicate exposure to oil constituents. For example, Woodin et al. (1997) measured P450 induction (specifically, the CYP 1A gene family) in intertidal fish collected from the field and from cages at various sites in PWS one year after the *Exxon Valdez* oil spill. They determined that P450 1A induction in fish from sites impacted by oil was significantly higher when compared to fish from areas unaffected by oil.

In this study, we measured P450 1A responses in harlequin ducks and Barrow's goldeneye (*Bucephala islandica*) from oiled and unoiled areas of PWS, eight to nine years after the spill, to assess potential continuing exposure of these seaduck species to *Exxon Valdez* oil. Due to their occurrence in nearshore habitats and consumption of benthic invertebrate prey, harlequin ducks and Barrow's goldeneyes are particularly susceptible to continued exposure to residual *Exxon Valdez* oil and, thus, are potentially vulnerable to subsequent physiological and population-level effects. In addition to oil-derived PAHs, certain polychlorinated biphenyl (PCB) congeners can induce cytochrome P450 systems. Therefore, we also measured congener-specific PCB concentrations in plasma from harlequin ducks overwintering in PWS to compare with P450 1A enzyme activity.

Methods

Field Collections

Barrow's goldeneyes and harlequin ducks were sampled from oiled and unoiled parts of PWS (Fig. 1) from 1996 through 1998. Samples from oiled sites were collected throughout the spill area. Samples also were collected from Montague Island, which was selected as an unoiled study site due to its proximity to the spill zone, thus limiting any geographic effects not related to the *Exxon Valdez* oil spill.

Barrow's goldeneyes were collected during December 1996 and February 1997 by shotgun from oiled Knight Island (Bay of Isles and Herring Bay) and unoiled Montague Island study areas (Fig. 1). Liver samples were collected to assess P450 induction by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity. Immediately upon retrieval of each carcass (within 10 minutes), approximately one gram of liver was dissected, wrapped in aluminum foil, and placed into liquid nitrogen.

Harlequin ducks were captured during March and April 1998, using a modified floating mist net trap (Kaiser et al., 1995) at Montague Island and (oiled) Crafton Island and Main Bay study sites (Fig. 1). Captured birds were placed under Isoflourane® anesthesia and livers were surgically biopsied to obtain a small (0.07 - 0.22 g, mean= 0.11 g) sample for EROD analysis. Immediately following biopsy, liver samples were placed in a cryogenic vial and frozen in liquid nitrogen. Following recovery from surgery, animals were released.

Three ml blood samples were collected into sodium heparinized glass evacuated tubes from each harlequin duck prior to surgery using 23 gauge, 1" needles and 5 cc syringes. Blood samples were centrifuged at approximately 1500 x g for 5 min, and plasma was decanted into 2 ml polypropylene microcentrifuge tubes. Plasma was frozen for biochemical and PCB congener analyses.

Laboratory Analyses

<u>EROD</u> <u>Activity</u>

Liver samples frozen in liquid nitrogen were shipped to Woods Hole for subsequent preparation and analysis. Individual liver pieces were homogenized in 7 ml final volume homogenizing buffer (0.05 M Tris, 0.15 M KCl, pH 7.4), and microsomes were sedimented by differential centrifugation as described previously (Stegeman et al., 1979). Microsomes were resuspended in approximately 2 ml per g tissue with resuspension buffer (0.05 M Tris, 0.1 mM EDTA, 1 mM DTT, 20% v/v glycerol, pH 7.4). Protein was determined in a 96 well plate using the micro-procedure of Smith et al. (1985).

7-Ethoxyresorufin-O-deethylase, the catalytic function of hydrocarbon-inducible CYP 1A, was measured using a kinetic modification of the plate-based assay of Kennedy et al. (1993). EROD activity was determined in duplicate in a 48 well plate at 20^0 C using a Cytofluor® fluorescent plate reader (Millipore, Bedford, MA). Each well contained 200 µl consisting of 1 µl of microsomes (4-15 µg protein), 2 µM 7-ethoxy resorufin in 50 mM Tris buffer, 0.1 M NaCl, pH = 7.8. Catalytic activity was initiated by the addition of NADPH in buffer to a final 1.67 mM

concentration. Fluorescence was determined at 1 min intervals over 6 min, and the linear slope (fluorescence per minute) was divided by the slope of the resorufin product standard curve (fluorescence per pmol) determined under the same conditions to yield pmol per minute per mg protein catalytic rates.

PCB Analysis

Harlequin duck plasma samples were analyzed for total PCB concentration and congener-specific concentrations of 93 congeners, including 12 known to induce P450 1A. To achieve a minimum sample volume of 0.5 ml, some samples were pooled based upon EROD values and capture sites (Table 1).

Plasma samples were prepared and analyzed using modified methods of Shoda (1997). Approximately 0.5 ml plasma was mixed with 5 ml hexane:diethylether (1:1) and shaken briefly. Two ml methanol was added, and the combined sample was mixed, shaken vigorously (by hand) and centrifuged for approximately 10 min. The extraction was repeated two more times with 5 ml hexane:diethylether. The combined hexane:diethylether extracts were concentrated to approximately 1 ml under a gentle stream of nitrogen. Sample clean-up was performed by passing the extract through a pasture pipette column containing (from bottom to top) glass wool, sand, silica gel, alumina and anhydrous sodium sulfate. The column was sequentially eluted with 5 ml of hexane and 10 ml of methylene chloride. The eluent was concentrated to 0.5 ml for analyses. Quantitative analyses were performed by capillary gas chromatography (CGC) with electron capture detector for PCBs (Wade et al., 1988). Some PCB congeners, including 114 and 157, co-elute during CGC and are indistinguishable by electron capture detection. These combined peaks were analyzed using a mass spectrometer detector in the SIM mode.

Statistical Analyses

All statistical analyses were conducted using SAS (SAS Institute Inc., Cary, North Carolina, USA). For each duck species, EROD activity was compared between areas using Student's T-test. For the PCB analysis we compared proportions of observations that were above the detection limits between areas (oiled vs unoiled) using Fisher's Exact test. For each area we had 10 samples and 93 congeners, thus the test compared numbers of positive values (above limit of detection) per 930 possible. We conducted the same analysis using only the 12 congeners known or suspected of inducing P450 1A (congeners 77, 105, 118/108/149, 126, 128, 138, 141, 156/171/202, 158, 167, 169, and 189). Multiple regression analysis was used to simultaneously assess effects of sample area and concentrations of specific PCB congeners on EROD activity.

Results

EROD Activity

Rates of EROD activity in Barrow's goldeneye liver samples averaged higher in birds from oiled Knight Island (94.3 pmol/min/mg protein; $n = 22$) than in those from Montague Island (49.5 pmol/min/mg protein; $n = 19$; $P = 0.0014$; Fig. 2). Hepatic EROD activities of wintering

harlequin ducks also were higher in samples from oiled areas (204.6 pmol/min/mg protein; $n = 19$) than in those from unoiled Montague Island (70.7 pmol/min/mg protein; $n = 18$; $P < 0.001$; Fig. 3).

PCB Analysis

Total PCBs were not measured above detection limits in any harlequin duck plasma sample; detection limits ranged from 0.03 to 0.13 ppm (averaged = 0.07 ppm; oiled areas = 0.07 ppm; unoiled areas = 0.06 ppm). Total PCB and congener concentrations are expressed on a wet weight basis and are not normalized to lipid.

Congener-specific analyses had lower detection limits, ranging from 0.14 to 0.50 ppb. Average congener-specific detection limits were 0.29 ppb for oiled areas, 0.24 ppb for unoiled areas, and 0.26 ppb overall. Of the 93 PCB congeners analyzed, concentrations measured above detection limits occurred in 8.9% of possible instances in birds from oiled areas and 11.9% in birds from unoiled areas. Frequency of values above detection limits was slightly higher at unoiled areas ($P = 0.04$). For congeners suspected of inducing P450 1A in birds, frequencies of observations above detection limits did not differ ($P = 0.82$) between oiled areas (8.3%) and unoiled areas (10.0%).

PCB congener 138 was measured above detection limits in all samples (range = 0.30 to 11.4 ppb), although concentrations did not differ ($P = 0.80$) between oiled (2.15; $n = 10$) and unoiled (1.79; $n = 10$) areas. In a multiple regression analysis, congener 138 concentration was positively related to EROD activity ($F = 53.86$, $P < 0.001$). However, after accounting for variation due to congener 138, birds from oiled areas had considerably higher EROD activity than those from unoiled areas ($F = 19.98$, $P < 0.001$) suggesting that congener 138 concentrations may influence P450 activity, but oiling history explained significant variation after accounting for any effect of congener 138. The relationship between congener 138 concentration and EROD activity was driven by 4 samples (1 from unoiled areas, 3 from oiled) with higher congener 138 values (Fig. 4). Without those samples in the model, there was no relationship between congener 138 and EROD activity, although the term for area was still highly significant ($F = 10.00$, $P = 0.008$).

Discussion

Cytochrome P450 1A activity was significantly higher in harlequin ducks and Barrow's goldeneye from areas of PWS originally impacted with *Exxon Valdez* oil than in birds from unoiled areas. Considerable evidence indicates that PAHs from residual *Exxon Valdez* oil were likely responsible for elevated EROD activities in seaducks and several other vertebrates in oiled areas of PWS (Marty et al., 1997; Woodin et al., 1997; Holland-Bartels, 1998); this suggests that some species of seaducks were still vulnerable to potential deleterious effects of oil exposure as long as 9 years following the oil spill.

Potential Sources of P450 1A-Inducing Compounds

Sources of P450 1A- inducing PAHs in PWS, other than oil from the *Exxon Valdez*, could include natural oil seeps and oil released in Valdez, Alaska, during the 1964 earthquake. However, Short and Babcock (1996) concluded that PAH concentrations in intertidal sediments and mussel (*Mytilus trossulus*) tissues were negligible in PWS immediately prior to the *Exxon Valdez* oil spill. Low concentrations of background hydrocarbons were detected in deep (> 100 m) benthic samples (Short et al., 1999), however, harlequin ducks and Barrow's goldeneyes are not deep foragers. Furthermore, the source of these deep sediment hydrocarbons are coal deposits in eastern PWS, which are not bioavailable (Short et al., 1999) and therefore, cannot induce P450 1A responses from biota. We conclude that background or natural hydrocarbon sources do not explain observed differences in P450 1A induction in seaducks between oiled and unoiled areas of PWS.

Other compounds potentially leading to P450 1A induction are certain PCB congeners. PCBs are ubiquitous throughout the environment, and several congeners are presumed to mediate their toxicity through the aryl-hydrocarbon (Ah) receptor, thereby inducing the CYP 1A gene family (Rattner et al., 1994). The most toxic PCB congeners and therefore, the most potent CYP 1A inducers are three planar congeners, 77, 126 and 169. These congeners were not measured above the limit of detection in any harlequin duck plasma sample, however, all samples contained measurable concentrations of PCB 138 (2, 2', 3, 4, 4', 5' hexachlorobiphenyl). PCB 138 is a di-ortho chlorine substituted analog of the more toxic planar PCB congeners. The two ortho-chlorine substitution decreases the planarity and toxicity of the congener, thereby reducing its potency as a CYP 1A inducer. In fact, the di-ortho analogs are thought to be 0.0001-0.00001 as toxic as the most potent CYP 1A inducer, 2,3, 7,8-TCDD (dioxin) (Safe, 1990).

PCB 138 is one of the most ubiquitous congeners measured in avian species. In Britain tissue analyses from 8 species, including sea birds, raptors and herons, indicated that congeners 138, 153 and 180 were most prevalent (Boumphrey et al., 1993). Threshold concentrations of PCB 138 in duck plasma necessary to induce CYP 1A expression are unknown, however concentrations reported here are low. Concentrations of PCB 138 in black-crowned night heron (*Nycticorax nycticorax*) embryos from a non-industrial reference site (Chincoteague National Wildlife Refuge, VA) had mean values of 7 ppb compared to 77 ppb from a contaminated site (Cat Island, Green Bay, WI) (Rattner et al., 1994). Hepatic EROD activity was 20-fold higher in herons from Cat Island than Chincoteague and positively correlated with total PCB concentrations; however, the contribution of individual congeners to EROD activity was unknown. The relationship between sample tissue type, PCB congener concentrations, and EROD induction has not been researched in birds. However, distribution of congeners in different tissue types appears to be consistent among 16 tissues measured in 3 waterbird species. For each bird, the relative contribution of individual congeners to total PCB concentrations was the same in each organ, although there were differences in total amount of PCBs among tissue type (Boumphrey et al., 1993).

Congener 138 concentration in harlequin duck blood plasma may explain some variation in EROD activity; the four samples with highest congener 138 concentrations also had highest EROD activity. However, this relationship was not sufficient to explain area differences in

EROD activity. Mean concentrations of congener 138 did not differ by area. Moreover, excluding the four samples with the highest congener 138 concentrations eliminated the positive relationship between EROD and congener 138 concentration. However, even this reduced data set showed dramatically different EROD activities between harlequin ducks from oiled and unoiled areas of PWS.

Vulnerability to Continued Oil Exposure

Life history characteristics of harlequin ducks and Barrow's goldeneyes make them particularly susceptible to continued oil exposure and, thus, any subsequent population-level consequences of exposure. These seaduck species occur in intertidal and shallow subtidal habitats in the nearshore environment, which are the same areas that received much of the oil spilled from the *Exxon Valdez* (Galt et al. 1991; Wolfe et al., 1994). In 1992, it was estimated that 15% of the oil spilled from the *Exxon Valdez* (1.65 million gallons) remained in intertidal shorelines and subtidal sediments (Wolfe et al., 1994). Much of this remnant oil was in sheltered bays or beneath beach surfaces (Hayes and Michel, 1999) thus inhibiting further weathering and dispersal. The continuous, but slow degradation of these remaining oil deposits makes continued oil exposure of birds that inhabit these areas plausible.

During winter, harlequin ducks and Barrow's goldeneyes feed almost exclusively on benthic invertebrates (Koehl et al., 1982; Vermeer, 1982; Goudie and Ankney, 1986; Goudie and Ryan, 1991). In the marine environment, bottom sediments and subsequently, benthic invertebrates, are often the final destination for oil constituents (Woodin et al., 1997). Benthic invertebrates do not rapidly metabolize PAHs (Boehm et al., 1996), so ingestion of contaminated prey could continually expose seaducks to low concentrations of oil which could, in turn, induce P450 1A responses. Mussels, a dietary component of both seaducks, in the oil spill zone had negligible concentrations of PAHs prior to the spill; however, accumulation of *Exxon Valdez* oil occurred in mussels throughout the spill-affected area (Short and Babcock, 1996). Similarly, other studies have also documented hydrocarbons in seaduck prey from immediately post-spill through 1995 (Patten et al., 1998; Babcock et al., 1996), suggesting that contaminated prey are a potential source of oil ingestion.

Potential Physiological and Population Consequences of Oil Exposure

Petroleum products are toxic to birds (see reviews by Leighton, 1993 and Leighton et al., 1985). Oil and oil-derived products can damage red blood cells, restrict uptake of nutrients, alter hormone balances, suppress the immune system, inhibit growth, and impair reproduction.

Polycyclic aromatic hydrocarbons are known to induce hepatic EROD activity in herring gulls (*Larus argentatus*) (Lee et al., 1985; Peakall et al., 1989), mallards (*Anas platyrhynchos*) (Gorsline and Holmes, 1981) and starlings (*Sturnus vulgaris*) (Trust et al., 1994). However, it is unclear whether PAH-induced P450 1A activity in birds causes additional toxicological effects (Leighton, 1993). Correlations have been made between early embryonic death and PAH content in crude oil applied to duck eggs (Hoffman, 1979). Additionally, Lee et al. (1986) demonstrated increased mortality with concomitant induction of P450 activity when minute amounts of Prudhoe Bay crude oil were applied to chicken eggs. However, they were uncertain whether

metabolism, and subsequent induction of MFO enzymes were necessary for toxicity. The metabolism of PAHs by the MFO system can produce highly reactive intermediate compounds that interact with other cellular constituents and cause the initiating event leading to mutagenesis or carcinogenesis (Fox, 1993). In laboratory mammals, compounds that bind to the Ah receptor and induce P450 1A responses also cause weight loss, promotion of tumors and immunotoxicity (Fox, 1993).

Oil ingestion and, particularly, external oiling of feathers can have severe metabolic consequences (Jenssen, 1994). Oil disrupts feather structure, reduces insulative properties of feathers, and can result in hypothermia and death. This is the main cause of immediate mortalities of marine birds following oil spills. However, even small amounts of external oil can increase costs of thermoregulation, thus metabolic costs of external oiling could be incurred as long as environmental oil is present. In PWS, oil sheening was observed as late as 1997 from beaches heavily oiled by the *Exxon Valdez* spill (Hayes and Michel, 1999), suggesting that external oiling and subsequent metabolic consequences for birds inhabiting nearshore environments are possible.

Potential physiological consequences of oil exposure could have population-level effects on seaducks. Many lab studies have suggested that oil exposure doesn't have toxic effects on waterfowl (almost always mallards) until high doses are ingested (Stubblefield et al., 1995). Such studies have been used to suggest that harlequin ducks should, similarly, be unaffected by residual *Exxon Valdez* oil spill (Boehm et al., 1996). However, these studies have typically been conducted for relatively short periods (weeks) under benign laboratory conditions. Other studies have documented that oil exposure is a physiological stressor that may not have toxic or demographic consequences in the absence of other stresses; however, with addition of other stressors such as cold temperatures, oiled ducks in the lab suffered considerably higher mortality than unoiled birds (Holmes et al., 1978; 1979). This may be a much more appropriate paradigm for wild seaducks chronically exposed to oil.

Data collected on harlequin ducks following the *Exxon Valdez* oil spill continue to demonstrate population-level effects from oil. Numbers of harlequin ducks surveyed during wing molt declined in oiled portions of Prince William Sound during 1995 to 1997, while populations were stable in unoiled areas (Rosenberg and Petrula, 1998). Winter survival of adult female ducks was lower in oiled areas compared to unoiled areas of PWS (D. Esler, unpubl. data); population model projections incorporating these survival rates matched the population trends observed by Rosenberg and Petrula (1998), suggesting that survival differences were responsible for observed population trends. Goudie and Ankney (1986) suggested that harlequin ducks were on the lower extreme of seaduck body mass necessary for surviving subarctic winters. Under predictable, natural conditions harlequin ducks should have high winter survival. However, harlequin ducks exist close to an energetic threshold, and survival rates may be compromised by even small physiological challenges. We acknowledge that links between oil exposure and population-level effects are speculative, but argue that these links are reasonable based on available information. We conclude that full recovery of some seaduck populations impacted by the *Exxon Valdez* oil spill may be constrained by exposure to residual oil and encourage further research on the mechanisms by which oil exposure may impact wild bird populations.

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TABLE 1. Harlequin duck blood serum samples used for PCB analysis, sorted by area and EROD activity.

Site	EROD Activity[a]	Sample ID	Avg. EROD[b]
Unoiled Area (Montague Island)			
Port Chalmers	4.0	M1	9.6
Stockdale Harbor	7.3	M2	14.55
Port Chalmers	15.2	M1	
Stockdale Harbor	21.8	M2	
Stockdale Harbor	24.0	M3	24.00
Stockdale Harbor	26.2	M4	27.85
Stockdale Harbor	29.5	M4	
Stockdale Harbor	30.3	M5	31.80
Stockdale Harbor	33.3	M5	
Stockdale Harbor	34.6	M6	40.80
Stockdale Harbor	47.0	M6	
Stockdale Harbor	48.9	M7	66.77
Stockdale Harbor	67.0	M7	
Stockdale Harbor	84.4	M7	
Stockdale Harbor	102.6	M8	102.60
Stockdale Harbor	141.5	M9	155.45
Stockdale Harbor	169.4	M9	
Stockdale Harbor	386.4	M10	386.40
Oiled Area			
Crafton Island	92.1	K1	97.65
Crafton Island	103.2	K1	
Main Bay	123.7	K2	123.70

Site	EROD Activity[a]	Sample ID	Avg. EROD[b]
Crafton Island	133.6	K3	139.30
Crafton Island	145.0	K3	
Crafton Island	156.8	K4	164.90
Crafton Island	173.0	K4	197.57
Crafton Island	179.9	K6	181.90
Main Bay	181.9	K5	
Crafton Island	195.2	K6	
Crafton Island	217.6	K6	283.50
Main Bay	263.6	K7	
Main Bay	303.4	K7	
Crafton Island	329.6	K8	353.15
Main Bay	368.5	K9	368.50
Crafton Island	376.7	K8	
Main Bay	n/a[c]	K10	

[a]pmol/min/mg protein
[b] Each line represents an individual bird; lines with common sample numbers were pooled to achieve minimum volume for analysis. Pooling was conducted based on EROD activity and site. Average EROD of pooled samples is presented at the first occurrence of each sample number.
[c]Recaptured bird with implanted radio; liver biopsy not collected.

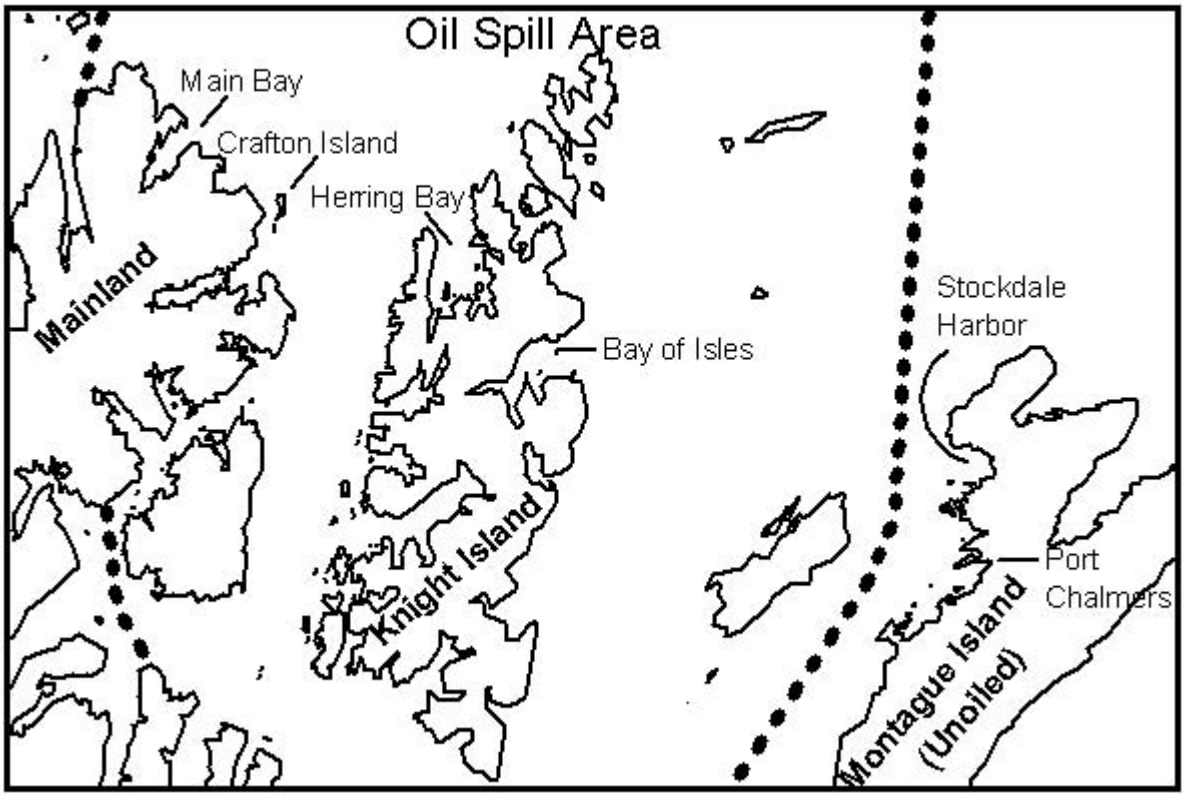

Figure 1. Oiled and unoiled areas of Prince William Sound, Alaska, used as sampling sites to measure hepatic P450 1A induction in harlequin ducks and Barrow's goldeneyes. The area bounded by bold, dotted lines is the area affected by the oil spill.

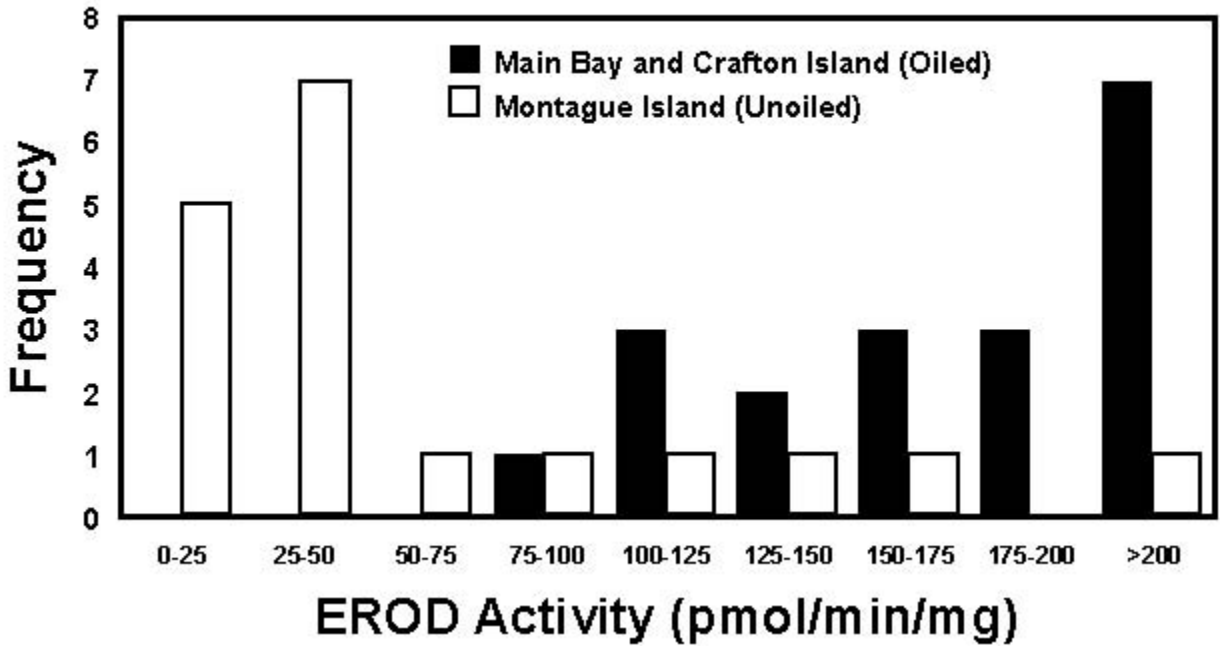

Figure 2. Comparisons of hepatic EROD activity of Barrow's goldeneyes collected from oiled and unoiled areas of Prince William Sound, Alaska.

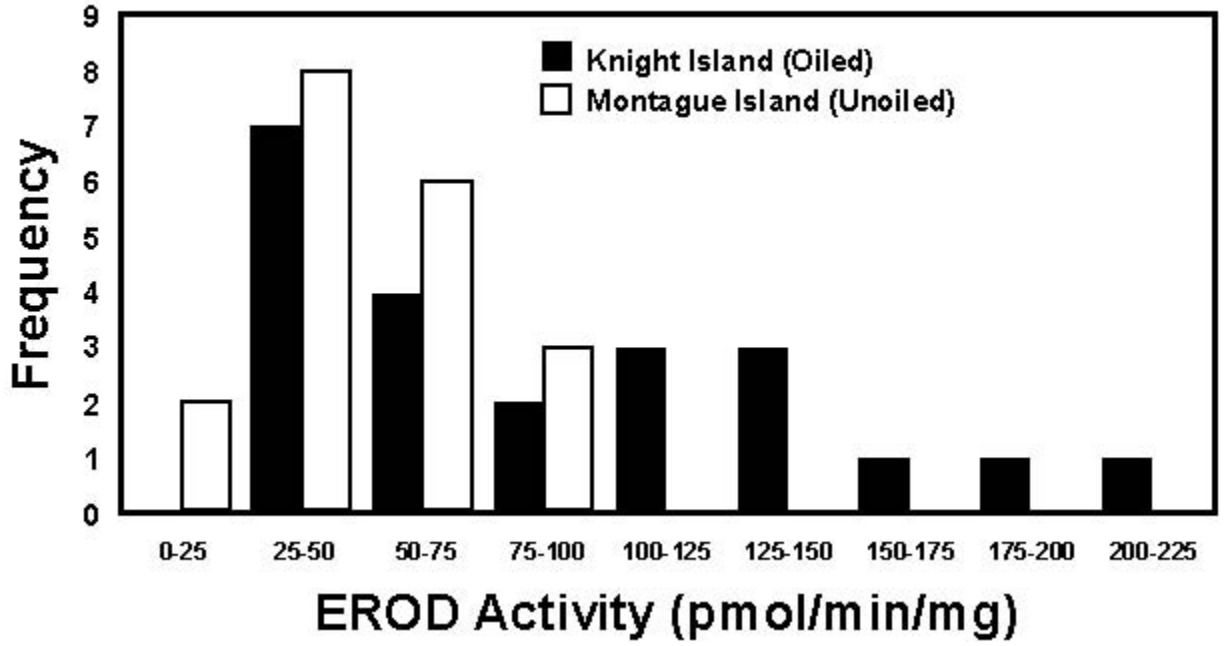

Figure 3. Comparisons of hepatic EROD activity of harlequin ducks captured from oiled and unoiled areas of Prince William Sound, Alaska.

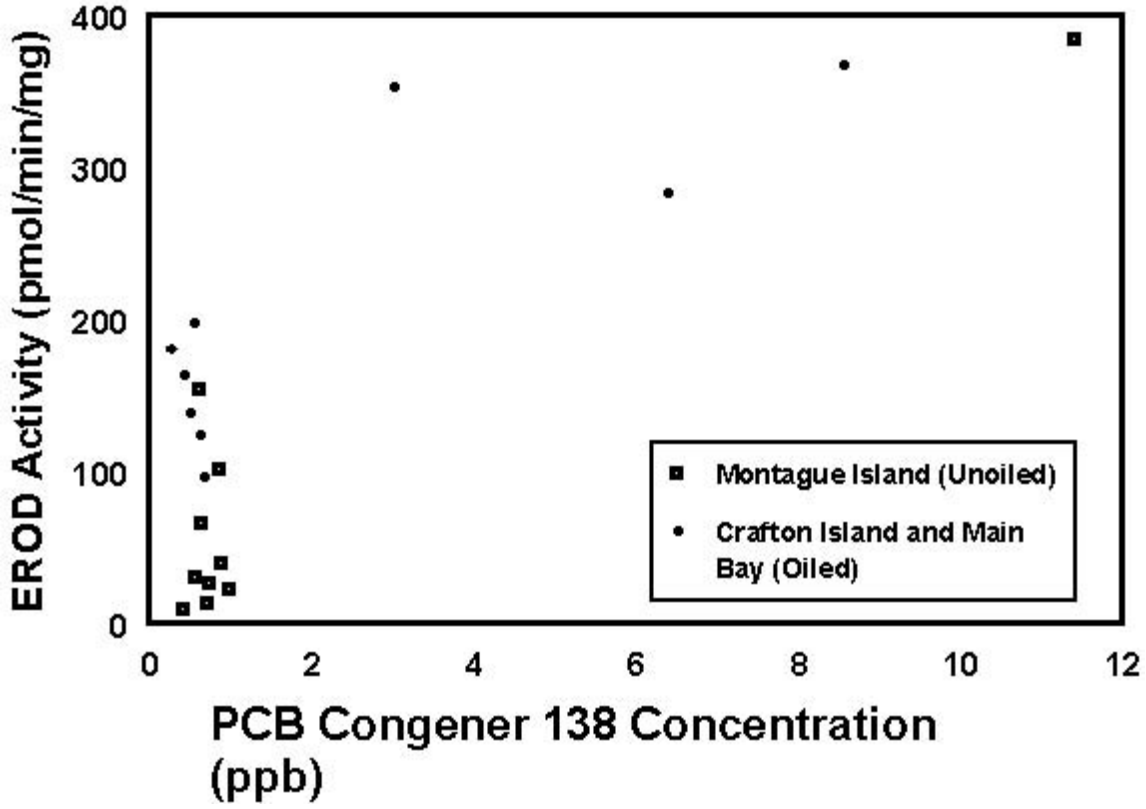

Figure 4. Scatterplot of EROD activity by concentrations of PCB congener 138 in blood plasma of harlequin ducks captured in oiled and unoiled areas of Prince William Sound, Alaska.

River Otter (*Lontra canadensis*) Appendix

(RO)

This River Otter Appendix illustrates the various published works on river otter that were funded in part by the NVP study.

RO-01 Ben-David, M., R. T. Bowyer, L. K. Duffy, D. D. Roby, and D. M. Schell. 1998. Social behavior and ecosystem processes: River otter latrines and nutrient dynamics of terrestrial vegetation. Ecological Society of America. Ecology 79(7):2567–2571.

Abstract: River otters (*Lutra canadensis* Schreber) inhabiting coastal environments scent-mark specific locations along the coast, known as latrine sites. In this study, we used stable isotope techniques to investigate the effects of this scent-marking behavior on terrestrial vegetation at the terrestrial-marine interface. Our analysis of stable isotope ratios of fur and feces indicated that river otters fed mainly on intertidal and subtidal fish. Eight different species of plants, growing in latrine sites of river otters, had significantly higher values of delta^{15}N compared with the same plant species growing on nonlatrine sites. Elevated N concentrations occurred only in grasses and mosses growing in latrine sites. Our results indicate that, through their scent-marking behavior, coastal river otters transfer marine-derived nitrogen into the beach-fringe forest and thus fertilize the terrestrial vegetation in the terrestrial-marine interface.

RO-02 Blajeski, A., L. K. Duffy, and R. T. Bowyer. 1996. Differences in faecal profiles of porphyrins among river otters exposed to the *Exxon Valdez* oil spill. Biomarkers 1:262–266.

Abstract: River otters (*Lutra canadensis*) living in marine environments of Prince William Sound, Alaska, exposed to crude oil from the *Exxon Valdez* spill in March 1989, showed significantly elevated levels of faecal porphyrin over those of otters from non-oiled areas (oiled mean = 48.2 and non-oiled mean = 34.5 nmol g^{-1} dry faeces). Profiles of uro-, hepta-, hexa-, penta-, copro-, and protoporphyrin profiles were qualitatively characterized by high-performance liquid chromatography. These findings suggest that river otters may serve as a suitable indicator species in which porphyrin profiles can be used to monitor the effects of marine and freshwater crude oil exposure. Also, this is the first model showing the effects of an oil spill on porphyrins on a free-ranging mammal using a non-lethal methodology. These effects were detectable 1 year after the spill and following a major effort to clean oil from the shorelines of Prince William Sound.

RO-03 Blundell, G. M., R. T. Bowyer, M. Ben-David, T. A Dean, and S. C. Jewett. 2000. Effects of food resources on spacing behavior of river otters: Does forage abundance control home-range size? Proceedings of the 15th International Symposium on Biotelemetry. (In press)

Abstract: We use three analytical techniques to examine home-range dynamics of river otters in Prince William Sound, Alaska, USA, from February 1997 to January 1998 and discuss problems with analysis of linear home ranges. River otters inhabiting marine environments where fish were abundant had smaller home ranges than animals living in freshwater systems with fewer prey, whereas otters using multiple salmon runs had larger home ranges than otters in other habitats.

RO-04 Blundell, G. M., J. W. Kern, R. T. Bowyer, and L. K. Duffy. 1999. Capturing river otters: A comparison of Hancock and leg-hold traps. Wildlife Society Bulletin 27 (1):184–192.

Introduction: The ability to live-capture study animals is essential to many research and management programs (Schemnitz 1994). An efficient and effective method to live-capture river otters (*Lontra canadensis*) is critical for the success of both theoretical (Ben-David et al. 1996, 1998) and applied studies (Erickson and McCullough 1987, Testa et al, 1994. Bowyer et al. 1995). Both Hancock and leg-hold traps have been used for such purposes. The number of trap nights required to capture a river otter in a Hancock trap ranged from 58 to 123 (Melquist and Hornocker 1983, Shirley et al. 1983, Woolington 1984). Rates of captures for various types of leg-hold traps ranged from 60 to 315 trap nights/otter captured (Shirley et al. 1983, Serfass et al. 1996).

Other potential differences between Hancock and leg-hold traps have been reported. Using Soft-catch[@] leg-hold traps resulted in a high rate of escape (43% of 51 potential captures; Serfass et al. 1996); modifications of the Hancock trap were thought to make it more efficient (Northcott and Slade 1976). However, the cumbersome size of Hancock live traps (95 × 59 × 40 cm; >11 kg) may limit the number of traps that can be transported efficiently or the locations or size of areas where those traps can be set appropriately. Moreover, river otter may learn to avoid capture in Hancock traps (Duffy et al. 1994a).

Few data are available on injuries to river otters from methods used to capture these large mustelids. Shirley et al. (1983) reported 16% of 30 river otters experienced a broken toe from being captured with an unpadded leg-hold trap, but most otters had only minor skin lacerations or suffered no injury. Serfass et al. (1996) compared injuries to otters from Softcatch[@] leg-hold traps with traps lacking padded jaws. Traps with padded jaws caused injury rates of 38% for canine teeth and 38% for appendages (n = 20 otters). Private trappers using unpadded traps caused much greater injuries to otters, but the types of traps and handling techniques varied markedly (Serfass et al. 1996). Hurbert et al. (1996) reviewed efficiency of traps to capture terrestrial carnivores and the injuries caused by those traps. No study has assessed injuries caused to otters by Hancock traps.

We evaluated capture success and injury rate for river otters live-captured in Hancock and unpadded leg-hold traps. We tested for differences in capture efficiency, rate of escape, rate of malfunction, and utility of those types of traps. Additionally, we tested for differences in severity and types of injuries to otters from Hancock and leg-hold traps.

RO-05 Duffy, L. K., M. K. Hecker, G. Blundell, and R. T. Bowyer. 1999. An analysis of the fur of river otters in Prince William Sound, Alaska: Oil-related hydrocarbons 8 years after the *Exxon Valdez* oil spill. Polar Biology 21:56–58.

Abstract: Approximately 8 years after the *Exxon Valdez* oil spill, river otters (*Lutra canadensis*) were trapped from the shoreline in both oiled (Knight Island) and nonoiled (Jackpot Bay) areas of Prince William Sound, Alaska. Captive river otters were wiped with isopropanol-soaked gauze and the gauze extracts were analyzed by gas chromatography with mass spectrometry detection. Differences in pentacosane (C-25) levels in the fur were observed between the oiled and nonoiled sites, while lower molecular weight aliphatics and aromatics were absent. These data are useful when evaluating the role of fur grooming in the long-term exposure of river otters to hydrocarbons and the expression of P450-1A in Knight Island otters.

RO-06 Hecker, M. K., L. K. Duffy, G. M. Blundell, and R. T. Bowyer. 1997. River otters as a sentinel species: Effect and detection of crude oil on the fur of river otters. Pages 100–102 *in* B. Jessup and J. Mazet, editors. Effects of oil on wildlife. Proceedings of the Fifth International Conference on Oil Spills.

Abstract: River otters (*Lutra canadensis*) have been used as a sentinel species in pollution studies throughout North America. A modified wipe test of river otter fur was developed to detect the presence of residual crude oil on the fur of river otters inhabiting Prince William Sound, Alaska. River otter pelts (both tanned and untanned) were used as models and exhibited differences in hair structure and absorption of crude oil. Immunochemical detection, as well as detection by mass spectrometry, after a methanol extraction of crude oil from the fur were compared. Our results showed that an immunoassay provides an inexpensive and reliable test for oil at concentrations greater than 1 ppm. Crude oil on the fur after extraction with methanol could also be detected in the 1 ppm range. Using the immunoassay wipe test, 17 river otters from oiled and nonoiled areas of Prince William Sound sampled in summer 1996 showed no detectable oil >1 ppm. Mass spectrometry can be used to increase the sensitivity of detection.

RO-07 Sauer, T. M., M. Ben-David, and R. T. Bowyer. 1999. A new application of the adaptive-kernel method: Estimating linear home ranges of river otters, *Lutra canadensis*. The Canadian Field-Naturalist 113. 6 pp.

Abstract: Standard techniques for estimating size of home range for semiaquatic mammals usually result in overestimating area because unused tracts of land and water are incorporated into calculations. For river otters (*Lutra canadensis*) that inhabited a narrow strip of habitat along the terrestrial-marine interface, linear length of shoreline previously was used as a measure of home-range size. Although that method produced a conservative estimate, selection of data points using that procedure did not provide any measure of probability or indication of core areas. We used the adaptive-kernel estimator and Geographic Information System for calculating linear length of home ranges for river otters inhabiting a marine environment and assessed the effect of reducing the bandwidth size on those home-range estimates. Using locations collected from four otters in Ester Passage, Prince William Sound, Alaska, USA, during summer 1991, we determined that adaptive kernel with 100% and 95% density contours resulted in a larger estimate than that produced by the previously used method. Decreasing bandwidth did not significantly alter the estimated linear distances of home range. In addition, the use of density contours of 65% delineated core areas; therefore, this technique provided a tool with which researchers can test hypotheses such as seasonal shifts in size and location of home ranges in relation to resource availability and distribution. Our technique may be useful for estimating home ranges of other animals approximating a linear distribution of locations.

Pigeon Guillemot (*Cepphus columba*) Appendix

(PG)

APPENDIX PG-01

MECHANISM OF IMPACT AND POTENTIAL RECOVERY OF PIGEON GUILLEMOTS (*CEPPHUS COLUMBA*) AFTER THE *EXXON VALDEZ* OIL SPILL[1]

A
THESIS

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Pamela Edith Seiser

Fairbanks, Alaska

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ABSTRACT

The abundance of pigeon guillemots in oiled areas of Prince William Sound, Alaska, failed to increase after the1989 *Exxon Valdez* oil spill. Population growth may be constrained by the physiological effects of oil exposure, food availability, and nest predation. I conducted a comparative study among unoiled, oiled, and pre-spill data sets, to provide insight on factors limiting population recovery in oiled areas. Blood samples from chicks in oiled and unoiled areas provided little evidence of physiological effects of exposure to oil. Pigeon guillemot diet, productivity, growth rates, and fledging weights in unoiled areas of southwestern Prince William Sound from 1994 to 1998 indicate oiled areas had a lower proportion of high-lipid fish in the chick diet and lower fledging weights, compared to unoiled and pre-spill studies. These results suggest that the lack of recovery in oiled areas is associated with a prey base that results in lower fledging weights, which may reduce juvenile survival.

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ACKNOWLEDGMENTS

My research was a component of a larger study, the Nearshore Vertebrate Predator Project, funded by the *Exxon Valdez* Oil Spill Trustees Council. I have many individuals to thank because my thesis included data from three separate study areas, Jackpot Island, Naked Island and Kachemak Bay, which in turn were supported by three different agencies: the Alaska Cooperative Fish and Wildlife Research Unit, the office of Migratory Bird Management of the U.S. Fish & Wildlife Service and the Alaska Biological Science Center of the U. S. Geological Survey, in Anchorage, Alaska.

First, I would like to thank my committee members: my major advisor Dr. Dave McGuire for his patient instructions in the art of technical writing; Dr. Larry Duffy for his advice on biomarkers; and Dr. Alan Springer for his insights on seabird ecology. I thank the unofficial members of my committee Dr. Dan Roby and Dr. Scott Newman DMV, for contributing their expertise on avian nutrition and physiology. Dr. Greg Golet and Lindsey Hayes collected blood samples from Naked Island and shared their insight on the recovery of pigeon guillemots. I thank *Cepphus* experts George Divoky and Alex Prichard for their helpful discussions. I would also like to acknowledge the contribution of Jackpot Island's 1994 and 1995 data set by Lindsey Hayes and Gail Blundell, respectively.

My study includes data collected over a five-year project by many hard working individuals. At Jackpot Island, they were Adrian Gall, Mike Grene, Phil Joy, Cynthia Restrepo, Kelsey Sullivan, Mike Walgren and Darcie Zeil. Ted Spencer of the Naked Island crew provided assistance and humor, as well as numerous other 'Naked boys and girls': Laura Ballock, Mary Cody, Bryan Duggan, Amy Hahn, Jim Hamon, Melissa Luanglue, Aly McKnight, Angela Palmer, Mark Russell, Scott Schaffer, Bev Short, Oliver Sternicki, Dave Tessler, and Ed Vorisek. At Kachemak Bay, Mike Litzow, April Nielsen, and Sadie Wright generously contributed their time and energy to collect blood samples for my project. Maps were produced by GIS expert and friend Debbie Nigro. Last, but not least, I thank my family, friends, and office mates for the encouragement and supported they provide through my graduate work.

1

CHAPTER ONE

OVERVIEW: THE RESPONSE OF PIGEON GUILLEMOTS TO THE 1989 *EXXON VALDEZ* OIL SPILL

OVERVIEW

In March of 1989, the oil tanker *Exxon Valdez* spilled 42 million L of crude oil into Prince William Sound (PWS). Between 100,000 to 375,000 birds were killed in the spill (Piatt *et al.,* 1990; Ford *et al.,* 1996; Piatt *et al.,* 1996). Negative impacts of the *Exxon Valdez* oil spill (EVOS) on the abundance and subsequent return of PWS avian species back into heavily oiled areas after the spill was still evident in pigeon guillemots, *Cepphus columba*, two years after the spill (Murphy *et al.,* 1997). The profound impact of EVOS on nearshore communities was related not only to the volume of the oil spill, but also to the fate of the spilled oil (Burger, 1993). Pigeon guillemots are tightly linked with the health of nearshore marine habitats (Ewins, 1993; Prichard, 1997). Among seabirds, pigeon guillemots are particularly vulnerable to contaminants in the vicinity of breeding colonies because of their limited foraging range during the chick rearing period (Ewins, 1993). Forty percent of EVOS oil was deposited on PWS shorelines in 1989 (Galt *et al.,* 1991). Despite beach cleaning efforts and natural biodegradation processes, Wolfe *et al*. (1994) estimated that 15% of the EVOS oil remained in intertidal and subtidal areas two years after the spill. The majority of prey base of pigeon guillemots is associated with subtidal and intertidal substrates (Ewins, 1993). While the greater portion of PWS avian communities demonstrated signs of population recovery 2 to 3 years after the spill (Wiens *et al.,* 1996), pigeon guillemot populations in oiled areas, such as Naked Island, have continued to decline below 1990 levels (Hayes and Kuletz, 1997).

From 1979 to 1981, Oakely (1981) and Kuletz (1983) studied the breeding and foraging ecology of Naked Island's pigeon guillemots. The *T/V Exxon Valdez* ran aground in 1989 within 30 km of Naked Island. The degree of oiling along the convoluted shoreline of Naked Island varied from negligible to heavy. When Oakely and Kuletz (1996) returned to Naked Island in 1990, they found 43% fewer pigeon guillemots as compared to their pre-spill censuses (1978 to 1981). Many researchers investigating the impact of EVOS on the PWS ecosystem pointed out that natural changes in the marine environment between pre-spill and post-spill studies may be as significant as the impact of EVOS (Oakely and Kuletz, 1996; Piatt and Anderson, 1996; Wiens and Parker, 1995).

Broad scale changes in climate and oceanographic conditions in the Gulf of Alaska (GOA) between the late 1970's and late 1980's resulted in a shift in the relative abundance and size classes of fish species (Royer, 1993; Piatt and Anderson, 1996; Anderson *et al.,* 1997; Fritz *et al.,* 1993). The Alaska Coastal Current brings GOA waters into PWS resulting in ocean conditions similar to GOA in the southern parts of

PWS (Niebauer *et al.*, 1994). There is evidence that this shift in forage fish species negatively affected GOA seabird survival and productivity. Black-legged kittiwakes, *Rissa tridactyla*, of the outer GOA colonies of Middleton Island, Semidi Islands, and Kodiak Island suffered numerous breeding failures in the 1980's and colony populations dropped in number (Hatch *et al.*, 1992; Springer *et al.*, 1993). As with the outer GOA colonies, kittiwake colonies in southern PWS also experienced low productivity from 1985 to 1989 (Irons *et al.*, 1999; Hatch *et al.*, 1992).

Pigeon guillemot abundance in PWS declined between the 1970's and 1980's in a manner parallel with wide scale declines in the abundance of surface schooling fish species and shrimp (Oakely and Kuletz, 1996; Agler *et al.*, 1999). Similar population responses were observed in other PWS bird and marine mammal species that consumed forage fish (Kuletz *et al.*, 1997; Piatt and Anderson, 1996). However, the declines observed at Naked Island and Knight Island between the eighties and the early nineties were greater along oiled shorelines than along unoiled shorelines (Oakley and Kuletz, 1996; Murphy *et al.*, 1997).

Hayes and Kuletz (1997) reported that the proportion of surface schooling fish in the diet of pigeon guillemot chicks had declined between pre-spill (1979 to 1981) and post-spill studies (1990 to 1996). Surface schooling fish, Pacific herring, *Clupea pallasi*, and Pacific sand lance, *Ammodytes hexapterus*, are important food items for breeding seabirds. Because their summer lipid stores translate to high energy meals for chicks, and their schooling behavior represents a concentrated food source for foraging adults, surface schooling fish represent a potentially high provision rate for chicks (Golet *et al.*, 2000).

Herring and sand lance spawn in the nearshore habitat. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal and spawning habitats (Murphy and Rice, 1999; Rice, 1999). Herring embryos exposed to oil yielded more physically deformed larvae than unoiled embryos (Kocan *et al.*, 1996; Hose *et al.*, 1996). Several studies have reported that EVOS oil in sediments induce xenobiotic responses, induction of cytochrome P450, or liver lesions in intertidal fish species, including high cockcomb, *Anoplarchus purpurescens*, walleye pollock, *Theragra chalcogramma*, and kelp greenling, *Hexagrammos decogrammus* (Woodin *et al.*, 1997; Collier *et al*., 1996; Jewett *et al.*, 1995; Holland-Bartels, 1998). The two potential routes that pigeon guillemots may be exposed to residual oil are through ingestion of contaminated food items, or contact with oil sheens while foraging in oiled areas (King and Sanger, 1979; Piatt *et al.*, 1990; Prichard, 1997).

Recovery of the pigeon guillemot population may be constrained by the physiological effects of oil exposure on chicks and adults, demographic limitations due to pigeon guillemot life history traits, food limitations, or other factors such as nest predation. In my thesis, I assess both the oil and non-oil related factors that may be constraining the post EVOS population growth of pigeon guillemots in PWS. In chapter two, I assess the impacts of residual oil on the clinical health of chicks and adults by comparing the hematological and plasma biochemical profiles of chicks and adults in oiled and unoiled areas. In chapter three, I examine demographic and food limitations. To gauge the current demographic limitations of populations in unoiled areas, I tracked

trends in population, productivity, and fledgling survival, at unoiled Jackpot Island in southwestern PWS, from 1995 to 1998. I compared productivity parameters and the relative quality of the chick diet among unoiled Jackpot Island, pre-spill Naked Island, and post-spill Naked Island studies. To assess quality of the chick diet, I examined the species composition of the chick diet and the frequency of meal deliveries. To evaluate food limitations, I compared the relative quality of the chick diet with chick survival rates, growth rates, and fledging weights. In my final chapter, I address the question 'Is it oil or is it food?' by providing a summary of my findings from chapters two and three. This eco-toxicology approach in assessing the health of post-spill pigeon guillemot populations is unique because few oil spill studies have tested for the lingering sub-lethal effects of residual oil, or gauged population response in accordance with available food resources.

2

CHAPTER TWO

COMPARISON OF PIGEON GUILLEMOT, *CEPPHUS COLUMBA*, BLOOD PARAMETERS FROM OILED AND UNOILED AREAS OF ALASKA, EIGHT YEARS AFTER THE *EXXON VALDEZ* OIL SPILL

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2.1 INTRODUCTION

Population estimates of pigeon guillemots, *Cepphus columba*, in Prince William Sound (PWS), Alaska, have declined from 15,000 individuals in 1972-73 to approximately 3,000 individuals in the mid-1990's (Dwyer *et al.*, 1976; Klosiewski and Laing, 1994; Agler and Kendall, 1997; Sanger and Cody, 1994). A large-scale regime shift in the Gulf of Alaska during the late 1970's (Piatt and Anderson, 1996) likely caused much of this decline, as high-quality forage fish were more widely available in the 1970's than in recent years (Hayes and Kuletz, 1997; Kuletz *et al.*, 1997). Pigeon guillemot populations in PWS were further impacted by the Exxon Valdez oil spill (EVOS; Murphy *et al.*, 1997), which occurred when the supertanker *Exxon Valdez* ran aground on 24 March 1989 and spilled 42 million L of crude oil into PWS. Approximately 40% of this oil was deposited on the shorelines of PWS (Galt *et al.*, 1991). Between 100,000 to 375,000 birds died in the spill, of which 1,500 to 3,000 were pigeon guillemots (Piatt *et al.*, 1990). Seven years after the spill, pigeon guillemots had not recovered to pre-spill numbers (Agler and Kendall, 1997; Oakley and Kuletz, 1996). It is not clear to what extent demography, food availability, or the physiological effects of lingering oil exposure may be constraining recovery of pigeon guillemots in PWS.

Pigeon guillemots are vulnerable to oil spills because they use the nearshore habitat (King and Sanger, 1979; Piatt *et al.*, 1990). They breed in small colonies along rocky coastlines, and roost on intertidal rocks. Guillemots spend much of their time on the sea surface or diving for surface schooling fish, demersal fish, and invertebrates associated with the intertidal and subtidal zones.

The prey of pigeon guillemots are also susceptible to oil contamination. There is evidence of longer-term toxic effects of oil to fish populations when oil persists in their natal habitats (Murphy and Rice, 1999; Rice 1999). For example, Pacific herring, *Clupea pallasii*, embryos exposed to oil yielded more physically deformed larvae than unoiled embryos (Kocan *et al.*, 1996; Hose *et al.*, 1996). Biomarkers of oil ingestion were noted

in PWS fish several years after EVOS. Walleye pollock, *Theragra chalcogramma*, collected from oiled Naked Island in 1990 and 1991, exhibited high levels of fluorescent aromatic compounds in their bile (Collier *et al.*, 1996). Jewett *et al.* (1995) reported that demersal fish in the oiled eelgrass beds of Herring Bay, PWS, demonstrated a high incidence of hemosiderosis lesions in the liver. Kelp greenling, *Hexagrammos decogrammus*, collected in 1996 showed significantly higher expression of P450 activity in oiled Herring Bay versus unoiled Jackpot Bay (Holland-Bartels, 1998). Research in the early 1990's demonstrated that oil exposure had detrimental effects on nearshore predators including river otters, *Lutra canadensis* (Bowyer *et al.*, 1994, 1995; Duffy *et al.*, 1993, 1994), and sea otters, *Enhydra lutris* (Loughlin *et al.*, 1996). Whether residual oil from the EVOS affected pigeon guillemots required further evaluation.

Acute toxic effects of petroleum hydrocarbons are well known (Leighton, 1993), but the lingering effects of chronic oil exposure have not been investigated fully in free ranging piscivorous birds (Fry and Lowenstine, 1985). Leighton (1993) provided an extensive review of avian studies of petroleum oil toxicity. Dosing experiments have shown that the effects of oil ingestion include: (1) lower hatch rate and altered yolk structure (Grau *et al.*, 1977; Szaro *et al.* 1978a); (2) reduced rate of growth (Szaro *et al.*, 1978b; Peakall *et al.*, 1982); (3) slower development and reduced survivorship of chicks (Trivelpiece *et al.*, 1984); (4) liver, kidney and intestine damage in long-term exposure (Khan and Ryan, 1991; Patton and Dieter, 1980; Fry and Lowenstine, 1985); and (5) Heinz-body hemolytic anemia associated with a substantial decrease in packed-cell volume (Leighton *et al.*, 1983).

Because guillemot chicks remain in their natal burrow until they fledge, oil contamination can occur through contact with the oiled feathers of an adult while in the egg or chick stage, or through ingestion of contaminated fish (Leighton, 1993; Peakall *et al.*, 1980). At nine days of incubation, avian embryos are extremely sensitive to oil contacting the egg shell. As little as 5 µl of Prudhoe Bay crude oil has been reported to cause embryo death at this stage (Albers, 1977; Szaro *et al.*, 1978a). Dosing studies of weathered crude oil on congeneric black guillemots, *Cepphus grylle*, suggest that oil ingestion may cause long-term physiological effects which could reduce a young bird's ability to survive at sea (Peakall *et al.*, 1980).

Payne et al. (1986) suggested that detecting simple changes in a biochemical or physiological response in a population may provide information on the presence of toxins. Hematological analyses (differential cell counts) may provide information about the immunological status of birds (Campbell, 1986a). Levels of plasma enzymes provide information on the function of organs, e.g. liver (Campbell, 1986a). Elevated levels of acute-phase protein haptoglobin indicate responses to exogenous toxins, bacterial or viral infections, and physical trauma (Silverman and LeGrys, 1987). Physiological changes occurring during the chick growth period have been suggested by many authors to influence blood parameters (Wolf *et al.*, 1985; Hoffman *et al*., 1985; Kostlecka-Myrcha, 1987; Starck, 1998; Work, 1996; Prichard *et al.*, 1997). To prevent age-dependent variation from biasing assessments, hematological and plasma biochemical profiles should be repeated on chicks at different stages of development.

To make an accurate assessment of clinical tests, reference values of healthy individuals are needed (Hawkey and Samour, 1988), but information on hematological and clinical chemistry on pigeon guillemots or other alcids is limited (Newman *et al*., 1997; Newman and Zinkl, 1998; Prichard *et al.*, 1997; Kosteleck-Myrcha, 1987). We assume therefore that colonies in the unoiled areas represent healthy populations. If oil contamination is limiting recovery of pigeon guillemots in PWS, we expected that blood chemistry and cell counts would differ between oiled and unoiled areas and these differences should be consistent with either toxic responses or lower fitness. In this study, we compare the hematological and plasma biochemical profiles between pigeon guillemot populations in an oiled area of PWS and in unoiled areas of PWS.

2.2 METHODS

During summer 1997, measurements of growth and blood samples from pigeon guillemot chicks were collected in areas oiled by the EVOS and in reference areas that were not oiled (Fig. 2.1). The oiled area we evaluated was Naked Island (60° 40' N, 147° 28' W) in central PWS. The prevailing winds and currents during spring of 1989 deposited oil predominately on the east and northwest shorelines of Naked Island (Galt *et al.*, 1991; Oakley and Kuletz, 1996). The combined colonies of Jackpot Island (60° 19' N, 148° 11' W) and Icy Bay (60° 14' N, 148° 17' W) in southwestern PWS were not oiled and represent the reference areas in this study. For evaluating adults, we also included a third reference area located in Kachemak Bay (59° 35' N, 151° 19' W), which is located in lower Cook Inlet, Alaska.

For each chick, mass and length of wing-chord were measured every five days until the chick fledged. When possible, two blood samples were collected from each chick at approximately 20 and 30 days after hatch. The hatching date of the chick was determined from either direct observation or was estimated by comparing wing-chord length for chicks of unknown age to wing-chord length for chicks of known age. Adults were captured either by noose traps placed on roosting rocks or with a dip net.

One cc of blood was collected from the brachial vein of chicks using a one cc tuberculin syringe with a 25 or 26 gauge needle. Adults were bled from the medial metatarsal vein. Fresh blood was used to make blood smears on glass slides. Two heparinized micro-hematocrit tubes were filled with blood from the puncture site, capped with clay, and stored in coolers. Whole blood was placed in microtainer tubes treated with lithium heparin. These samples were centrifuged within two hours of collection. After centrifuging, plasma was removed with a disposable pipette and divided between two snap-top plastic vials. Vials were frozen in propane freezers. Blood smear slides, micro-hematocrit tubes and one vial of plasma were placed in chilled insulated boxes and shipped to the Avian and Exotic Laboratory of Redondo Beach, California, within 48 hours of collection. The following parameters were measured: red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), hemoglobin (Hp), mean cell hemoglobin content (MCHC), counts of white blood cells (WBC), heterophils,

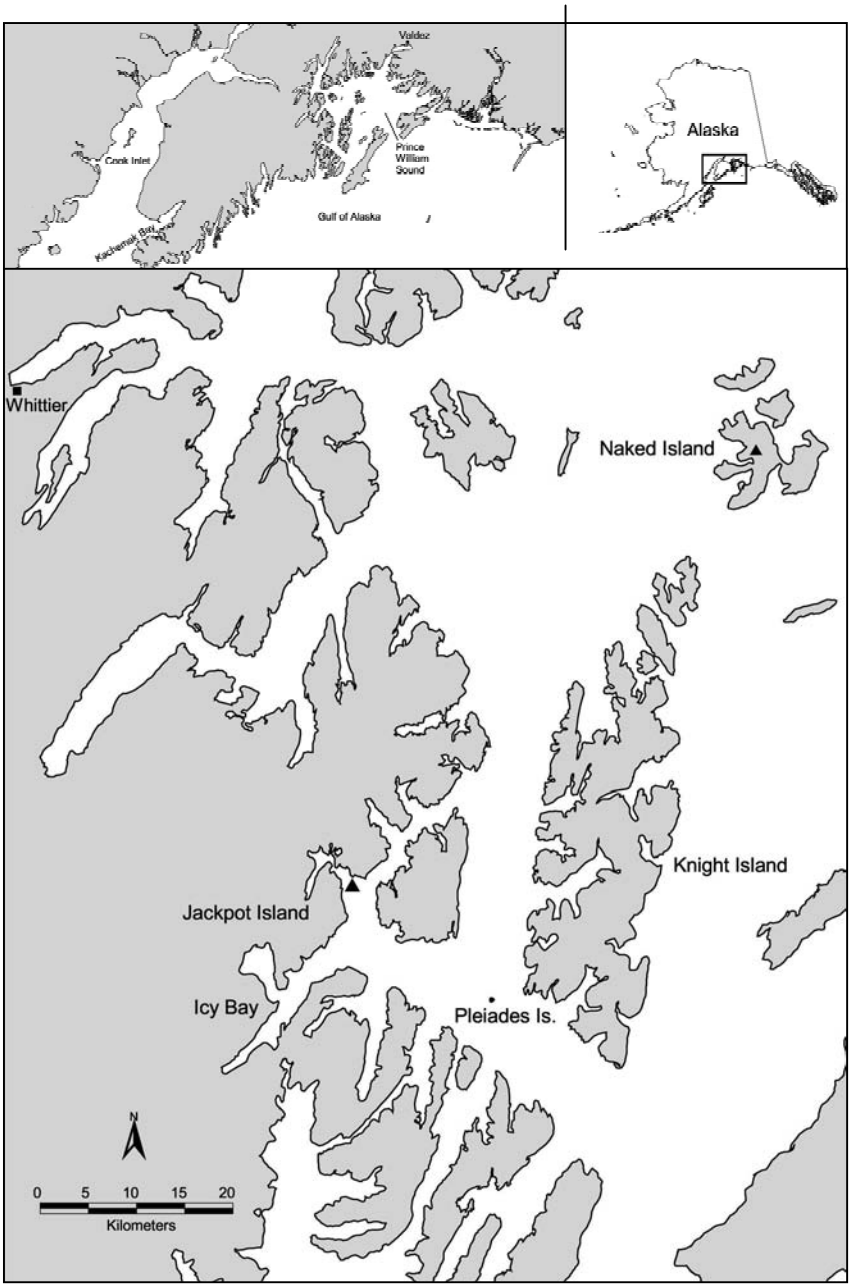

Figure 2.1 Location of the oiled and unoiled reference areas in Prince William Sound and Kachemak Bay, Alaska. The oiled area for this study included several pigeon guillemot colonies on Naked Island in central Prince William Sound. The unoiled reference areas for this study included pigeon guillemot colonies at Jackpot Island and Icy Bay in southwestern Prince William Sound, and pigeon guillemot colonies in Kachemak Bay.

lymphocytes, eosinophils, basophils, activity of creatine phosphokinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alkaline phosphatase, gamma-glutamyl transferase (GGT), concentration of calcium, uric acid, plasma protein, total protein, alpha-1 macroglobulin, alpha-2 macroglobulin, beta globulin, gamma globulin, albumin, albumin to gamma globulin ratio, bile acid, phosphorus, and sodium. A second vial of frozen plasma was sent to the University of Alaska Fairbanks for measurement of haptoglobin concentration with electrophoresis kits (Helena Laboratories, Beaumont Texas, USA). Plasma was applied to agarose gels and electrophoresed at 100 volts for one hour. Agarose plates were then fixed with 7.5% trichloroacetic acid and stained with o-dianisidine to detect the Hp-hemoglobin complex. The Hp-hemoglobin complex was quantified by densitometry and results are reported in mg hemoglobin binding capacity per 100 ml of plasma (Duffy *et al*., 1994). Enzyme immunoassay wipes were used to evaluate the presence of polyaromatic hydrocarbon molecules on the plumage of adults. The plumage of adults was wiped with a one-ply section of 5 by 5 cm gauze pad saturated with isopropanol. The gauze pad was then placed in aluminum foil and frozen until analysis. Levels of phenathrene, pentacosane, and hexacosane from the wipes were measured with the EnSysEnviroGard™ Polynuclear Aromatic Hydrocarbon test kit 70608, produced by Millpore Corporation (Bedford, Massachusetts, USA), or detected with gas chromatography-mass spectrometry (Duffy *et al*., 1999).

Data were tested for normality and equal variance with the Kolmogorov-Smirnov test with Littiefors correction and with the Levene median test, respectively. To test the hypothesis that there was no difference between samples collected at 20 days of age and 30 days of age, we used the paired *t*-test or the Friedmans test on ranks, a nonparametric test for a repeated measures design, on the samples collected in the reference area. Blood parameters with significantly different values between sampling ages are considered to be influenced by the development stage of the chicks. A *t*-test or Mann-Whitney test was used, when appropriate, to detect differences in blood parameters between oiled and unoiled areas, and between 30-day post hatch chicks and adults in the reference areas.

2.3 RESULTS

2.3.1 Effects of age: nestlings

We found several age-related differences in the blood samples. For chicks in southwestern PWS, significant differences between the blood samples of chicks 20 and 30 days after hatching included PCV ($P = 0.014$), RBC ($P = 0.002$) and alkaline phosphatase activity ($P = 0.001$). Differences in phosphorus concentrations were marginally non-significant ($P = 0.063$). The mean (± SD) wing-chord lengths of the 20-day and 30-day age groups were 92.8 ± 7.6 cm and 128.7 ± 6.3 cm, respectively. A multiple logistic regression model using variables RBC, PCV, and alkaline phosphatase activity correctly predicted the age group in 18 of 22 blood samples with a concordance of 82% (likelihood ratio test = 7.7, $P = 0.051$). Variables correlated with the nestling wing-chord length of chicks included PCV ($r = 0.59$, $P = 0.001$, $n = 26$), RBC ($r = 0.58$,

P = 0.001, n = 24), alkaline phosphatase (r = 0.57, P = 0.003, n = 24), phosphorus (r = -0.39, P = 0.059, n = 24) and Hp (r = 0.56, P = 0.004, n = 24).

2.3.2 Effects of age: adults versus nestlings

The blood profiles of the adult birds from reference areas of Jackpot Island, Icy Bay and Kachemak Bay were distinct from the blood profile of the chicks from the reference area of Jackpot and Icy Bay. The age-related differences among chicks, which included PCV, RBC, alkaline phosphatase, and phosphorus, extended to our comparison between adults versus chicks. By the time a chick fledges, which occurs between 33 and 54 days of age, its weight is comparable to that of an adult, but its wing growth is not complete (Ewins, 1992; Ewins, 1993). For adults from southwestern PWS, the mean (± SD) for wing-chord length and body weight were 184 ± 4 cm and 508 ± 50 g, respectively. The wing-chord length at 20 days and 30 days after hatching was 49% and 70%, respectively, of wing-chord length in adults. The body mass at 20 days and 30 days after hatching was 66% and 86%, respectively, of the adult body mass. Because we had only samples from four adults in southwestern PWS, we incorporated adults from Kachemak Bay (n=3) into our sample of adults from unoiled areas. In the unoiled areas, adults had higher PCV (P = 0.001), RBC (P = 0.003), Hp (0.004), AST (P = 0.010), and albumin concentrations (P = 0.011), and lower alkaline phosphatase (P < 0.001) and lower phosphorus concentrations (P < 0.001) than 30-day old chicks in southwestern PWS. Adults also tended to have lower WBC (P=0.072), calcium concentration (P = 0.063), and bile acid concentration (P = 0.094) than chicks.

2.3.3 Oiled vs. Unoiled Populations: nestlings

In the 20-day age group, chicks sampled from the oiled population at Naked Island had lower calcium (P = 0.002), plasma protein (P = 0.008), and alkaline phosphatase activity (P = 0.025), and a higher lymphocyte count (P = 0.006) than chicks in the unoiled area of southwestern PWS (Table 2.1). In the 30-day age group, Naked Island chicks had significantly lower calcium (P = 0.043) and MCV (P = 0.015) than chicks from southwestern PWS (Table 2.2).

2.3.4 Oiled vs. Unoiled Populations: adults

Our sample size of adults was small. The number of adult blood samples from Naked Island, southwestern PWS, and Kachemak Bay were 10, 4 and 3, respectively. Adults at Naked Island were captured between 29 July and 3 August. Three of the adults in the reference areas were captured in June and two in August. Adults captured in the oiled area had significantly higher AST activity (P = 0.017), lower RBC (P = 0.006), Hp (P = 0.004) and GGT (P = 0.015) than adults in the reference areas (Table 2.3). The AST activity for the adults in the oiled area was nearly double the levels for the adults in the

reference areas. The plumage wipes from adults at Naked Island (n = 10) indicated low levels of phenathrene, pentacosane and hexacosane (mean ± SD: 0.004 ppm ± 0.002, 0.178 ppm ± 0.059, and 0.202 ppm ± 0.047, respectively).

Table 2.1 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks sampled at 20 days of age. Samples were collected in 1997 from oiled Naked Island colonies and unoiled Jackpot Island & Icy Bay colonies, in Prince William Sound, Alaska.

	Oiled Area Naked Island			Unoiled Area Jackpot Island & Icy Bay		
	mean	SD	n	mean	SD	n
Red Blood Cells (cu mm^{-3})	2.6	0.4	14	2.58	0.4	17
Packed Cell Volume (%)	44	4	15	43	6	18
Mean cell volume (cu mm^{-3})	159	16	14	160	13	17
Hemoglobin (g dl^{-1})	12.7	1.6	14	11.6	1.6	16
MCHC (g dl^{-1})	30.7	5.4	14	28	4.3	16
White Blood Cells (10^3 mm^{-3})	13	5	14	16	6	18
Heterophil *	49	12	14	61	10	17
Lymphocytes *	49	12	14	37	10	17
Eosinophil	0.6	1.3	14	0.7	1.3	16
Basophil	1.4	1.3	14	1.1	1.4	18
Calcium (mg dl^{-1})*	8.9	1.9	14	11.0	1.2	18
CK (u l^{-1})	530	233	14	776	541	17
LDH (u l^{-1})	937	234	14	897	471	18
AST (u l^{-1})	277	106	14	221	119	16
Uric Acid (mg dl^{-1})	18.3	8.7	14	20.0	11.2	16
Plasma Protein (g dl^{-1})*	3.1	0.5	14	3.8	0.6	18
Total Protein (g dl^{-1})	4.5	0.6	14	4.8	0.8	18
Alpha-1 (g dl^{-1})	0.39	0.11	14	0.44	0.18	18
Alpha-2 (g dl^{-1})	0.70	0.31	14	0.75	0.32	18
Beta (g dl^{-1})	0.88	0.21	14	0.91	0.35	18
Gamma Globulin (g dl^{-1})	0.70	0.16	14	0.75	0.15	18
Albumin (g dl^{-1})	1.86	0.33	14	1.94	0.51	18
Albumin/Gamma Globulin (g dl^{-1})	0.72	0.17	14	0.68	0.15	18
Bile Acid Assay (umol l^{-1})	38.8	35.6	14	61.9	105	14
Alkaline phosphatase (u l^{-1})*	372	151	14	279	82	17
GGT (u l^{-1})	25.2	12.5	14	20.6	14.8	13
Phosphorus (mg dl^{-1})	9.6	4.8	13	6.2	1.7	17
Sodium (mmol l^{-1})	129	17	11	141.0	5	13
Haptoglobin (Hp binding dl^{-1})	109	40	15	124	51	16

*Means significantly different ($P < 0.050$) between chicks sampled at Naked Island and Jackpot-Icy Bay.

Table 2.2 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of pigeon guillemot chicks at 30 days of age. Samples were collected in 1997 from oiled Naked Island colonies and unoiled Jackpot Island & Icy Bay colonies, in Prince William Sound, Alaska.

	Oiled Area Naked Island			Unoiled Area Jackpot Island & Icy Bay		
	mean	SD	n	mean	SD	n
Red Blood Cells (mm^{-3})	3.16	0.40	24	2.95	0.42	13
Packed Cell Volume (%)	48	4	25	47	6	15
Mean cell volume (cu mm^{-3})*	148	13	24	160	10	13
Hemoglobin (g dl^{-1})	13.8	1.6	22	13	1.8	14
MCHC (g dl^{-1})	29	5	22	27	4	14
White Blood Cells (10^3 mm^{-3})	13	6	24	12	5	17
Heterophil	62	11	24	56	12	17
Lymphocytes	36	11	24	42	12	17
Eosinophil	0.3	0.5	24	0.2	0.4	17
Basophil	1.1	1.0	24	1.3	1.8	17
Calcium (mg dl^{-1})*	9.0	1.8	19	10.3	1.3	15
CK (u l^{-1})	613	528	20	554	221	15
LDH (u l^{-1})	863	482	21	863	325	15
AST (u l^{-1})	313	169	19	304	233	15
Uric Acid (mg dl^{-1})	12.3	11.1	21	16.7	8.7	14
Plasma Protein (g dl^{-1})	3.5	0.8	22	4.0	1.5	17
Total Protein (g dl^{-1})	5.0	1.7	22	4.6	0.9	15
Alpha-1 (g dl^{-1})	0.50	0.37	22	0.40	0.19	15
Alpha-2 (g dl^{-1})	0.68	0.40	22	0.72	0.39	15
Beta (g dl^{-1})	0.98	0.35	22	0.90	0.49	15
Gamma Globulin (g dl^{-1})	0.75	0.38	22	0.73	0.19	15
Albumin (g dl^{-1})	0.75	0.17	22	0.70	0.21	15
Albumin/Gamma Globulin (g dl^{-1})	2.14	0.75	22	1.84	0.50	15
Bile Acid Assay (umol l^{-1})	38	45	15	106	158	14
Alkaline phosphatase (u l^{-1})	502	367	18	443	152	15
GGT (u l^{-1})	16	15	14	16	11	13
Phosphorus (mg dl^{-1})	7.4	4.5	21	5.6	1.9	15
Sodium (mmol l^{-1})	133	16	16	142	13	13
Haptoglobin (Hp binding dl^{-1})	99	38	20	122	44	14

*Means significantly different ($P < 0.050$) between chicks sampled at Naked Island and Jackpot-Icy Bay.

Table 2.3 Mean, standard deviation (SD) and sample size (n) of the hematological and plasma chemistry of adult pigeon guillemots sampled in 1997 from oiled Naked Island, Prince William Sound and unoiled areas of Jackpot Island & Icy Bay, Prince William Sound and Kachemak Bay, Lower Cook Inlet in Alaska.

	Oiled Area Naked Island			Unoiled Area Jackpot Island, Icy Bay and Kachemak Bay		
	mean	SD	n	mean	SD	n
Red Blood Cells (cu mm^{-3})*	3.01	0.35	10	3.76	0.59	6
Packed Cell Volume (%)	53	5	10	58	6	7
Mean cell volume (cu mm^{-3})	168	9	10	163	10	6
Hemoglobin (g dl^{-1})	18.3	3.3	10			
MCHC (g dl^{-1})	34.3	7.12	10	33.2	11.4	4
White Blood Cells (10^3 mm^{-3})	8	2	10	8	1	7
Heterophil	58	13	10	64	13	7
Lymphocytes	37.9	8.8	10	33.4	12.1	7
Eosinophil	0	0	10	0	1	7
Basophil	4	5	10	3	2	7
Calcium (mg dl^{-1})	8.6	1.6	9	9.1	1.2	7
CK (u l^{-1})	244	168	9	375	339	7
LDH (u l^{-1})	892	296	10	915	143	7
AST (u l^{-1})*	979	816	10	461	199	7
Uric Acid (mg dl^{-1})	14.85	5.83	10	14.6	6.5	7
Plasma Protein (g dl^{-1})	4.7	2.3	10	3.9	0.7	6
Total Protein (g dl^{-1})	5.5	0.98	10	5.6	1.7	7
Alpha-1 (g dl^{-1})	0.45	0.24	10	0.43	0.29	7
Alpha-2 (g dl^{-1})	0.67	0.42	10	0.90	0.45	7
Beta (g dl^{-1})	0.90	0.58	10	0.71	0.30	7
Gamma Globulin (g dl^{-1})	0.69	0.17	10	1.02	0.97	7
Albumin (g dl^{-1})	2.75	0.71	10	2.63	0.71	7
Albumin/Gamma Globulin (g dl^{-1})	1.03	0.30	10	0.94	0.27	7
Bile Acid Assay (umol l^{-1})	40.3	74.5	7	2.05	2.6	7
Alkaline phosphatase (u l^{-1})	93	70	8	137	102	6
GGT (u l^{-1}) *	3	5	9	10.8	8.2	7
Phosphorus (mg dl^{-1})	2.2	1.8	8	1.7	0.8	7
Sodium (mmol l^{-1})	138.6	17.1	7	143.8	9.3	4
Haptoglobin (Hp binding dl^{-1})	122	28	8	93	50	7

*Means significantly different ($P < 0.050$) between adults sampled at oiled areas and unoiled areas.

2.4 DISCUSSION

The clinical hematology and biochemistry of seabirds is not as well known as for waterfowl, poultry or pet species (Newman and Zinkl, 1998). Blood parameters vary among species according to life history patterns, diet, and activity level. Pigeon guillemots differ from more commonly studied birds in that they have rapidly growing semi-precocial chicks, their diet is composed of marine fish, and they are adapted to diving to depths greater than 20 m (Ewins, 1993). Interpreting our results is also made difficult because of the paucity of biochemical studies on this species. The few reference values for this species are from studies with sample sizes of less than ten individuals (Newman and Zinkl, 1998; Newman *et al.*, 1997; Prichard *et al.*, 1997; Haggblom *et al.*, 1988; Bradley and Trefall, 1974). Our study extends the biochemical information for chicks of this species by providing reference values for different stages of development that are based on larger sample sizes.

2.4.1 Effects of Development

Physiological changes occurring during post hatch development of chicks affect many hematological and biochemical parameters (Starck, 1998; Vinuela *et al.*, 1991; Kostlecka-Myrcha, 1987). Age-related variation in blood parameters is an important consideration when collecting samples from pigeon guillemot colonies, because the range in chick ages may be as great 42 days (Drent, 1965). This is caused by asynchronous nesting or the laying of replacement clutches (Ewins, 1993; Drent, 1965). It has been well documented in many avian species that adults have higher PCV, RBC, and Hp than immature birds (Work, 1996; Wolf *et al.*, 1985; Kostlecka-Myrcha, 1987; Fairbrother *et al.*, 1990), but there is little documentation of the changes in these parameters within the nestling period for free-living species (Kostelecka-Myrcha, 1987). Anemia has been associated with oil contamination (Hartung and Hunt, 1966; Szaro *et al.*, 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Leighton *et al.*, 1983). Clinical signs of anemia are low PCV, RBC, MCV or MCHC. Therefore it was critical for us to identify these age-specific differences in red blood cell parameters before evaluating the health of immature birds. During the nestling period, there are dramatic changes in the profile of the red blood cells as embryonic forms, natal forms, and adult forms replace one another (Schenk *et al.*, 1978). Kostelecka-Myrcha (1987) documented PCV increases and MCV decreases during the nestling period of the little auk, *Plautus alle*, as smaller sized adult red blood cells replaced the red blood cells after hatching. The greatest increases in RBC occurred during the first 10 days after hatch (Kostelecka-Myrcha, 1987; Hoffman *et al.*, 1985). Post-hatch development of erythropoietic tissue is closely related to growth of body mass. As the chick approaches adult size or asymptotic body mass, bones are ossifying in preparation for flight and erythropoietic tissue decreases to adult levels (Starck, 1998). Pigeon guillemot chicks reach asymptotic growth between 30 and 40 days of age (Ewins, 1993). Kostelecka-Myrcha (1987) noted a non-significant

increase in Hp level during the latter half of the nestling period. Our study and the study of Haggblom *et al.* (1988) confirm that similar age-related changes in Hp occur in pigeon guillemots chicks. We expect subtle changes in red blood cells and Hp to continue after chicks fledge.

Elevated alkaline phosphatase (AP) activity in birds is associated with increased osteoblastic activity such as skeletal growth and repair, egg production, or nutritional deficiencies (Lumeij, 1994). Therefore the normal range of AP activity in rapidly growing chicks is higher than in adults (Wolf *et al*., 1985; Hoffman *et al*., 1985; Vinuela *et al.*, 1991; Work, 1996). We found AP activity nearly doubled between the samples for chicks 20 days and 30 days after hatching. The activity of AP reported by Newman and Zinkl (1998) for fledglings were similar to the AP activity for 20-day old chicks in our study. In red kites, *Milvus milvus*, Vinuela *et al.* (1991) reported that AP activity peaked at 38 days after hatch, when the growth of long bones were near completion. Pigeon guillemot chicks also had higher phosphorus and marginally higher calcium levels than adults. Vinuela *et al.* (1991) noted that increases in calcium and phosphorus levels correlated with increases in AP activity during the nestling period of red kites. In brown pelicans, *Pelecanus occidentalis*, Wolf *et al.* (1985) found that AP activity and phosphorus concentration were highest during the first 10 months of development and remained moderately elevated through the first two years of life. Pigeon guillemots are smaller than pelicans, but their skeletal growth continues after fledging for at least two months (Ewins, 1992). These patterns suggest that AP activity, phosphorus and calcium concentrations of guillemot chicks will peak prior to fledging then gradually drop to adult range within the first six months of life.

Elevated WBC is a symptom of infection. Interpretation of elevated WBC in juvenile birds is difficult because their normal range is variable and higher than adults (Fudge, 1996). For terns, shearwaters, and petrels, Work (1996) reported that older chicks tend to have higher WBC than adults. Puerta *et al.* (1990) reported similar results for common cranes. We could not detect differences in WBC between 20-day and 30-day old chicks, but these chicks had higher WBC than adults.

Similar to our results, Prichard *et al.* (1997) and Work (1996) reported that chicks had lower AST activity than adults. Newman and Zinkl (1998) found that young pigeon guillemots between five and ten weeks old have AST activity greater than or equal to the activity in adults. Elevated AST activity is associated with hepatocellular damage, septicemia and muscle injury. Bollinger *et al.* (1989) studied the effect of different capture methods on waterfowl AST activity and reported that AST activity becomes elevated with physical exertion. We suggest that chicks have lower AST activity than adults because they are sedentary and their muscles are less developed. Compared to adults, chicks offer little resistance to capture and are less likely to experience muscular exertion and injury.

Age-related differences in Hp concentration have been documented in mammals. Stellar sea lion, *Eumetopias jubatus*, pups that are less than 15 days old have significantly lower haptoglobin (Hp) levels than adults (Zenteno-Savin *et al.*, 1997). In humans, neonates do not have detectable levels of Hp until two months of age (Henry, 1991). Prichard and co-workers (1997) reported that pigeon guillemot chicks had significantly

lower Hp levels than adults. Adults in our study had lower mean Hp levels than reported by Prichard (1997), which may explain why we did not find similar age-related differences. Prichard (1997) noted that Hp was correlated with the rate at which adults deliver meals to the nest. In our study Hp was significantly correlated with the rate of weight gain immediately prior to the drawing of blood from chicks. This relationship supports Prichard's speculation that Hp is sensitive to the nutrition of chicks. We also documented a positive correlation between Hp and RBC, which suggests that Hp levels may be linked to the development of erythropoietic tissue during chick development.

2.4.2 Comparison between populations in oiled and unoiled areas

Various oil-dosing studies have been conducted on birds, but the symptoms of toxicity of oil ingestion have varied with species, age, the chemical composition of the oil, the dosing levels, and the presence of additional stress factors (Hartung, 1995; Leighton, 1993). Ingestion of sublethal levels of crude oil may constitute a nonspecific stressor for birds and render them more vulnerable to stress factors such as persistent cold temperatures and bacterial diseases (Holmes *et al.*, 1979). To evaluate the presence of injury at the oiled colonies in this study, we measured blood parameters that were indicators of physiological health of organ systems that involve the liver function, kidney function, the haematopoietic system, immune function, and electrolyte balance.

The avian liver responds to oil ingestion with hypotrophic activity (Szaro *et al.*, 1978b; Patton and Dieter, 1980; Stubblefield *et al*., 1995) and induction of hepatic cytochrome P-450 (Peakall *et al*., 1989; Lee *et al*., 1985). Enlargement of the liver may be a compensatory response to metabolize the high burden of toxic material introduced in experimental diets (Patton and Dieter, 1980; Stubblefield *et. al*, 1995) or an inflammation response to cell injury. Hepatocellular damage and necrosis are associated with elevation in the activity of plasma liver enzymes (Lewandowski *et al*., 1986). In Leighton's (1993) review of oil toxicity research, he found that the evidence of injury to the liver was inconsistent among studies, which may be associated with enzyme responses that are specific to species (Franson *et. al*, 1985). Our indicators of liver injury were elevated bile acid, AST and LDH activity in the plasma. In pigeons, *Columba livia*, elevated levels of bile acid (Lumeij, 1988) and AST are the most sensitive indicator of experimentally induced liver injury (Lumeij, 1988; Campbell, 1986b). Ingestion stimulates the release of bile acid. Fasted peregrine falcons, *Falco peregrinus*, experienced a three-fold increase in plasma bile acid concentration after ingestion of meat (Lumeij and Remple, 1992). During our study adults fed their nestlings at a rate of 0.4 to 1.0 fish h^{-1}. We did not control the food intake of chicks and this would explain some of variation in bile acid concentrations between individuals. Post-prandial increases in bile acid concentration represent 1-fold to 2-fold increases, while hepatobilary disease results in 5-fold to 10-fold increases relative to the reference range (Lumeij, 1991). Elevated levels of bile acid concentration (exceeding 200 micro mol l^{-1}) indicate persistent loss of hepatic function (Fudge, 1996). The bile acid concentrations of chicks at Naked Island were in the ranges reported for pigeons and peregrine falcons (Lumeij, 1988; Lumeij and Remple, 1992).

While AST and LDH are considered non-specific because they occur in many tissues, Campbell (1986b) found that AST and LDH were sensitive indicators of liver disease in carnivorous birds including red tail hawks, *Buteo jamicensis*, and great horned owls, *Bubo virginianus*. Elevated BA, AST or LDH concentrations were uncommon among chicks in both the oiled and unoiled areas, and we did not observe a significant difference in mean activity of BA, AST or LDH between chicks of Naked Island and southwestern PWS. Other researchers working with weathered Prudhoe Bay crude oil found no effect of oil dosing on liver enzyme responses of alcid chicks (Leighton, 1993; Prichard, 1997) and mallards, *Anas platyrhynchos* (Rattner, 1981; Stubblefield *et al.*, 1995). The blood variables associated with liver function and hepatocellular damage do not indicate deleterious effects on livers of chicks at Naked Island.

Renal tubular necrosis was documented in Cassin's auklets, *Prychoramphus aleuticus*, after oil was applied to their breast feathers (Fry and Lowenstine, 1985). Increases in uric acid in the plasma may indicate adverse effects on renal function (Allen, 1988; Fudge, 1996). In veterinary practices uric acid levels greater than 20 mg dl^{-1} are abnormal (Allen, 1988; Fudge, 1996). Newman and coworkers (1997) noted that uric acid levels in adult piscivorous marine birds are typically higher than in other avian species. They suggest that high protein diets combined with the osmoregulation demands of living in a marine environment causes higher concentrations of serum uric acid. In our study, both chicks and adults had uric acid levels that were below 20 mg dl^{-1}, which is within the reference range previously reported for adult pigeon guillemots (Newman and Zinkl, 1998; Newman *et al.*, 1997). Therefore, the uric acid levels of chicks in the oiled area of our study do not appear to indicate the presence of impaired renal function or damage.

Anemia was documented in several species of birds following exposure to oil (Hartung and Hunt, 1966; Szaro *et al*., 1978b; Pattee and Franson, 1982; Fry and Lowenstein, 1985; Fry and Addiego, 1987; Leighton *et al*., 1983). Reduced PCV and Heinz-body hemolytic anemia was documented in young herring gulls, *Larus argentatus*, and Atlantic puffins, *Fratercula arctica*, after experimental ingestion of crude oil (Leighton *et al.*, 1983). Yet, ingestion of high doses of Prudhoe Bay crude oil did not result in anemia in both adult rhinoceros auklets, *Cerrorhinca monocerata*, (Newman, personal communication) and mallards (Stubblefield *et al*., 1995). Hemolytic anemia was documented in adult white-winged scoters, *Melanitta fusca*, rescued from an oil spill, but blood samples were taken several days after the birds were captured (Yamato *et al*., 1996). The decrease in physical activity, the stress of handling, and the change in diet associated with captivity may influence erythropoiesis in adult alcids (Newman, personal communication). Anemia is the result of reduced erythropoiesis, accelerated erythrocyte destruction (hemolytic anemia), or blood loss. Clinical signs of anemia are low PCV, RBC, MCHC or MCV. There is little variation in PCV among species, and values below 32% are considered diagnostic of anemia (Hawkey and Samour, 1988). In our study, the values for PCV, MCHC and hemoglobin were within the ranges that are normal for immature birds, which indicates that there was probably no anemia for chicks in the oiled area of our study. The MCV values for 30-day old chicks at Naked Island were significantly less than the MCV for chicks in southwestern PWS and in Kachemak Bay,

Alaska (Seiser, unpublished data). It is not clear why MCV values are lower in the oiled area.

Immunosuppression has been noted in various oil dosing studies (Leighton, 1993). Reduced lymphocytes and reduced resistance to bacterial pathogens have been recorded in mallards (Holmes *et al*., 1979; Rocke *et al*., 1984). In adult rhinoceros auklets, ingestion of crude oil elicited no inflammatory response in WBC or differential cell counts, but young alcids may respond differently (Newman, personal communication). Leighton (1986) reported morphological changes to the lymphoid glands of young Atlantic puffins and herring gulls. In our study, WBC and differential cell counts (lymphocytes, heterophils, eosinophils and basophils) were our indicators of the state of the immune system. The ratio of lymphocytes to heterophils for the 20-day old chicks at Naked Island was significantly different from the ratio for chicks in southwestern PWS, but this pattern did not persist for the 30-day old chicks. We found that Naked Island did not have significantly lower values of WBC or differential cell counts than the unoiled area in southwestern PWS, which suggests that the immune system was not stressed or impaired in a way that would influence cell production.

Hypertrophy of salt glands has been documented in marine birds dosed with crude oil (Peakall *et al.,* 1980, 1982, 1983; Miller *et al*., 1978). Osmoregulatory impairment can be accompanied by increases in plasma sodium levels. Peakall et al. (1980) noted a transient rise in plasma sodium levels in black guillemot chicks dosed with 0.1 ml and 0.2 ml of Prudhoe Bay crude oil. Similar results have been found in herring gulls (Miller *et al*., 1978) and mallards (Eastin and Rattner, 1982). In contrast, Prichard (1997) found sodium levels of pigeon guillemot chicks did not respond to dosing with 0.2 ml of weathered Prudhoe Bay crude oil. The sodium levels for chicks in the unoiled area of our study were similar to levels for the control chicks in the study by Prichard *et al.* (1997). Because the sodium levels for the chicks at Naked Island were not significantly different from the levels for chicks in southwestern PWS, we conclude that there is no evidence for hypertrophy of salt glands.

The results reported here also extend the data base for Hp levels in pigeon guillemots. Haptoglobin is an acute phase protein that has been widely used in human and other mammal medical practices as an indicator of inflammatory diseases, infectious diseases, trauma or stress. Gevaert and co-workers (1991) demonstrated that Hp concentrations increased after the pigeons were infected with salmonellosis. Although Hp has been employed to assess potential stressors in compromised wildlife populations (Duffy *et al*., 1993; Duffy *et al*., 1994; Zenteno-Savin *et al*., 1997; Prichard *et. al.,* 1997), it has not been widely used for assessing health in free-ranging birds. The recovery of river otters from the initial impact of the EVOS was documented with the use of Hp (Duffy *et al*., 1993: Duffy *et al*., 1994). In comparisons between declining and stable populations of pinnipeds, significantly higher Hp concentrations were associated with the declining populations of harbor seals, *Phoca vitulina*, and sea lions (Zenteno-Savin *et al*., 1997). Prichard et al. (1997) examined the use of Hp as a potential biomarker of oil ingestion in pigeon guillemot chicks, but found that variation in growth rates and feeding rates among chicks from different colonies confounded their interpretation of Hp response to the ingestion of weathered crude oil. In our study, there was no evidence of

poor health identified by our suite of health indicators, which is consistent with the similar Hp levels we observed in chicks from oiled and unoiled areas.

Because nearly all the chicks that were sampled for blood in our study ultimately fledged, we conclude that our handling and blood sampling did not affect survival. This observation also supports our diagnosis of clinically healthy chicks. In contrast, the overall fledging success (fledglings per hatchling) for Naked Island and Jackpot Island was 46% and 68%, respectively. In Kachemak Bay, Prichard (1997) also noted that the majority of nestling mortality occurred in the first 12 days after hatch. Predators or food shortages are the most common sources of mortality of young chicks (Hayes and Kuletz 1997, Nelson, 1987). Mink, a major predator of nestlings in PWS, was not present on Jackpot Island in 1997, but was at Naked Island. The shoreline of Naked Island suffered both oil contamination and physical disturbance from efforts to clean beaches after the spill. Both events tend to have negative effects on the prey base of pigeon guillemots. Therefore, we limit our conclusions on the health of chicks to the latter half of the nesting period. Currently, hematological and biochemical variables of the pigeon guillemots we studied provide little evidence of oil-related injury for chicks that hatched in 1997, eight years after the Exxon Valdez oil spill. In contrast to chicks, the pilot study we conducted on adult health suggests that the issue of oil-related injury in pigeon guillemot adults cannot be dismissed without further study.

Pigeon guillemot adults have greater opportunities for exposure to oil than nestlings. Adults feed on invertebrates including crabs, shrimps, and bivalves (Oakley 1981; Kuletz, 1983; Sanger, 1987), but rarely provision their chicks with invertebrates (Oakley, 1981; Ewins, 1993). In the winter, invertebrate consumption may increase because of seasonal changes in distribution of prey fish. Pacific sand lance, *Ammodytes hexapterus*, are inaccessible because they are burrowed in the sediment, and young cod move to deeper waters (Oakley, 1981; Sanger, 1987). Bioaccumulation of polynuclear aromatic hydrocarbons (PAH) is greater in invertebrates than fish. Invertebrates cannot metabolize PAH as efficiently as fish, because of differences in the activity of mixed function oxgenenase enzymes and metabolic rate between invertebrates and fish (Gibson, 1977). Therefore, adults potentially have a greater dietary source of PAH's than nestlings (Bolger *et al.*, 1996; Baumard *et al.*, 1998).

It is important to recognize that our sample of adults is small and was obtained opportunistically. The majority of the samples from the unoiled areas were obtained in June, while the samples from the oiled area were collected in late July and early August. Also, we do not know the sex of the birds we sampled. Sex and reproductive condition have been documented to affect plasma biochemistry (Wolf *et al.*, 1985; Fairbrother *et al.*, 1990; Gee *et al.*, 1981). Because interpretation of differences between blood parameters for adults from the oiled and unoiled areas in our study is complicated by sampling issues, the interpretation we present is preliminary and should be viewed with some caution.

In comparison to adults in the unoiled area of our study, GGT activity was significantly lower for adults in the oiled area. GGT activity is commonly measured in mammal clinical practices to detect cholestatic diseases of the liver or the consumption of drugs and other toxic substances that induce the microsomal enzyme system (Henry,

1991). For example, fungi infested feed produces elevated plasma GGT activity in domestic chickens (Espada *et al*., 1994). GGT activity is not a sensitive indicator of avian hepatocellular injury (Campbell, 1986b). Egg laying also appears to elevate serum GGT activity. In domestic mallard hens, Fairbrother *et al.* (1990) observed that serum GGT activity was 10-fold higher during the egg-laying period compared to the incubation period. Newman and Zinkl (1998) measured the mean serum GGT activity for several seabird species, and reported a mean GGT activity of 16.5 IU l^{-1} with a range of 0 to 60 IU l^{-1} for five pigeon guillemot adults captured during the egg laying period. These values were slightly higher than the values we observed for adults in the unoiled areas of our study, which were also sampled early in the breeding season. For adults at Naked Island, which were sampled late in the breeding season, the GGT activity was within the range previously reported for adult rhinoceros auklets, *Cerorhinca monocerata*, common murre, *Uria aalge,* incubating western gull, *Larus occidentalis*, and non-breeding white pelicans, *Pelecanus onocrotalus* (Newman and Zinkl, 1998; Puerta *et al.*, 1991). It is not clear if the lower GGT activity we observed for adults in the oiled area represents a normal seasonal trend in GGT activity for adult pigeon guillemots.

The AST activity of adults in the oiled area was significantly higher and nearly double the AST activity of adults in the unoiled areas of our study and double the AST activity of adult pigeon guillemots observed in other studies (Newman *et al*., 1997; Newman and Zinkl, 1998). Elevated AST activity is associated with both hepatocellular damage and muscle injury (Bollinger *et al*., 1989). Muscle injury associated with capture causes elevated CK or LDH activity in waterfowl species (Bollinger *et al.,* 1989; Franson *et al.,* 1985; Fudge, 1996). We did not observe significant differences in CK or LDH between adults in oiled and unoiled areas of our study. Because similar capture methods were used in the oiled and unoiled areas of our study, we suggest that the elevated AST concentrations in the adults from the oiled area are more consistent with hepatocellular injury than muscle injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Because adults have greater opportunities for exposure to residual oil than nestlings, we recommend additional studies to fully evaluate the health of adults residing in oiled areas.

3

CHAPTER THREE

POPULATION TRENDS AND REPRODUCTIVE BIOLOGY OF PIGEON GUILLEMOTS IN UNOILED AREAS OF PRINCE WILLIAM SOUND, ALASKA, BETWEEN 1994 & 1998: INSIGHTS ON THE RECOVERY OF PIGEON GUILLEMOTS IN OILED AREAS

3.1 INTRODUCTION

Pigeon guillemots were impacted by the *Exxon Valdez* oil spill (EVOS). Piatt *et al.* (1990) estimated between 1,500 to 3,000 pigeon guillemots were killed in 1989. When the pigeon guillemot population of oiled Naked Island began to decline in 1993, concern arose that it was a symptom of sub-lethal effects of chronic exposure to residual oil. However, several factors may cause the population to decline and limit growth, such as physiological effects of oil on chicks and adults, food shortages, and nest predators. At Naked Island, Hayes and Kuletz (1997) correlated population declines between pre- and post-spill periods with declines in the abundance of sand lance, an important prey species of pigeon guillemots.

The decline in sand lance abundance may be attributed to either localized oiling or broad scale changes in climatic-oceanographic conditions. In the Gulf of Alaska, many piscivorous seabird and marine mammal populations dependent on high-quality forage species declined during the 1970's and 1980's (Agler *et al.*, 1999; Piatt and Anderson, 1996; Springer *et al.*, 1993). Declines in high-quality forage species, such as herring, sand lance and capelin were associated with climatic and oceanographic changes in the Gulf of Alaska in the late 1970's (Piatt and Anderson, 1996). To provide insight concerning the role of oil and food in constraining recruitment at oiled areas in PWS, I conducted a five-year study to document population trends, breeding success, and chick diet at an unoiled area in southwestern PWS.

3.2 STUDY AREA

Jackpot Island (60° 19' N, 148° 11' W) is located near the mouth of Jackpot Bay in southwestern PWS, Alaska (Fig. 3.1). The oiled area study site, Naked Island (60° 40' N, 147° 28' W) is in central PWS, approximately 55 km NE of Jackpot Island. Descriptions of Naked Island are found in Oakely and Kuletz (1996), Golet *et al.* (2000) and Galt *et al.* (1991).

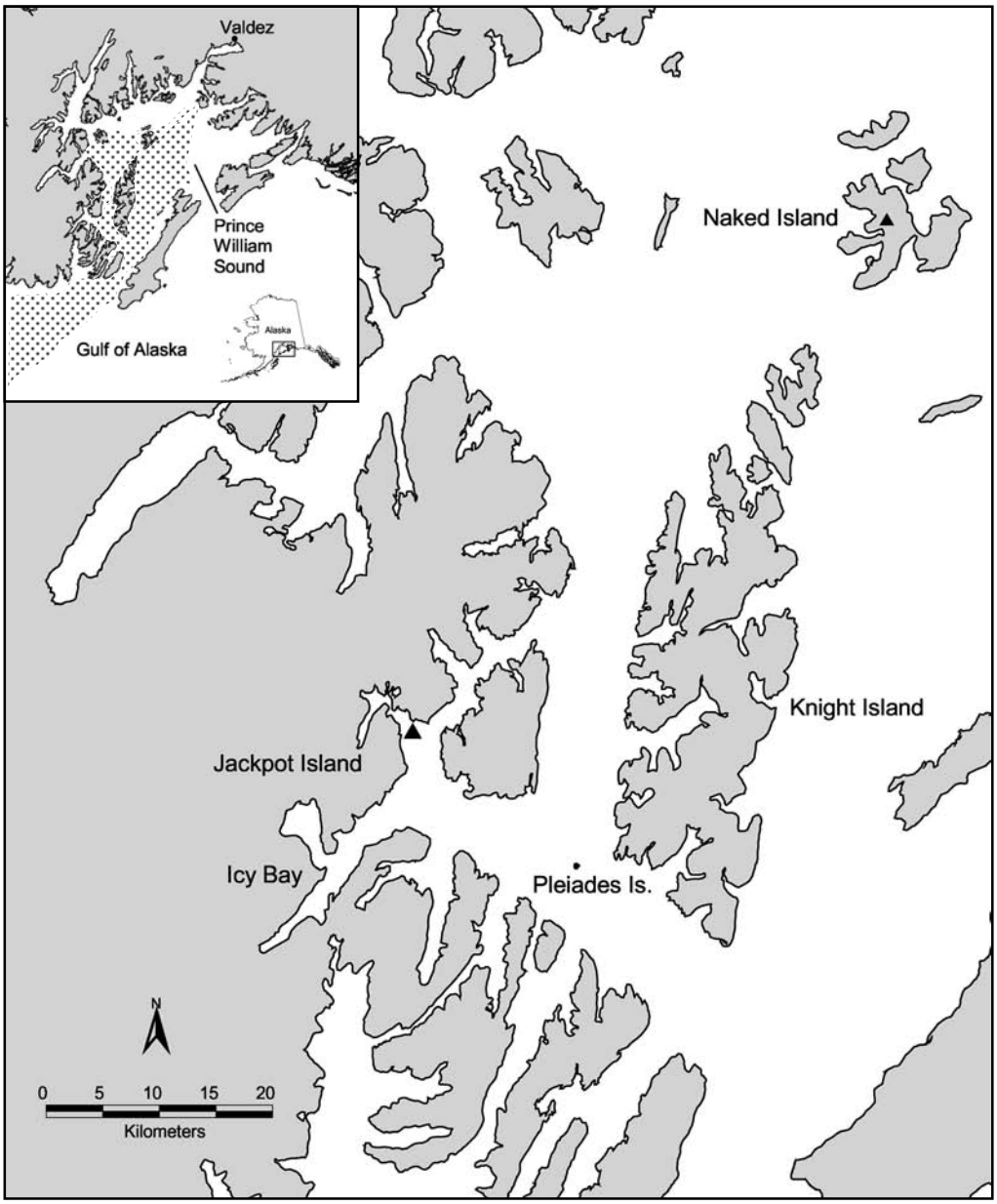

Figure 3.1 Location of the Jackpot Island and Naked Island study areas in Prince William Sound, Alaska. The inserted map of Prince William Sound shows the area oiled by the 1989 *Exxon Valdez* oil spill.

In 1993, Sanger and Cody (1994) noted that the density of breeding pigeon guillemots at Jackpot Island (103 birds km^{-1}) was the highest in PWS. Jackpot Island is separated from the mainland by 0.9 km of water. Deep waters (> 120 m) surround Jackpot Island, but to the north, Dangerous Passage and its associated bays offer shallower water (< 40 m) and to the south, guillemots forage at the submarine moraine at the mouth of Icy Bay. The large land mass of Chenega Island shielded Jackpot Island and its adjacent guillemot foraging areas from direct oiling in the aftermath of the EVOS (Galt *et al.*, 1991).

Jackpot Island (1.6 ha) is vegetated with Sitka spruce, *Picea sitchensis*, and western hemlock, *Tsugu heterophylla*. Pigeon guillemots on Jackpot Island predominantly nest in earthen burrows that are located under tree roots that jut out from the edges of cliffs, approximately 3 to 7 m above the mean high tide line. Other burrow nesters on the island include horned puffins, *Fratercula corniculata,* and common mergansers, *Mergus merganser*. Jackpot Bay is an important nursery area for Pacific herring, *Clupea pallasii* (Stokesbury *et al.*, 1997). Prey species common to the area include Pacific sand lance, *Ammodytes hexapterus,* crescent gunnel, *Pholis laeta,* northern ronquil, *Ronquilis jordani,* arctic shanny, *Stichaeus puctuatus,* Pacific cod, *Gadus macrocephalus,* Pacific tomcod, *Microgadus proximus,* walleye pollock, *Theragra chalocogramma,* and several species of salmon (Salmonidae) and sculpins (Cottidae).

3.3 METHODS

In this chapter, I compare productivity parameters and diets among three PWS pigeon guillemot studies: post-spill unoiled Jackpot Island, post-spill oiled Naked Island and pre-spill Naked Island. I collected the Jackpot Island (1994-1998) data using methods described below. Similar methods were used at the Naked Island study site. The pre-spill Naked Island (1979-1981) data were obtained from Oakely and Kuletz (1996) and post-spill oiled Naked Island data (1994-1997) from Hayes and Kuletz (1997) and Golet *et al.* (2000).

From 1994 to 1998, I documented the number of the birds attending the Jackpot Island colony, the phenology of the nesting season, the survival of eggs and chicks, the composition of the chick diet, the delivery rate of food to chicks, and the growth rate and fledging weight of chicks. In early June, during the morning high tide cycle, I conducted boat-based counts of the number of pigeon guillemots attending the Jackpot Island colony following census methods commonly used for guillemots (Ewins 1985; Drent, 1965; Kuletz, 1983). In 1997 and 1998, I also censused colonies at the Pleiades Islands, Gage Island, Flemming Point, Point Countess, West Arm of Whale Bay, and two locations in Icy Bay (denoted Icy Bay 2040 and Icy Bay 2035). The locations of all seven colonies are listed in the Beringian Seabird Colony Catalog (USFWS, 1999).

My estimates of productivity were restricted to nests found during the egg stage. Hatching dates of chicks were determined from direct observations or were estimated by comparing the wing length for chicks of unknown age to the wing length recorded for chicks of known age (Thoresen and Booth, 1958; Oakely, 1981). I calculated laying date

by subtracting 32 days (Drent, 1965) from my estimated hatching dates. For the majority of fledglings, my estimated fledge date was within 2 days of the actual date that the fledglings evacuated the burrow.

I began inspecting nest sites in late June, coinciding with hatch dates reported by Oakely and Kuletz (1996). Because incubating guillemots are sensitive to human disturbance (Drent *et al.*, 1964; Vermeer *et al.*, 1993; Cairns, 1980), I restricted the number of visits during the incubation period. Active nests were visited every third day in 1995 and every fifth day in the other years. To determine fledging weight and date, I increased my visitation rate to every other day after chicks reached the age of 30 days or when wing length became greater than 120 mm. All previously used nest sites were checked for re-occupation. During the incubation period nests are cryptic, but during the chick rearing period we could easily detect nests because of fecal stains at the burrow entrance, vocalization of the chick, or delivery of fish by adults. There were very few nest sites on Jackpot Island for which I could not physically or visually assess the presence of chicks.

On each visit to a nest, I measured the body mass of each chick to the nearest 1 g using a hand-held spring scale and measured the maximum flattened wing-length to the nearest 1 mm. I found the linear phase of growth for PWS chicks was 8 to 20 days post hatch (~ 40 to 90 mm wind length), similar to Emms and Verbeek (1991) and Koelink (1972). I conducted regression analyses between mass and age during the linear growth phase and used the slope of the relationship for my estimate of growth rates (g day^{-1}). To examine the effects of brood size and sibling competition on growth rate and fledging weight, I classified chicks as singleton chicks (chicks in one-chick broods), alpha chicks (first hatching chicks of two-chick broods) or beta chicks (second hatching chicks of two-chick broods).

I conducted provisioning watches to determine composition of the chick diet and the rate that adults provision their chicks with food (delivery rate). My observation platform was a boat anchored approximately 30 m offshore. During these 16-hour provisioning watches I recorded the time that the adult brought a prey item to the nest and identified the prey item to the lowest possible taxon. My hourly delivery rates were based on the 16-hour observation periods (0600 h to 2200 h). Annual mean delivery rates were computed from the mean delivery rate of individual nests.

Prey items were also classified into 4 groups: surface schooling fish (herring, sand lance, smelt, or salmon), gadids (Pacific cod, Pacific tomcod, or walleye pollock), non-schooling fish (such as gunnels, ronquil, and sculpins) or other species (uncommon species not included in the 3 previous groups, such as flatfish, lingcod, *Ophinod elongatus,* and greenling, *Hexogrammos* spp.). The composition of the chick diet was based on the total sum of identified chick meals recorded during all provisioning watches.

All the chicks I handled were marked for future identification with a unique combination of two colored bands on the right leg and a single colored band on the left leg. The color of the left leg band represented the hatch year of the chick. Pigeon guillemots first breed at the age of 3 or 4 years (Drent, 1965; Nelson, 1991). In 1998, when I expected to observe the 1994 and 1995 cohorts of fledglings return to the colony to breed, I dedicated five days to observing banded birds. Observations of banded birds

were also noted during provisioning watches. I estimated the percentage of the 1997 and 1998 fledglings surviving to their third year based on the number of banded fledglings observed at Jackpot Island during the 1997 and 1998 breeding season.

I tested data for normality and equal variance with the Kolmogorov-Smirnov goodness of fit test and the Levene median test, respectively. I tested for significant differences among years using analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test, as appropriate. When these tests resulted in significant differences, I identified significant differences among groups by conducting multiple pair-wise comparisons with the Bonferroni t-test or the non-parametric Dunn's method. I assumed statistical significance if $P < 0.05$. Means are reported with standard deviation.

3.4 RESULTS

3.4.1 Population trends and nesting effort

The number of pigeon guillemots attending the Jackpot Island colony increased 36% from 74 to 101 birds over the five-year study period (Fig. 3.2). For seven colonies in southwestern PWS, comparison between my 1998 census and the 1993 census conducted by Sanger and Cody (1994) indicates population increases at six colonies and no change at one colony (Table 3.1). Based on the report of 78 birds at Jackpot Island in 1993 by Sanger and Cody (1994), the annual changes in the population at Jackpot Island between 1993 and 1998 were –5%, 7%, 9%, 0% and 17%, respectively.

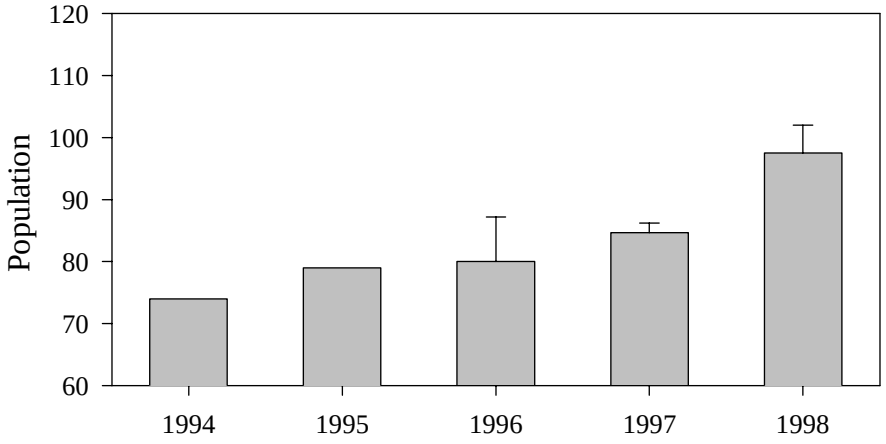

Figure 3.2 The number of pigeon guillemots attending the Jackpot Island colony in Prince William Sound, Alaska, from 1994 to 1998. Multiple census were conducted in 1996, 1997 and 1998.

There was little variation in number of nesting attempts among the five years (Fig.3.3); nesting attempts varied between 36 in 1996 and 40 in 1995. Nesting effort, which is defined as active nests per number of pairs in the June census, was higher during the first two years (97% in 1994, 100% in 1995) than nesting effort observed in the last

three years (81% in 1996, 88% in 1997, 72% in 1998). Over the five-year study period, we found 184 clutches dispersed over 72 different nest sites. New nest sites were discovered each year, but more commonly, nests from the previous year were re-occupied. Between 1995 and 1998, the percentage of current active nest sites occupied in the previous year ranged from 55% to 72%. The three nests where I found adults killed by mink were not occupied the following year. Of the 36 nest sites discovered in 1994, eleven were not occupied the following year, six were occupied for five consecutive years, five for four consecutive years, nine sites for three consecutive years, and five nest sites for two consecutive years.

Table 3.1 June census counts for eight pigeon guillemot colonies in southwestern Prince William Sound for 1993, 1997 and 1998.

Year	Jackpot Island	Pleiades Islands	Gage Island	Flemming Point	Point Countess	Whale Bay West arm	Icy Bay 2040	Icy Bay 2035
1993[a]	78	48	16	8	6	8	6	6
1997	86	48			7	8	7	6
1998	100	76	22	15	9	11	9	6

[a] Source: Sanger and Cody (1994)

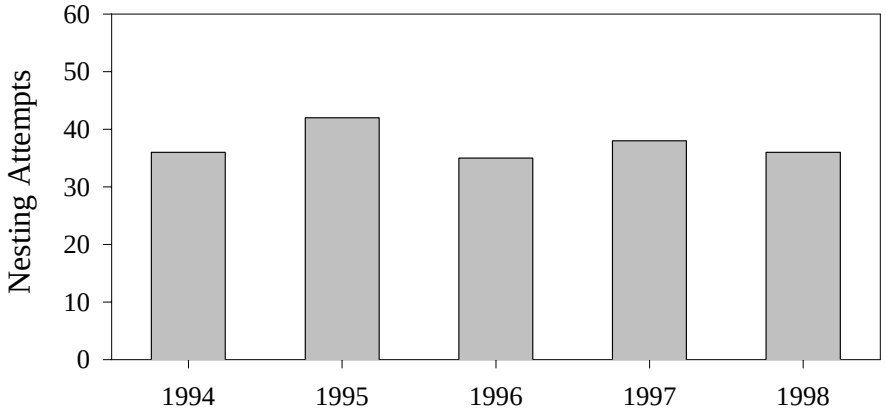

Figure 3.3 The number of active pigeon guillemot nests found on Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998.

3.4.2 Productivity

Estimates of median laying and hatch dates varied 15 days over the course of this study, and the 1995 and 1996 dates were earlier than the median dates for the other 3 years (Table 3.2). The 1995 median fledging date was significantly earlier than the

median fledging date in each of the other three years (Table 3.2; Kruskal-Wallis test, H = 19.7, P = < 0.001, df = 3).

Table 3.2 Estimates of median laying, hatching and fledging dates for the pigeon guillemot colony on Jackpot Island, Alaska, from 1994 to 1998.

Year	Median Laying Date			Median Hatch Date			Median Fledging Date		
	mean	SD	nests	mean	SD	nests	mean	SD	nests
1994	1 June	6	18	3 July	6	18	9 August	5	24
1995	24 May	4	14	26 June	4	14	4 August	5	12
1996	26 May	6	15	25 June	6	15			
1997	2 June	7	12	2 July	7	12	10 August	6	15
1998	6 June	6	9	8 July	6	9	12 August	3	9

Among the five years, mean clutch size, hatching success, fledging success and productivity were 1.82 ± 0.09 eggs $nest^{-1}$, 0.55 ± 0.18 chicks egg^{-1}, 0.48 ± 0.30 fledglings $chick^{-1}$, and 0.27 ± 0.22 fledglings egg^{-1}, respectively (Table 3.3). The 1994 breeding season was the most productive of the five years, with the highest number of hatchlings per nest, fledglings per nest, fledglings per egg laid, and total fledglings produced (Fig. 3.4 and Fig. 3.5a).

Table 3.3 Mean clutch size, hatching success and nestling survival for pigeon guillemot nests found during the egg stage on Jackpot Island, Alaska, from 1994 to 1998.

Year	Nests	Clutch Size (eggs/nest)	Hatching Success (chicks/egg)	Nestling Survival (fledglings/chick)	Productivity (fledglings/egg)
1994	24	1.92	0.80	0.76	0.61
1995	29	1.90	0.56	0.45	0.25
1996	21	1.73	0.61	0.00	0.00
1997	31	1.74	0.44	0.68	0.31
1998	28	1.79	0.33	0.53	0.18
5-year mean		1.82 ± 0.09	0.55 ± 0.18	0.48 ± 0.30	0.27 ± 0.22

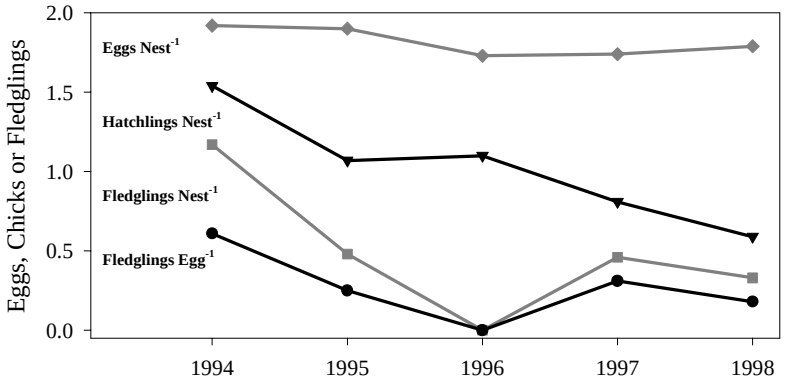

Figure 3.4 The mean clutch size, hatchlings per nest, fledglings per nest and productivity (fledglings egg^{-1}) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. Means are based on nests that were found during the egg stage.

Between 1994 and 1998, the percentage of nests with at least one fledgling was 75%, 41%, 0%, 26%, and 22%, respectively. Thus, Jackpot Island supported twice as many successful breeding pairs during the first two years than the last two years. The 1995 breeding season was less productive than the 1994 breeding season, the 1996 breeding season was a failure because of predation, and few fledglings were produced during 1997 and 1998 because of low hatching success. Hatching success was lower in the last two years because of nest abandonment during the incubation stage (Fig. 3.5b). With the exception of 1996, losses to predators were low on Jackpot Island (Fig. 3.5c). Predation losses in 1995 were attributed to a pair of northwestern crows nesting on the island, and the catastrophic losses in 1996 were caused by the presence of mink on the island. In 1997 and 1998, there was little evidence of nest predation as abandoned eggs and dead chicks remained in the burrows the entire breeding season.

3.4.3 Chick Diet

Surface schooling fish, which include herring and sand lance, formed at least one third of the diet of Jackpot Island chicks in four out of the five years (Fig. 3.6). Herring was the dominant species of schooling fish in the diet, and composed 42%, 29%, 20%, 0.4% and 41% of the number of fish delivered between 1994 and 1998, respectively.

Over the five year period, sand lance ranged from 0.5% to 13% of the diet. Non-schooling demersal fish, which include gunnels, pricklebacks, and sculpins, were as common as surface schooling fish in chick diet. In 1997 there was a major shift in the composition of the chick diet, in which schooling fish were rare and adults provided chicks with higher numbers of non-schooling demersal fish (Fig. 3.6). During 1994 gadids formed nearly one quarter of the chick diet, but in the following years gadids were less frequent. Other prey fish species, which include flatfish and greenlings, were uncommon and comprised no more than 3% of the chick diet in any given year.

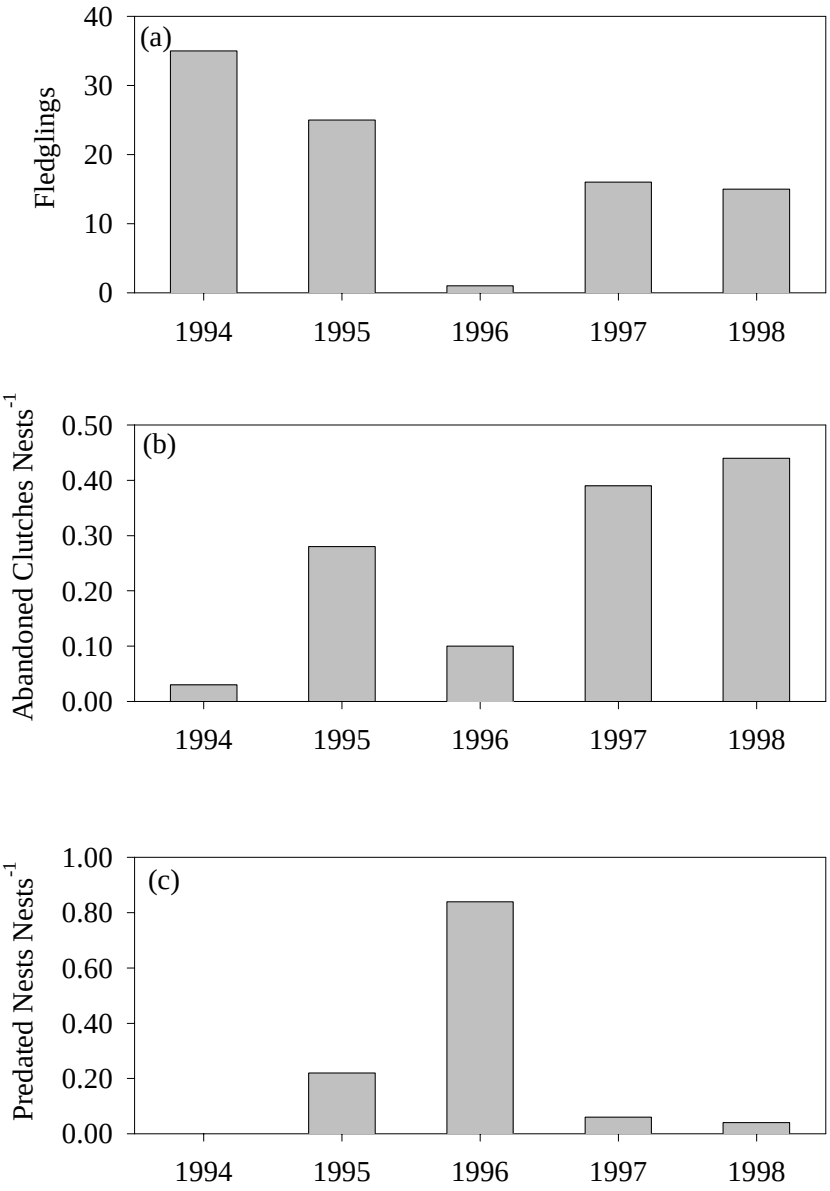

Figure 3.5 Total fledglings (a), abandonment rate (b), and predation rate (c) for the pigeon guillemot colony at Jackpot Island, Prince William Sound, Alaska, from 1994 to 1998. The total number of pigeon guillemot fledglings included all nesting attempts; the percentage of pigeon guillemot nests abandoned during the incubation stage is based on all nesting attempts; and the percentage of pigeon guillemot nests experiencing predation is based on nests found during the egg stage.

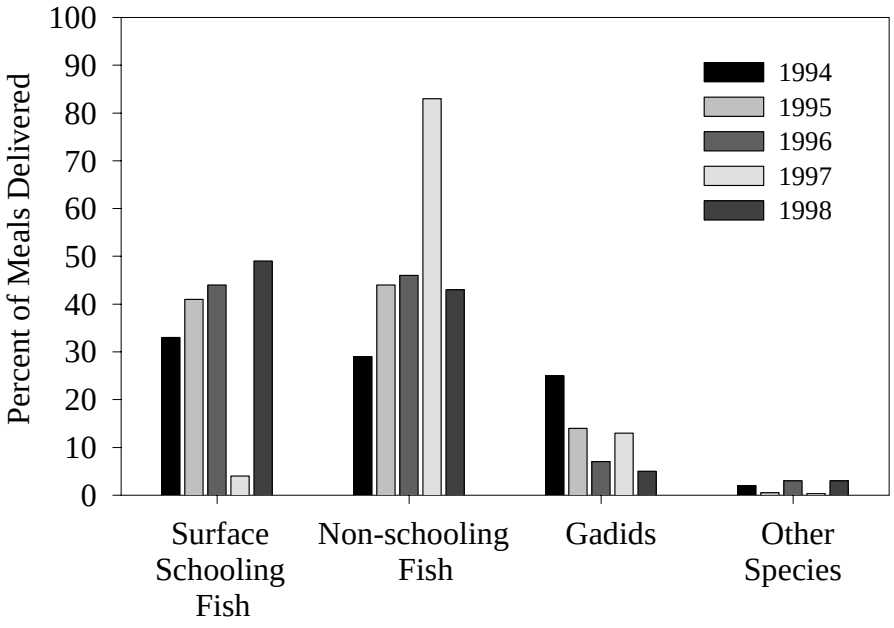

Figure 3.6 Composition of the pigeon guillemot chick diet at Jackpot Island, Alaska, from 1994 to 1998. Surface schooling fish include Pacific herring (*Clupea pallasi*) and Pacific sand lance (*Ammodytes hexapterus)*. Non-schooling fish include pricklebacks (Stichaeidae), gunnels (Pholidae), ronquils (Bathymasteridae) and sculpins (Cottidae). Gadidae include Pacific cod (*Gadus macrocephalus*), Pacific tomcod (*Microgadus proximus*) and walleye pollock (*Theragra chalocogramma)*. Other species represent food items not included in the three previous groups, such as flatfish (Bothidae and Pleuronectidae) and greenling (Hexagrammidae).

3.4.4 Delivery Rates

The sample of delivery rates in 1994 included only one observation period, and I have no data on delivery rates for chicks older than 8 days in 1996 because of mink predation during that year. Therefore, I eliminated both the 1994 and 1996 data from the following analyses of delivery rate variability among years and between brood size. Although delivery rates per chick were not significantly different among the 1995, 1997, and 1998 breeding seasons (Two-way ANOVA, year effect $F = 2.379$, $P = 0.114$, $df = 2$), delivery rates tended to be higher in 1995 (Fig. 3.7). Delivery rates per chick were significantly higher for nests with one chick than nests with two chicks (Fig. 3.7; Two-way ANOVA, brood size effect $F = 15.707$, $P < 0.001$, $df = 1$). The interaction between year and brood size was not significant ($P = 0.559$). Similar to analysis for delivery rates per chick, delivery rate per nest for 1995 (0.86 ± 0.28 fish nest^{-1} hr^{-1}, $n = 23$ nests), 1997 (0.79 ± 0.25 fish nest^{-1} hr^{-1}, $n = 16$ nests), and 1998 (0.75 ± 0.20 fish nest^{-1} hr^{-1}, $n = 7$ nests) was not significantly different among years (One-way ANOVA, $F = 1.270$, $P = 0.296$, $df = 3, 46$).

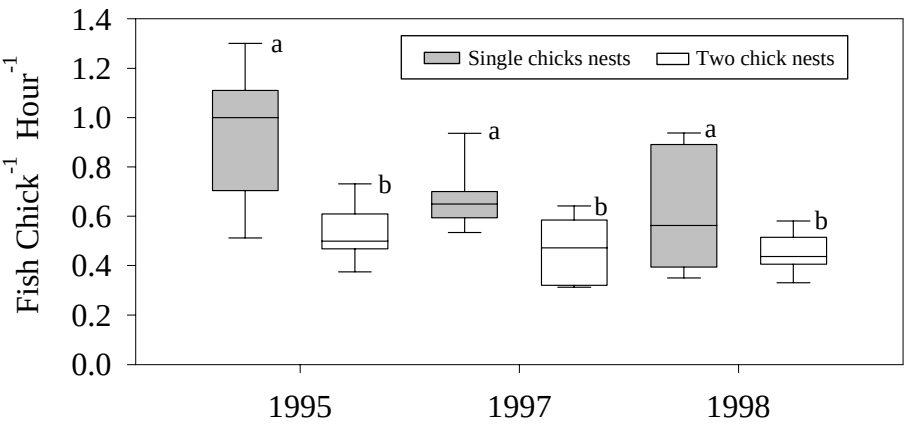

Figure 3.7 Comparison of the mean delivery rates per chick for the pigeon guillemot colony at Jackpot Island, Alaska, among the 1995, 1997 and 1998 breeding seasons and between nests with one and two chicks. Lines in the box plots indicate the median, and the 5th, 25th, 75th and 95th percentiles. Letters identify significantly different groups ($P < 0.05$).

3.4.5 Growth Rates and Fledging weights

The growth rate of chicks was not significantly different among years (Fig. 3.8a; (One-way ANOVA; $F = 0.619$, $P = 0.651$, $df = 4, 58$). Growth rates between nest mates were significantly different (Paired t-test, $t=3.12$, $P=0.008$, $df = 12$): single chicks grew significantly faster than the alpha and beta chicks of 13 pairs of siblings (One-way ANOVA; $F = 3.087$, $P = 0.53$, $df = 2, 60$). Beta chicks grew at slower rates (14.7 ± 3.5 g d^{-1}, $n = 13$) than their alpha siblings (16.4 ± 2.8 g d^{-1}, $n = 13$) or singleton chicks (17.6 ± 2.7 g d^{-1}, $n = 21$). Fledging weight in 1994 (500 ± 37 g) tended to be high in comparison with 1995 (467 ± 46 g), 1997 (463 ± 41 g), and 1998 (482 ± 42 g) (Fig. 3.8b; One-way ANOVA, $F = 2.021$, $P = 0.121$, $df = 3, 56$). Fledging wing-length in 1994 (141 ± 6 mm) tended to be smaller than 1995 (145 ± 7 mm), 1997 (144 ± 7 mm), and 1998 (145 ± 3 mm) (One-way ANOVA, $F = 2.336$, $P = 0.083$, $df = 3, 57$). Although fledging weight was significantly correlated with growth rate ($r = 0.336$, $P= 0.032$, $n = 41$), growth rate explains only 11% of the variation in fledging weight. Compared to their nest mate, beta chicks spent more days in the nest and tended to have slightly lower fledging weights (Paired t-test, $t=1.974$, $P = 0.072$, $df = 12$) and wing lengths (Paired t-test, $t = 1.818$, $P = 0.092$, $df = 13$).

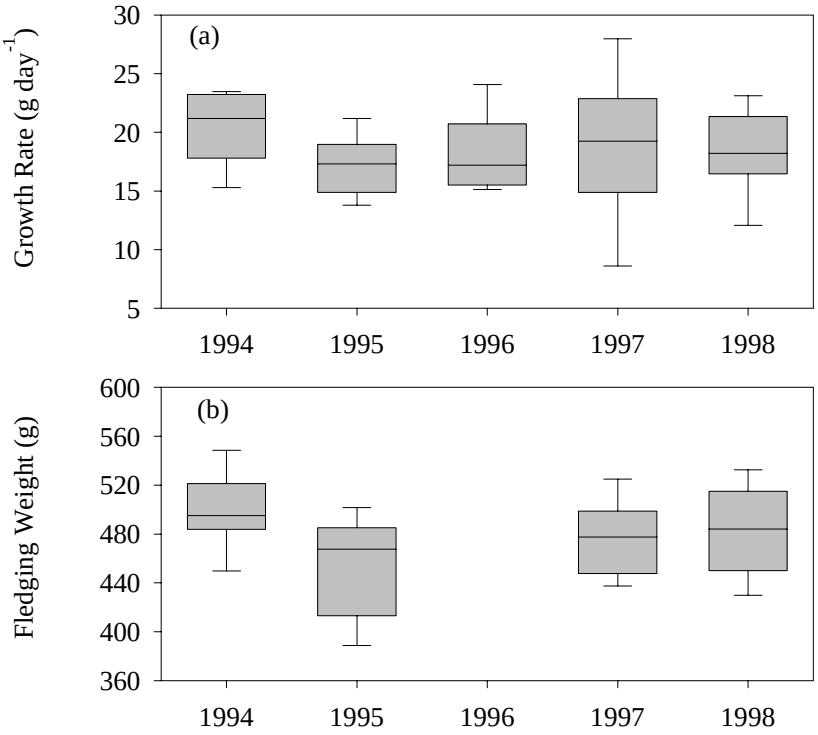

Figure 3.8 The linear growth rate (a) and fledging weight (b) of pigeon guillemot chicks on Jackpot Island, Alaska, from 1994 to 1998. Lines in the box plots indicate the median, and the 5th, 25th, 75th and 95th percentiles.

3.4.6 Fledgling Survival

I banded 28, 22, 0, 16 and 15 fledglings, in 1994, 1995, 1996, 1997 and 1998, respectively. In 1997, I located eight of the birds banded in 1994. Six of the birds were observed at Jackpot Island, one bird was observed at the Pleiades Islands and one bird was observed at a colony in Icy Bay. The following year, the searched area was limited to Jackpot Island. In 1998, I recorded two additional birds from the 1994 hatch year and five birds from the 1995 hatch year. My observations indicate that at least 36% of fledglings banded in 1994 survived to their third year. My conservative estimates of the proportion of fledglings returning to their natal colony in their third year is 21% for the 1994 cohort and 23% for the 1995 cohort. I documented four banded birds breeding at 3 years of age.

3.5 DISCUSSION

Recent increasing population trends at colonies in southwestern PWS suggest that favorable environmental conditions exist in Prince William Sound for the expansion of pigeon guillemot populations. However, at the end of this 5-year study, the abundance of pigeon guillemots at oiled Naked Island remained below their 1994 levels (G. Golet personal communication). These contrasting population trends suggest one or more demographic parameters, such as productivity, fledgling survival or adult survival, varies between the oiled and unoiled areas. Population growth at Naked Island may be limited by physiological effects of oil exposure, quality of diet, or other factors such as predators.

I could find no evidence that recovery was constrained by the physiological effects of oil exposure on chicks, although this hypothesis still needs to be rigorously evaluated for adults (Seiser *et al.,* 2000). The abundance of high-quality fish during the breeding season is a factor of particular interest because Hayes and Kuletz (1997) observed pre-spill and post-spill differences in the proportion of high-quality fish delivered to chicks at Naked Island. Sand lance and herring are surface schooling fish and are noted for their rich lipid stores and relative high energy density (kJ g^{-1}) (Anthony *et al.,* In Press). Temporal or regional differences in diet may represent limitation to population development if associated with lower productivity or survival rates (Carins, 1987). Declines in the relative abundance of these surface schooling fish in the chick diet has been associated with lower breeding success for Atlantic puffin, *Fratercula arctica,* (Lid, 1981; Anker-Nilseen, 1987) and arctic terns, *Sterna paradisaea* (Montevecchi, 1993).

I evaluated the hypothesis that availability of high-quality food is playing a role in constraining population growth in oiled areas by comparing diet and survival of chicks from unoiled Jackpot Island and pre-spill Naked Island to that of oiled Naked Island. In my discussion, I restricted the post-spill Naked Island data set to the five-year period coinciding with my Jackpot Island study. My pre- and post-spill comparison of Naked Island differs from Hayes and Kuletz (1997), because I do not include the two breeding seasons immediately following the grounding of Exxon Valdez oil tanker. Oakley and Kuletz (1996) examined the acute effects of EVOS on pigeon guillemot breeding success and diet, whereas I focus on a period when pigeon guillemot breeding success and diet may be influenced by chronic effects of EVOS to the nearshore community.

I ranked the relative quality of diet of chicks based on the proportion of high-quality fish in the diet and the rate that chicks received fish. I then examined nestling survival rate, growth rates and fledgling weight of the various colonies to determine if the observed difference in diet of chicks may have affected demographic parameters of productivity and fledgling survival rates.

3.5.1 Food Constraints

Diet of Chicks

Herring and sand lance accumulate lipid stores during summer months to sustain themselves during winter fasting periods (Blaxter and Holiday, 1963). Because of these substantial lipid stores, herring and sand lance tend to have higher energy density (kJ g^{-1}) than the other food items in the chick diet (Van Pelt *et al.,* 1997; Hislop *et al.,* 1991; Paul *et al.,* 1998; Anthony *et al.,* In press). I compare the proportion of surface schooling fish, demersal fish, gadids and other fish delivered to chicks at Jackpot Island to the proportions delivered to pre- and post-spill chicks at Naked Island to evaluate whether differences in diet composition have the potential to constrain recovery of pigeon guillemots in oiled areas of PWS.

Based on data in the literature, I have ranked the whole-body energy content among four categories of fish from high to low, as follows: surface schooling fish, demersal fish, gadids, and other fish. Paul and Paul (1998) reported significant regional, seasonal and annual variation in whole body energy content of PWS forage fish. Other researchers are currently addressing these diet issues for PWS pigeon guillemots (D. Roby, personal communication). In my evaluation of the quality of chick diet, I assume whole body energy of fish is constant over time and region. The validity of this assumption will be addressed in other studies.

The composition of the diet varied among the unoiled Jackpot Island colony reported here and oiled and pre-spill colonies at Naked Island (Chi-square; $\chi^2 = 31.8$, $P < 0.001$, $df = 8$). Compared to the diet of pre-spill chicks at Naked Island, I documented a lower abundance of sand lance and greater abundance of gadids in the diet of Jackpot Island chicks, which was similar to Hayes and Kuletz (1997) report on the diet of post-spill chicks at Naked Island. However, I found the abundance of herring in the diet of Jackpot Island chicks was significantly higher than the abundance of herring in the diet of both pre-spill and post-spill chicks at Naked Island (One-way ANOVA: $F = 5.568$, $P = 0.024$, $df = 2, 10$). In contrast to the Naked Island studies, the majority of surface schooling fish delivered to Jackpot Island chicks were herring rather than sand lance. Surface schooling fish represented 33 ± 16% of the Jackpot Island chick diet, which is intermediate between the proportion at Naked Island during the pre-spill period (48 ± 11%) and post-spill period (21 ± 7%) (One-way ANOVA: $F = 4.704$, $P = 0.036$, $df = 2, 10$). During the warm-water year of 1997, herring sharply declined in the diet of Jackpot Island chicks, while the Naked Island chicks experienced a modest gain in the abundance of surface schooling fish in their diet. With the exception of 1997, chicks at Jackpot Island had a greater proportion of schooling fish in their diet (40 ± 5%) than the chicks at post-spill Naked Island (T-test, $t = 6.277$, $P < 0.001$, $df = 6$).

During the warm-water year of 1997, adults delivered more non-schooling demersal fish to chicks at Jackpot Island compared to other years. Among the other years, the proportion of non-schooling demersal fish delivered to chicks at Jackpot Island (39 ± 8%) was similar to the proportion delivered to Naked Island chicks during the pre-

spill years (38 ± 5%), but less than during the post-spill years (58 ± 9%, n = 3). Gadids occurred in similar frequency in the diet of chicks at Jackpot Island (15 ± 7%, n = 5) and post-spill Naked Island (16 ± 13%, n = 4). In both areas, the abundance of gadids declined after 1994. However, the post-spill abundance of gadids in the chick diet remained greater than pre-spill diets at Naked Island (4 ± 4%). Other fish species comprised a minor proportion of diets of post-spill chicks: Jackpot Island (2 ± 2%) and Naked Island (5 ± 2%). The proportion of other fish species (10 ± 9%) was slightly higher for pre-spill chicks at Naked Island.

The lower abundance of high-quality fish in chick diets in oiled areas of PWS has the potential to constrain growth rates and survival of juvenile pigeon guillemots if delivery rates do not compensate for the lower energy content of chick meals. During periods of food shortages, brood reduction will offset the effect of low delivery rates. Single chicks received a significantly higher number of meals than individuals in two chick nests at Jackpot Island as well as in other studies (Prichard, 1997). I did not test the effect of brood size on delivery rates because information on brood size was not available for Naked Island delivery observations. The mean delivery rates I observed at Jackpot Island (0.86 ± 0.18 fish $nest^{-1}$ hr^{-1}, n = 4) and pre-spill Naked Island (0.90 ± 0.20 fish $nest^{-1}$ hr^{-1}) were not significantly higher than the post-spill Naked Island (0.74 ± 0.13 fish $nest^{-1}$ hr^{-1}; One-way ANOVA; F = 1.256, P = 0.330, df = 2, 9).

The observation of lower abundance of high-quality fish at post-spill Naked Island without a significant change in rate of fish delivered to chicks to compensate for the lower quality suggests lower energy content in the diet of Naked Island chicks during the post-spill years. The lower quality of the chick diet found at Naked Island can not be interpreted as a population limitation unless it is associated with lower productivity or fledgling survival (Cairns, 1988).

Productivity

Food limitations at the egg laying, incubation and chick rearing periods occur at different temporal scales or at different levels of prey supply (Carins, 1988). Therefore I examined the three components of productivity individually for evidence of food limitation (Table 3.4). The mean clutch size at Jackpot Island over the five-year study period (1.82 ± 0.09 eggs $nest^{-1}$) is only slightly higher than the mean clutch sizes observed at Naked Island during the pre-spill period (1.69 ± 0.14 eggs $nest^{-1}$) and during the post-spill period (1.72 ± 0.07 eggs $nest^{-1}$). Because there are no significant differences in the clutch size between these studies (One-way ANOVA; F = 1.766, P = 0.216, df = 2, 11), egg production is not impeding post-spill population growth.

During this study, I documented high abandonment rates at the Jackpot Island colony in two out of five years. Kuletz (1983) reported unusually low hatching rates at Naked Island in one out of three years. However, during the post-spill years Naked Island colonies experienced little interannual variation in hatching rates. Therefore, mean hatching success at Jackpot Island (0.55 ± 0.18 chicks egg^{-1}) was lower than the mean

Table 3.4 Comparison of clutch size, hatching success, fledgling success and productivity among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies. Means are based on pigeon guillemot nests found during the egg stage.

Period (years)	Study Area	Clutch Size (eggs/nest)	Hatching Success (chicks/egg)	Fledgling success (fledglings/chick)	Productivity (fledglings/egg)
Post-spill 1994-1998	Jackpot Island (unoiled)	1.82 ± 0.09	0.55 ± 0.18	0.48 ± 0.30 n = 5 0.61 ± 0.14 n = 4	0.27 ± 0.22 n = 5 0.35 ± 0.21 n = 4
Post-spill 1994-1998	Naked Island[a] (oiled)	1.72 ± 0.07	0.62 0.13	0.42 ± 0.17 n = 5	0.35 ± 0.15
Pre-spill 1979-1981	Naked Island[b]	1.69 ± 0.14	0.78 ± 0.07	0.77 ± 0.19[d]	0.47 ± 0.15
ANOVA	All	P = 0.216	P = 0.056	P = 0.041	P = 0.530

[a]Source: Golet *et al.* 2000 for 1994-1997 data and G. Golet contributed the 1989 data.
[b]Source: Oakley and Kuletz 1996, Golet *et al.* 2000
[c]Four year mean excludes 1996 breeding season at Jackpot due to high mink predation.
[d]Bonferroni t-test pair-wise comparisons ($P < 0.05$)

hatching success observed at Naked Island during both the pre-spill years (0.62 ± 0.13 chicks egg^{-1}) and the post-spill years (0.78 ± 0.07 chicks egg^{-1}; One-way ANOVA; $F = 3.790$, $P = 0.056$, $df = 2, 11$). Low hatching rates for guillemots has been associated with food shortages, presence of mammalian predators or frequent disturbance by humans (Ainley *et al.*, 1990; Drent *et al.*, 1964; Drent 1965; Hodder and Graybill, 1983; Emms and Morgan, 1987).

At Naked Island, Kuletz (1983) captured adults in their burrows during the 1980 nesting season and reported the high abandonment rate for birds she disturbed. At Jackpot Island, I avoided capturing incubating adults. The pattern of high nest abandonment in the last two years of the 5-year study suggests that factors besides our presence on the island prompted the birds to abandon their nests. There is indirect evidence that availability of prey during the incubation period may have declined in the last two years of this study. During the 1997 nesting season I observed substantial abandonment of nests coupled with a scarcity of herring among the fish delivered to chicks. The scarcity of juvenile herring in 1997 was associated with higher than average sea surface temperatures in the Gulf of Alaska that lasted from May 1997 to March 1998 (calculated from records of the National Data Buoy Center, NOAA). However, birds nesting at the Naked Island colonies did not experience similar food limitations. Sand lance in the Naked Island areas responded to warm waters by forming surface schools earlier in 1997 than observed in the two previous years (Brown, 1997).

In contrast, the abandonment I observed in 1998 occurred when herring were abundant in the chick diet. However, precipitation was twice as high in June of 1998 compared to the four other years of the study, and the second highest recorded in 16 years at Main Bay weather station (WRCC, 1999). High rainfall in June of 1998 may have represented poor foraging conditions for the birds at Jackpot Island. Kuletz (1983) documented that the rate adults provisioned chicks declined during periods of poor weather.

Thus, the abandonment I observed in both 1997 and 1998 may have been caused by an overall scarcity of food or poor foraging conditions. This interpretation is consistent with observations at the Farallon Islands by Ainley *et al.* (1990), who noted that low hatching success of pigeon guillemots was associated with warm-water years and low abundance of primary prey species, rockfish, *Sebates* spp., and that high hatching success was associated with cold water years that resulted in exceptional food availability.

Evaluating the role of food in the lower post-spill nestling survival at Naked Island is confounded by reports of increased nest predation (Oakley and Kuletz 1996). Mink are a major nest predator in PWS. The failure of the 1996 breeding season at Jackpot Island was caused by mink predation. The colonies at Naked Island suffered losses to mink predation on an annual basis and poor nesting success in 1998 was attributed to mink predation. Of the three pair-wise comparisons of nestling survival, only the comparison between pre-spill Naked Island and post-spill Naked Island was significantly different (One-way ANOVA; $F = 4.471$, $P = 0.041$, $df = 2, 10$; Bonferroi t-test, $t = 2.978$, $P = 0.042$).

Productivity was similar between Jackpot Island (0.35 ± 0.21 fledgling egg^{-1}, n = 4 years) and post-spill Naked Island (0.35 ± 0.15 fledgling egg^{-1}) because the lower hatch rates at Jackpot Island were balanced by lower survival of nestlings at Naked Island. The productivity of pre- spill Naked Island birds (0.47 ± 0.15 fledgling egg^{-1}) was not significantly greater than that of post-spill Naked Island birds (One-way ANOVA; F = 0.678, P = 0.530, df = 2, 10). Because of the small range observed between pre-spill and post-spill productivity, I suggest that other demographic factors, such as juvenile survival to breeding age or adult survival rates, are responsible for the post-spill populations trends at Naked Island.

Growth Rates, and Fledging Weight

In comparison to the measurement of fledglings per egg, growth rates and fledgling weights (Table 3.5) may be better measurements of overall reproductive performance because of their influence on post-fledgling survival (Greenwood *et al.,* 1993). From 1994 to 1998, the mean linear growth rate of chicks at Jackpot Island (18.6 ± 1.1 g^{-1} day-^{1}) was not significantly different from the pre-spill growth rate at Naked Island (20.4 ± 2.3 g^{-1} day^{-1}), or the post-spill growth rate at Naked Island (18.2 ± 2.6 g^{-1} day^{-1}) (One-way ANOVA; F = 1.123, P =0.363, df = 2, 10). In contrast, the mean fledging weight of chicks at Jackpot Island (482 ± 18 g chick^{-1}) was similar to the pre-spill fledging weight at Naked Island (480 ± 40 g;) and significantly greater than the post- spill fledging weight at Naked Island (446 ± 14 g) (One-way ANOVA; F = 9.788, P < 0.001, df = 2, 10). Post spill chicks at Naked Island fledged with similar wing length but at lighter weights than Jackpot Island and pre-spill chicks.

Table 3.5 Comparison of growth rates and fledging weights among unoiled Jackpot Island, oiled Naked Island, and pre-spill Naked Island pigeon guillemot studies.

Period (years)	Study Area	Growth Rate (grams /day)	Fledging Weight (grams)
Post-spill 1994-1998	Jackpot Island (unoiled)	18.6 ± 1.1	482 ± 18
Post-spill 1994-1998	Naked Island[a] (oiled)	18.2 ± 2.6	446 ± 14[c]
Pre-spill 1979-1981	Naked Island[b]	20.4 ± 2.3	480 ± 40
ANOVA	All	P = 0.363	P = 0.001

[a]Source: Golet *et al.* (2000) for 1994-1997 data and G. Golet contributed the 1989 data. [b]Source: Oakley and Kuletz (1996) and Golet *et al.* (2000). [c]Bonferroni t-test pair-wise comparisons (P < 0.05)

Guillemot chicks that are fed predominantly sand lance (>50%) have higher growth rates (Prichard, 1997, Golet *et al.*, 2000) and peak fledging weights (Golet *et al.*, 2000) than chicks that are fed predominantly gadids or non-schooling demersal fish. Similar results have been noted for rhinoceros auklets, *Cerorhinca monocerata,* (Bertram and Kaiser, 1993; Wilson and Manuswal, 1986) and Atlantic puffins, *Fratercula arctica,* (Harris and Hislop, 1978). Romano *et al.* (1999) reported that tufted puffins, *Fratercula cirrhata,* raised on schooling fish diets had greater fat reserves than birds raised on walleye pollock diets. In field studies at the Farallon Islands, Shultz and Sydeman (1997) reported that low fledging weights were associated with years of low food abundance. During the post-spill period, the combination of similar linear growth rates at Jackpot Island and Naked Island with the greater fledging weight at Jackpot Island suggests that food constraints were primarily realized late in the chick-rearing period and most likely associated with development of fat reserves.

Food limitations for fledglings are different than those of nestlings because fledglings do not receive food from their parents and fledglings are less experienced at capturing prey than adults. Fledgling survival may be increased through greater energy reserves and advanced development (Thompson and Flux, 1988). Fledging weight has been positively related to the survival of juveniles in many species (Manx shearwaters, *Puffinus puffinus,* Perrins *et al.*, 1973; South African gannet, *Sula capensis,* Jarvis, 1974; blue tit, *Parus caeruleus*, Nur, 1984; black-legged kittiwake, *Rissa tridactyla,* Coulsen and Porter, 1985; blackbird, *Turdus merula,* Magrath 1991; but see Harris and Rothery, 1985 on Atlantic puffins, *Fratercula arctica*.) For the 1994 and 1995 year-classes of guillemots, I found no significant difference in fledging weights between birds I observed in later years and birds I assumed dead. However, these fledglings were produced during a summer of high food abundance as indicated by delivery rates and abundance of schooling fish in the chick diet. Harris and Rothery (1984) suggested fledging weight is not critical to survival of puffins when post-fledging food resources are abundant. Although fledging weight has been suggested as an index of fledgling survival, this has not yet demonstrated for pigeon guillemots.

3.5.2 Demographic Limitations to Recovery

During my study, I observed a 36% increase in the number of adults attending the Jackpot Island colony, but annual population growth was not consistent among years. The 1998 increases in populations observed for several other southwestern and central PWS colonies suggest that conditions favorable for breeding success and fledgling survival existed throughout PWS in the mid-1990's. This pattern of colony growth may represent recruitment of a strong year-class after several years of lower recruitment.

Demographic factors that could contribute to lack of recovery at Naked Island compared to Jackpot Island, include lower production, higher net emigration, higher post-fledging mortality, or a combination of these factors. Because the production at Naked Island and Jackpot Island are similar, production does not appear to be the factor responsible for the lack of recovery at Naked Island, which is in agreement with the observations of Hayes and Kuletz (1997). With respect to emigration, we have one

documented case of a chick banded at Naked Island that subsequently nested at Jackpot Island in 1997 and 1998. Although this observation suggests the potential for emigration, we do not know the relative difference in emigration rates of pigeon guillemots between Jackpot Island and Naked Island. The higher fledging weights I observed at Jackpot Island in comparison to Naked Island suggest that recovery at Naked Island may be constrained through reduced fledging survival. It is also possible that recovery at Naked Island may be constrained through reduced adult survival.

Factors that contribute to the mortality of adult seabirds include predators, entanglement in fishing nets, food shortages, the long-term effects of oil exposure and disease. I observed mortality of nesting pigeon guillemot caused by mink predation, similar to that reported by others working in guillemot colonies (Petersen, 1981; Folkestad, 1982; Barrett and Vader, 1984,), however we do not know if adult predation is higher at Naked Island than colonies in unoiled areas. Similarly, we have no reason to believe that there are differences between Naked Island and unoiled populations in adult mortality caused by gillnet fisheries; gillnets are known to be a significant source of mortality for seabirds (DeGange *et al.,* 1993; Carter and Sealy, 1982; Takekawa *et al.,* 1990). The solitary foraging habits and moderate diving depth of guillemots may make them less susceptible to gillnet losses unless the nets are in the vicinity of colonies (Evans and Nettleship, 1985). Gillnets are used in PWS herring and salmon fisheries. During this study the herring fisheries was closed for three years. Wynne (1990, 1991) reported no mortality of pigeon guillemots associated with the PWS Copper River salmon gillnet fisheries.

Naked Island could also be experiencing higher adult mortality because of the long-term effects of oil exposure. In chapter two I presented preliminary evidence that adults from oiled areas have elevated aspartate aminotransferase concentrations, which is consistent with hepatocellular injury. Confirmation of hepatocellular injury requires histological examination of liver tissue. Additional studies to fully evaluate the health of adults residing in oiled areas would help to evaluate the issue of whether adult mortality caused by the long-term effects of oil exposure plays a role in the lack of recovery at Naked Island.

Several studies have found declines in seabird populations associated with declining abundance of herring or sand lance (Atlantic puffins: Lid, 1981; Harris and Wanless, 1991; black-legged kittiwakes: Heubeck and Mellor, 1994). The higher fledging weights I observed at Jackpot Island compared to Naked Island, suggest that food limitation may be expressed in the later part of the breeding season. If late-season food shortages affect body condition of adults, then these shortages may affect survival. I recommend that adult survival and late-season body condition of adults be monitored for breeding birds at Naked Island and Jackpot Island to determine whether late-season food shortages have the potential to cause higher adult mortality.

For an injured population to return to their initial levels after a major mortality event, such as an oil spill, environmental conditions must favor breeding success, survival of juveniles to breeding age and survival of breeding adults. My analysis indicates that lack of recovery of pigeon guillemot populations in oiled areas of PWS is likely associated with lower quality prey, which results in lower fledging weight, and

which may constrain recovery through reduced fledgling survival. Food shortages and the long-term effects of oil exposure may also constrain recovery if they result in lower adult survival.

CONCLUSIONS

1. From 1994 to 1998, I observed a positive trend in the Jackpot Island pigeon guillemot population. For four consecutive years, the number of birds at Jackpot Island met or exceeded the previous year's counts.

2. Jackpot Island experienced high rates of nest abandonment in 1997 and 1998. High sea-surface temperatures in June of 1997 and high rainfall in June of 1998 may have caused poor foraging conditions during the incubation period. These factors may have contributed to the high abandonment rates observed during those two years.

3. Productivity losses to mink predation occurred only in one out of five breeding seasons at Jackpot Island. The presence of mink on the island in 1996 resulted in higher morality rates for both adults and nestlings. The relative isolation of Jackpot Island from mink predation may explain the difference in nesting density between Jackpot Island and the shoreline of the mainland.

4. The proportion of high-lipid fish in the diet of Jackpot Island chicks was higher than the post-spill Naked Island, but lower than pre-spill Naked Island. The abundance of herring was significantly higher in the diet of Jackpot Island chicks compared to pre- and post-spill Naked Island chicks. Delivery rates were not significantly different among the three studies. Therefore, the quality of chick diet was higher at Jackpot Island than post-spill Naked Island, but not pre-spill Naked Island.

5. Mean fledgling success, productivity rates and growth rates at Jackpot Island and post-spill Naked Island were similar. However, fledging weights were significantly higher at Jackpot Island. This observation suggests that food limitations at Naked Island was experienced in the later stages of the nesting period. Lower fledging weights at Naked Island may lead to a lower post-fledgling survival rate.

6. Population trends at unoiled Jackpot Island and oiled Naked Island did not exhibit similar temporal patterns. Because mean productivity levels did not differ between the two areas, the disparity in fledging weight may partially account for these varying population trends. It is unknown if food limitation at Naked Island extends to breeding adults. I recommend comparative studies on adult survival rates and late summer body condition.

4

CHAPTER FOUR

STATUS OF RECOVERY OF PRINCE WILLIAM SOUND'S PIGEON GUILLEMOT POPULATION.

Pigeon guillemots and their foraging areas were impacted by the 1989 *Exxon Valdez* oil Spill (EVOS) (Piatt *et al.*, 1990; Spies *et al.*, 1996). In the years immediately following EVOS, no significant increase in pigeon guillemot abundance were reported at several spatial scales within Prince William Sound (Oakely and Kuletz, 1993; Murphy *et al.*, 1995; Agler and Kendall, 1996). The pigeon guillemot population had not recovered according to the conventional measurement of recovery, the numeric replacement of individuals directly killed by oiling. This definition assumes that current environmental conditions support recruitment. Oakley and Kuletz (1996) pointed out that survey estimates prior to the spill (1972, 1984-85) indicated that the abundance of pigeon guillemots in PWS were declining. Present oceanic conditions may not support population growth. Therefore, comparing population trends in oiled areas to adjacent unoiled areas might be a more suitable indicator of recovery than comparing post-spill populations to pre-spill abundance levels. My observations at unoiled Jackpot Island and several other colonies in southwestern PWS indicate that it was possible for pigeon guillemot populations to substantially expand in the post-spill period, between 1994 and 1998. However, during the same period, the abundance of pigeon guillemots at oiled Naked Island dropped below the population level measured in 1994. Several factors may constrain population growth in oiled areas: the physiological effects of oil exposure on guillemots, food limitations, predation, and other factors, such as disease. I assessed the role of oil exposure and food limitations in the recovery of pigeon guillemot populations affected by the EVOS.

Before this study, information on the breeding success and diet of pigeon guillemots in PWS was only available for Naked Island colonies in central PWS. In 1979, ten years prior to the spill, Naked Island supported 1,200 pigeon guillemots (Oakely and Kuletz, 1996). Since the spill, the annual counts of pigeon guillemots along Naked Island shorelines have oscillated between 400 to 700 birds (Hayes and Kuletz, 1997). The pre-spill (1979 to1981) and post-spill studies (1989 to 1991, 1994 to 1998) of Naked Island pigeon guillemots represent 12 years of data on breeding success and diet. Because of the time laps between studies and oiling of Naked Island, researchers did not have the information necessary to determine whether the lack of population growth in the nineties would have occurred in the absence of EVOS or not (Oakley and Kuletz, 1996; Hayes and Kuletz, 1997; Golet *et al.*, 2000).

To provide insight on mechanisms behind the population trend in oiled areas, I collected information on the health, food habits and population dynamics of pigeon guillemots at Jackpot Island. This unoiled reference site is located in southwestern PWS. To make comparisons between an oiled area and a reference site, the reference site must

meet three criteria: (1) the foraging habitats of the two areas must be similar except for oiling; (2) the movement of birds between the reference site and the oiled site must be limited, and (3) the reference site must have an adequate number of accessible nests for logistical and statistical purposes.

Jackpot Island was selected as a reference site because it offered a large concentration of nests located at a fair distance (55 km) from oiled Naked Island. Selecting reference sites with similar oceanographic conditions to oiled study sites is difficult because oil was not randomly distributed in PWS, nor was the degree of oiling consistent along shorelines. Wind and current patterns responsible for the distribution of oiling along PWS shorelines may also be correlated with other less obvious habitat variables (Laur and Haldorson, 1996). I found that birds in the two areas feed on similar prey species, but other measurements of foraging habitat varied. The shoreline density of nests in the greater Jackpot area was low, compared to Naked Island's. Breeding densities may be related to the quality of the forage area, availability of nesting sites, or predator densities. The lower shoreline density of pigeon guillemots in unoiled areas is an unavoidable weakness in my study. Despite these limitations for a direct comparison to Naked Island, the diet of Jackpot Island birds typifies the oil exposure levels and foraging habits of breeding birds in the unoiled area of Prince William Sound.

To evaluate the health of birds in 1997, I compared the hematological and plasma biochemical profiles among populations of pigeon guillemots in oiled and unoiled areas. If the effect of chronic exposure to residual oil is significant enough to limit the recovery of pigeon guillemots in PWS, then I expected the blood parameters to differ between populations in oiled and unoiled areas in a pattern that would be consistent with toxic responses. I examined chicks and adults separately, because adults have greater opportunities for exposure to residual oil than nestlings residing in burrows. With the 30-day old chick, I found calcium and mean cell volume were significantly different between populations in oiled and unoiled areas. However, these blood biomarkers provided little evidence of continuing oil injury to chicks. Preliminary data from adults indicated elevated aspartate aminotransferase activity (AST) for adults in the oiled area, which is consistent with hepatocellular injury. These findings indicate that exposure to residual oil elicited a physiological response in pigeon guillemots. The consumption of invertebrates by adults (Oakely, 1981; Ewins, 1993) may contribute to difference in biomarker responses between adults and chicks. Bioaccumulation of polynuclear aromatic hydrocarbons is greater in invertebrates than fish (Gibson, 1977). The energetic costs of this physiological response and its influence on adult survival and productivity are unknown. I recommend studies that fully evaluate health and survival rates of adults residing in oiled areas.

Alcid productivity is affected by availability and abundance of food in the vicinity of their nests (Evans and Nettleship, 1985). To evaluate food resources and limitations during the breeding season, I examined the diet, survival, growth performance and fledging weight of chicks. Hayes and Kuletz (1997) suggest that the availability of high-lipid fish was limiting the growth of the population at Naked Island. They reported a decline in the abundance of high-lipid fish in the diet of chicks between the pre-spill (1979-81) and post-spill (1989-90, 1994-96) studies. In PWS, distribution of pigeon

guillemot colonies in non-glaciated waters, overlap with the summer distribution of juvenile herring and sand lance documented by Brown (1997, 1998). Thus populations in both oiled and unoiled areas of PWS would be influenced with changes in abundance of high-lipid fish. Little is know about the abundance of fish in areas with tidewater glaciers, because silt and ice prevent aerial and sonar detection of surface schooling fish. I expanded on the pre-and post-spill comparisons of Hayes and Kuletz (1997) to include post-spill data for an unoiled area. I also truncated the post-spill Naked Island data set to the 5-year period corresponding to the data from the unoiled area.

I evaluated the quality of chick diet based on the proportion of the meal that were high-lipid fish and the frequency at which meals were delivered to the chicks. I found that the proportion of high-lipid fish in the diet of Jackpot Island chicks was higher than post-spill Naked Island chicks, but not pre-spill Naked Island chicks. Yet, delivery rates were not significantly different among the three studies. Similar to the observations by Hayes and Kuletz (1997), I concluded that the quality of post-spill diet was lower than the pre-spill diet. However, the quality of the post-spill diet was higher for chicks at Jackpot Island than for the chicks at Naked Island. The abundance of herring at Jackpot contributed to post-spill regional differences in diet quality. Although food limitation was not expressed in the linear growth rates of chicks, the difference in diet quality between Jackpot Island and Naked Island was expressed later in the chick rearing period and translated into lower fledging weights for Naked Island chicks. Fledging weight has been suggested as an index of fledgling survival (Perrins *et al.,* 1993; Jarvis 1974). Consequently, the lower fledging weights at Naked Island suggest that recovery at Naked Island may be constrained through reduced juvenile survival.

Populations increase only by recruitment of natal juveniles and immigration. I noted that mean productivity rates over the five-year period were not significantly different between Jackpot and Naked Island, but lower fledging weights at Naked Island may lead to regional differences in recruitment rates. Martin (1987) suggested that productivity be defined by both the number of fledglings that survive to breed and by the negative effects of the breeding effort on the parent. Tinbergen *et al*. (1984) reported that the cost of reduced survival for adults was expressed in years when winter food abundance was low. Guillemot species experience seasonal shifts in their diet from a fish-dominated diet in the summer to a mixed diet of fish and invertebrates in the winter (Vermeer *et al.,* 1987). I recommend that winter food limitations be examined for pigeon guillemots in oiled and unoiled areas.

Cairnes and Elliot (1987) theorize that the rate of population recovery from a large mortality event such as an oil spill depends on the size and location of neighboring colonies. The spatial distribution of breeding pigeon guillemots in PWS does not present a high immigration potential for Naked Island. According to the colony surveys of Sanger and Cody (1994), the density of pigeon guillemots in the area surrounding Naked Island and its associated islands in central PWS, is much lower than the Naked Island complex. The observation by Murphy *et al.* (1997) that the abundance of pigeon guillemots occupying oiled shorelines did not increase the first two years after the spill supports the notion that immigration potential in PWS is low. The failure of the Naked Island population to maintain levels above the 1990 census level (723 birds) suggests that

recruitment as well as immigration are insufficient to compensate for adult morality and emigration losses.

To maintain a stable population, breeding pairs must produce at least two fledglings over the course of their lifetime. Based on present production (0.6 fledgling per nest) and fledgling survival (36%) rates at Jackpot Island, a breeding pair would have nest 9 years to insure two of their fledglings survived to breeding age, an annual survival rate of 90%. However, lower adult survival rates lower reported in the literature for pigeon guillemot (80%; Nelson, 1981) and black guillemot (85%; Asbirk 1979; 89%; Frederiksen, 1998). Lower abundance of high-lipid fish in the oiled area and the long-term effects of oil exposure are potential mechanisms to create regional differences in adult survival rates. Again, I recommend future research on adult survival rates.

Herring was the major contributing factor to the high-lipid diet at Jackpot Island. The herring population in PWS peaked in 1989 and crashed in 1993, just prior to this study. In 1989 herring composed 25% of the diet of Naked Island chicks (Oakely and Kuletz, 1996). After 1993, only 5% fish in the Naked Island chick diet was herring (G. Golet personal communication). In contrast, a third of fish delivered to chicks at Jackpot Island were herring. The local abundance of juvenile herring is attributed to the size of local spawning biomass as well as favorable spring wind and current patterns for retention of planktonic larva (Stokesbury *et al.,* 1997). Few herring spawn in Southwest PWS and the source of Jackpot Bay's juvenile herring population has not been traced (Stokesbury *et al.,* 1997). Naked Island has both an intermittent spawning population (Funk, 1995) and a favorable location for receiving larva from other spawning areas (B. Norcross, personal communication). Differences in the abundance of herring may be attributed to physical differences in nearshore habitats, natural cycles in herring populations, or to the lingering effects of EVOS.

The difference in abundance of herring and sand lance at Jackpot Island and Naked Island was also noted in the diet of marble murrelets (Kuletz and Kendall, 1999), and in Brown's (1997, 1998) aerial surveys of surface schooling fish. Little is known about historic population trends in the abundance of sand lance because lack of commercial interest in the species. The abundance of high-lipid fish in the diet of oiled Naked Island chicks continued to remain low from 1990 to 1998. Declines in high-lipid fish abundance between the pre-spill and post-spill studies may be related to declines in primary productivity (Bertram *et. al.,* 1991), diseases and oiling (Hose *et al*., 1996, Carls *et al.,* In press).

If the effect of EVOS on the pigeon guillemot populations was limited to the 1989 mortality event, then I would expect by 1994, that the population dynamics in oiled and unoiled areas would follow similar trends. In fact population trends differed between oiled Naked Island and unoiled Jackpot Island, which suggests regional differences in one or more demographic parameters. Productivity was similar between the two areas but indirect measurements suggest potential for lower juvenile and adult survival rates for populations residing in oiled areas. Blood biomarkers provided evidence that residual oil in the nearshore environment is a potential health risk to adult birds. But, I lack information to determine at what scale oil toxicity currently inhibits adult survival or productivity. I presented evidence that food limitations at Naked Island may lead to

lower fledgling survival. The recovery of pigeon guillemots from EVOS depends on the status of food resources in the nearshore habitat. The low abundance of high-lipid fish during the breeding season was one mechanism limiting population expansion in oiled areas. Current environmental conditions in the oiled areas of PWS do not appear to support growth and recovery of pigeon guillemot populations.

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Biosketches of
Nearshore Vertebrate Predators Team
(B)

PERSONNEL

Dr. Brenda Ballachey is a Research Physiologist at the U.S. Geological Survey (USGS), Alaska Biological Science Center. She was Project Leader for sea otter National Resource Damage Assessment studies from 1990 through 1996 and has been involved in all aspects of post-spill research on sea otters. She has authored or coauthored more than 25 peer-reviewed publications and is currently a co-principal investigator for the Nearshore Vertebrate Predator (NVP) project, examining effects of residual oil on health and recovery of sea otters and other NVP study species.

Mr. Jim Bodkin, Research Wildlife Biologist, is the Team Leader for studies of coastal marine research at the USGS Alaska Biological Science Center in Anchorage. He has 22 peer-reviewed scientific publications and directs an active sea otter research program. He has studied and published articles on sea otter population biology, natural history, and community ecology since 1988. Jim has been a principal investigator in *Exxon Valdez* oil spill related research since March 1989.

Dr. R. Terry Bowyer, Professor of Wildlife Ecology, is the Deputy Director of the Institute of Arctic Biology at the University of Alaska Fairbanks. Dr. Bowyer has an extensive publication record (more than 80 scientific articles). He has conducted extensive research on river otters and impacts of *Exxon Valdez* oil spill on this species.

Dr. Thomas A. Dean is the President of the ecological consulting firm Coastal Resources Associates, Inc., in Vista, California. He has over 20 years of experience in the study of nearshore ecosystems and has authored more than 20 publications, including several papers dealing with sea urchin and kelp interactions. He has extensive experience in long-term monitoring studies with marine plants and invertebrates. He has had a major role in both the shallow subtidal and intertidal *Exxon Valdez* oil spill investigations since 1989.

Dr. Lawrence Duffy, Professor of Chemistry and Biochemistry at the University of Alaska Fairbanks, has been working in the area of toxicology for 17 years and is a member of the International Society of Toxicology. He has studied various bacterial and mammalian toxins. Since the *Exxon Valdez* oil spill, he has published several papers related to developing biomarkers. He is currently on the editorial board of the Science of the Total Environment. At the University, he teaches “Environmental Biochemistry and Biotechnology” and is a member of the Environmental Chemistry Program and Mammal Group.

Dr. Daniel Esler, Research Wildlife Biologist with the USGS, Alaska Biological Science Center, is now a University Research Associate with the Centre for Wildlife Ecology, Simon Fraser University. He has conducted waterbird research in arctic and subarctic regions of Alaska and Russia for the past 11 years. Since 1995, he has served as Project Leader for harlequin duck studies as part of the *Exxon Valdez* Oil Spill Trustee Council-sponsored NVP project. He has authored more than 20 peer-reviewed journal publications and numerous reports and presentations addressing research and issues in waterbird conservation.

Dr. Leslie Holland-Bartels is the former head of the Marine and Freshwater Ecology Research Program for the Alaska Biological Science Center and current Director of the USGS Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin. In Alaska, she directed the research of 17 senior scientists in the areas of seabirds, marine mammals, anadromous fisheries, and associate habitat and population issues. She has 24 years experience in aquatic ecology and more than 30 publications in national scientific journals on subjects including contaminants, ecology of invertebrates, fisheries, water quality, and aquatic ecology.

Dr. Stephen C. Jewett currently serves as a Research Professor and the Scientific Diving Officer at the School of Fisheries and Ocean Science, University of Alaska Fairbanks (UAF), since 1975. While at UAF, he has been involved in numerous benthic and intertidal investigations throughout Alaska that emphasize assessment and/or monitoring. He has authored more than 30 publications in scientific journals and books. In addition to his role in the NVP project, he was co-principal investigator on the *Exxon Valdez* oil spill shallow subtidal investigations (1989–95) in Prince William Sound and is currently examining cytochrome P450 in nearshore fishes in the Sound.

Dr. Lyman McDonald is a Senior Biometrician and the President of Western EcoSystems Technology, Cheyenne, Wyoming. He has 30 years of comprehensive experience in the application of statistical methods to design, conduct, and analyze environmental and laboratory studies. He has designed and managed both large and small environmental impact assessment and monitoring programs.

Dr. A. David McGuire is an Assistant Professor of Biology and Wildlife and an Assistant Leader of the Alaska Cooperative Fish and Wildlife Research Unit at the University of Alaska Fairbanks. His research interests include operation of ecological processes at large spatial scales, ecological modeling, and global change biology.

Dr. Charles E. O'Clair, Fishery Research Biologist, is now retired from the National Marine Fisheries Service, Auke Bay Laboratory, in Juneau, Alaska. He has more than 16 peer-reviewed scientific publications. His research experience includes 9 years of damage assessment and restoration process research related to the *Exxon Valdez* oil spill. Other research experience includes 12 years of field and laboratory work on the effects of oil pollution and logging practices on marine benthic invertebrates and research on the ecology and behavior of Dungeness, King, and Tanner crabs.

Dr. Alan Rebar is the Dean of the School of Veterinary Medicine and the Professor of Veterinary Clinical Pathology at Purdue University. He is internationally recognized as an expert in the field of clinical pathology and toxicology. He has been involved in *Exxon Valdez* oil spill studies of sea and river otters since 1991.

Dr. Paul W. Snyder is an Assistant Professor of Pathology and Immunotoxicology and the Director of the Clinical Immunology Laboratory of the Department of Veterinary Pathobiology,

Purdue University. He is also a Diplomat of the American College of Veterinary Pathologists. His research interests are in the area of mechanism-based studies on the pathology and immunology of xenobiotics on biological systems.

Dr. Glenn R. VanBlaricom is an Assistant Unit Leader (Wildlife), Washington Cooperative Fish and Wildlife Research Unit, and an Associate Professor of Fisheries in the School of Aquatic and Fishery Sciences, University of Washington. He has conducted research on coastal ecosystems since 1970 and has been involved in research on sea otters and their ecosystems for 22 years. He has more than 30 peer-reviewed scientific publications.

Cooperators:

Mr. Timothy D. Bowman is a Wildlife Biologist for the U.S. Fish and Wildlife Service, Migratory Bird Management Project. He was principal investigator for the *Exxon Valdez* oil spill damage assessment study on bald eagles and has conducted aerial and ground surveys of waterfowl and seabirds throughout Alaska. He has nine publications in national peer-reviewed journals.

Dr. Gregory H. Golet is a Wildlife Biologist for the U.S. Fish and Wildlife Service. He has studied seabirds in Prince William Sound since 1989 and has published in national peer-reviewed journals.

Dr. John Stegeman is a Research Scientist at Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. He is internationally recognized as an expert in the area of Cytochrome P450 biomarkers of hydrocarbon exposure.