

*Exxon Valdez* Oil Spill  
Restoration Project Final Report

Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators  
Restoration Project 030423  
Final Report

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December 2003

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## Restoration Project //423

### Final Report

**Study History:** This project began in April 1999 with the approval of a 5 year plan by the *Exxon Valdez* Oil Spill (EVOS) Trustee Council. The project is an extension of Restoration Project 93043-2, designed to develop an aerial survey method for sea otters in 1993, and the Nearshore Vertebrate Predator Project, 95-99025, designed to assess recovery of the nearshore ecosystem affected by the *Exxon Valdez* oil spill. This project has focused on recovery of sea otter and harlequin duck populations. Sea otter research has included surveys of sea otter abundance in Prince William Sound (including the western Sound and oiled and unoiled intensive study areas), collection of beach-cast sea otter carcasses for estimation of age specific mortality, evaluation of biomarkers of oil exposure (2001), and estimates of the density and sizes of green sea urchins from the intensive study areas. The sea otter components have been closely linked to Restoration Projects 01534, 03585, and //0620 (continuing in FY04), which have further addressed questions of bioavailability of lingering oil and sea otter population recovery. Harlequin duck research included assessment of survival rates and biomarker induction, addressed through both field studies and captive experiments at the Alaska SeaLife Center in Seward.

**Abstract:** Sea otters (*Enhydra lutris*) and harlequin ducks (*Histrionicus histrionicus*) are two species for which there was strong evidence that (1) population recovery from the spill had not occurred by 1998, (2) hydrocarbon exposure was higher in oiled areas of Prince William Sound than in unoiled areas, and (3) demographic differences between areas, particularly survival, were a likely mechanism explaining lack of full recovery. This study was initiated to continue to track the progress of population recovery and to more closely examine links between demography and oil exposure toward understanding the process of population change.

Between 1993 and 2003 we detected an increase of nearly 600 sea otters in western Prince William Sound. During this same period sea otter abundance in Prince William Sound has remained relatively stable at about 9,000 individuals. At northern Knight Island, where the effects of the *Exxon Valdez* oil spill were greatest, we have failed to detect any increase in sea otter abundance since we began surveys in 1993, and the population in this area has declined by 66% since 2001. During this same period, we have seen a significant increasing trend in population size at unoiled Montague Island from about 300 in 1993 to more than 600 in 2003. From 1999-2003, we collected 173 sea otter carcasses from beaches in western Prince William Sound. The proportion of prime age animals in our sample was 35%, compared to 17% prime age carcasses recovered from the same area prior to the spill, implicating adult mortality as a factor contributing to delayed recovery. In July 2001-2003, blood and liver samples were collected from sea otters at northern Knight and Montague Islands. Livers from the oiled area had a higher incidence and severity of alterations, and cytochrome P4501A induction continued to be higher in the oiled area, similar to results from 1996-98, indicating ongoing exposure to aromatic hydrocarbons. The elevated cytochrome P4501A values and liver pathologies observed at northern Knight Island suggests exposure to hydrocarbons continues to constrain recovery of

sea otters, although a declining trend in cytochrome P4501A values over time may indicate diminishing exposure.

As part of harlequin duck studies, we conducted experiments with captive birds at the Alaska SeaLife Center to evaluate responses to oil ingestion and plumage oiling that could lend insight into mechanisms leading from oil exposure to mortality in Prince William Sound. Also, we conducted field studies in which we measured cytochrome P4501A induction as a measure of exposure to residual Exxon Valdez oil, and quantified survival probabilities of radio-marked females in oiled and unoiled areas of Prince William Sound.

In our captive bird experiments we found, predictably, that oil ingestion resulted in significant cytochrome P4501A induction relative to controls. The levels of induction were very similar to levels observed in wild birds, leading us to believe that results from our experiments have strong inference for understanding potential mechanisms constraining wild populations of harlequin ducks in Prince William Sound. We found that feeding and maintenance behaviors were not strongly affected by oil ingestion. However, plumage oiling resulted in important changes to behavior; in particular, feeding activity decreased and maintenance increased with increasing plumage oiling. We suggest that the behavior changes associated with plumage oiling would not be sustainable under natural conditions and, hence, that external exposure to residual oil would most likely have significant demographic consequences, i.e., mortality.

During field studies, we documented significantly higher cytochrome P4501A induction in oiled areas of Prince William Sound relative to unoiled during November 2000. However, during Novembers of 2001 and 2002, cytochrome P4501A induction was becoming more similar between oiled and unoiled areas, with point estimates nearly indistinguishable in 2002. We found that relative survival in oiled and unoiled areas closely tracked cytochrome P4501A results; point estimates for female winter survival differed between areas during winter 2000-01, but were similar during winters 2001-02 and 2002-03. These data are critical for understanding the process of recovery of harlequin duck populations, and suggest that exposure to residual Exxon Valdez oil, and the associated demographic consequences, are waning and nearly gone. However, for return to pre-spill abundance, numbers in the oil spill area must increase via the processes of recruitment and immigration. To consider the process of immigration, we evaluated dispersal probabilities within and between years using banded and radio-marked females. We found very high site fidelity (low dispersal) at relatively small scales (lo's of kilometers), suggesting that full demographic recovery, despite abatement of direct effects of oil, may be a very long (decadal-scale) process.

**Key words:** cytochrome P4501A, demography, *Enhydra lutris*, Exxon Valdez, harlequin ducks, *Histrionicus histrionicus*, oil spill, population status, Prince William Sound, sea otters.

**Project Data:** Data will be kept in digital format (MS Excel) at the USGS Alaska Science Center in Anchorage.

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## INTRODUCTION

The nearshore environment of Prince William Sound (PWS) received about 40% of the oil spilled after the *Exxon Valdez* ran aground (Galt et al. 1991). Recent studies have identified unanticipated quantities of residual *Exxon Valdez* oil remaining along PWS shorelines that were most affected by the 1989 spill (Short et al. 2003). Further, the lingering oil has been protected from weathering, and remains toxic and bio-available to organisms that occupy intertidal habitats, particularly in the northern Knight Island Archipelago (Short et al. 2003).

Concerns about ecosystem processes, ecosystem recovery from the spill, and restoration needs resulted in a suite of studies sponsored by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC), including the 1995-99 Nearshore Vertebrate Predator project (NVP; Holland-Bartels 2002). Principal NVP findings include an apparent lack of recovery to pre-spill levels of population abundance among sea otters and harlequin ducks, both invertebrate feeders in the nearshore ecosystem (Bodkin et al. 2002, Esler et al. 2002). Available evidence suggests that sea otters from the spill area suffered higher rates of mortality (Monson et al. 2000), and their exposure to hydrocarbons was elevated compared to unoiled areas (Ballachey et al. 2002b), through at least 1998. Additionally, several sea otter prey species showed increased proportions of large individuals in areas where sea otter populations had failed to recover, a pattern consistent with reduced predation (Dean et al. 2000, Dean et al. 2002). Harlequin ducks had lower survival in oiled areas of PWS relative to unoiled areas through 1998 (Esler et al. 2000c), densities were lower on oiled areas than expected based on habitat attributes (Esler et al. 2000a), and hydrocarbon exposure was higher in oiled areas (Trust et al. 2000). For both sea otters and harlequin ducks, these data suggested that continuing effects of the EVOS were constraining full population recovery almost a decade after the spill.

In 1999, we implemented Restoration Project //423, *Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators*, applying the components of previous research that were most effective and statistically powerful at identifying if and how recovery was constrained among sea otter and harlequin duck populations in western PWS (WPWS). For sea otters, our efforts have included aerial surveys of distribution and abundance, collection of beach-cast carcasses to evaluate relative survival of different age classes, and biological sampling to monitor health and biomarkers of oil exposure. We have also examined ecological interactions between sea otters and green urchins, a preferred invertebrate prey. For harlequin ducks, we have monitored winter survival and biomarkers of oil exposure to evaluate the process of recovery. We also have collected data to assess mechanisms by which oil could affect harlequin duck survival, through both field studies and experiments with captive birds. Herein, we report our findings from the //423 studies conducted during 1999-2003, and review our findings in the context of prior results, primarily reported under the suite of NVP (99925) restoration studies, and resulting publications. The report is structured into two sections, one on sea otters and green urchins and one on harlequin ducks. There is a common introduction, conclusion, acknowledgement, and list of references.

## **PROJECT OBJECTIVES**

### Sea otters

- A. Estimate and compare sea otter abundance and population trends over time between oiled and unoiled areas within WPWS, over all of WPWS, and over all of PWS.
- B. Collect age at death data from sea otter carcasses found beach cast in WPWS.
- C. Measure and compare CYP1A levels in blood samples from sea otters in oiled and unoiled areas of WPWS.

### Sea urchins

- A. Estimate abundance and size class composition of green sea urchins in oiled and unoiled study sites.

### Harlequin ducks

- A. Estimate winter survival rates of harlequin ducks in relation to area (history of oil contamination) and indices of oil exposure (CYP1A induction).
- B. Monitor progress of harlequin duck population recovery via tracking of survival rates and CYP1A induction in oiled and unoiled areas.
- C. Quantify behavioral and CYP1A responses to oil exposure under controlled, captive conditions.

## **STUDY AREA**

This research is focused on WPWS, the site of NVP studies. We surveyed sea otter population sizes at three geographical scales, PWS, WPWS, and an oiled and unoiled area within WPWS. The PWS survey area included all of Prince William Sound, with the exception of Orca Inlet, near Cordova. The WPWS study area includes all oiled areas of Prince William Sound as well as areas that are contiguous to oiled areas (Figure 1). Intensive survey areas include an oiled area identified as the shorelines of the northern Knight Island archipelago between NW Herring Bay and SE Bay of Isles (Figure 1). Oiling was heaviest here, and population levels of sea otters are generally lower here than in other areas of PWS that were not oiled. The unoiled area is along the northwestern shore of Montague Island between Graveyard Point and southern Stockdale harbor. Collections of beach-cast sea otter carcasses occurred only along or adjacent to WPWS shorelines oiled in 1989. Capture and sampling of sea otters was at northern Knight Island and Montague Island, in the same areas as included in the intensive surveys. Sampling of sea urchins took place in the oiled area at Herring Bay and Bay of Isles on Knight Island and within the shoreline surveyed for sea otters at Montague Island. Harlequin duck study sites also were those used in previous NVP work: unoiled Montague Island and oiled Green Island,

Knight Island, Crafton Island, Main Bay and Foul Bay. Captive studies on Harlequin ducks were done at the Alaska SeaLife Center in Seward, Alaska.



Figure 1. Prince William Sound, Alaska. Dashed black line delineates the Western Prince William Sound (WPWS) study area. Solid red and yellow lines depict transects flown for aerial sea otter surveys.

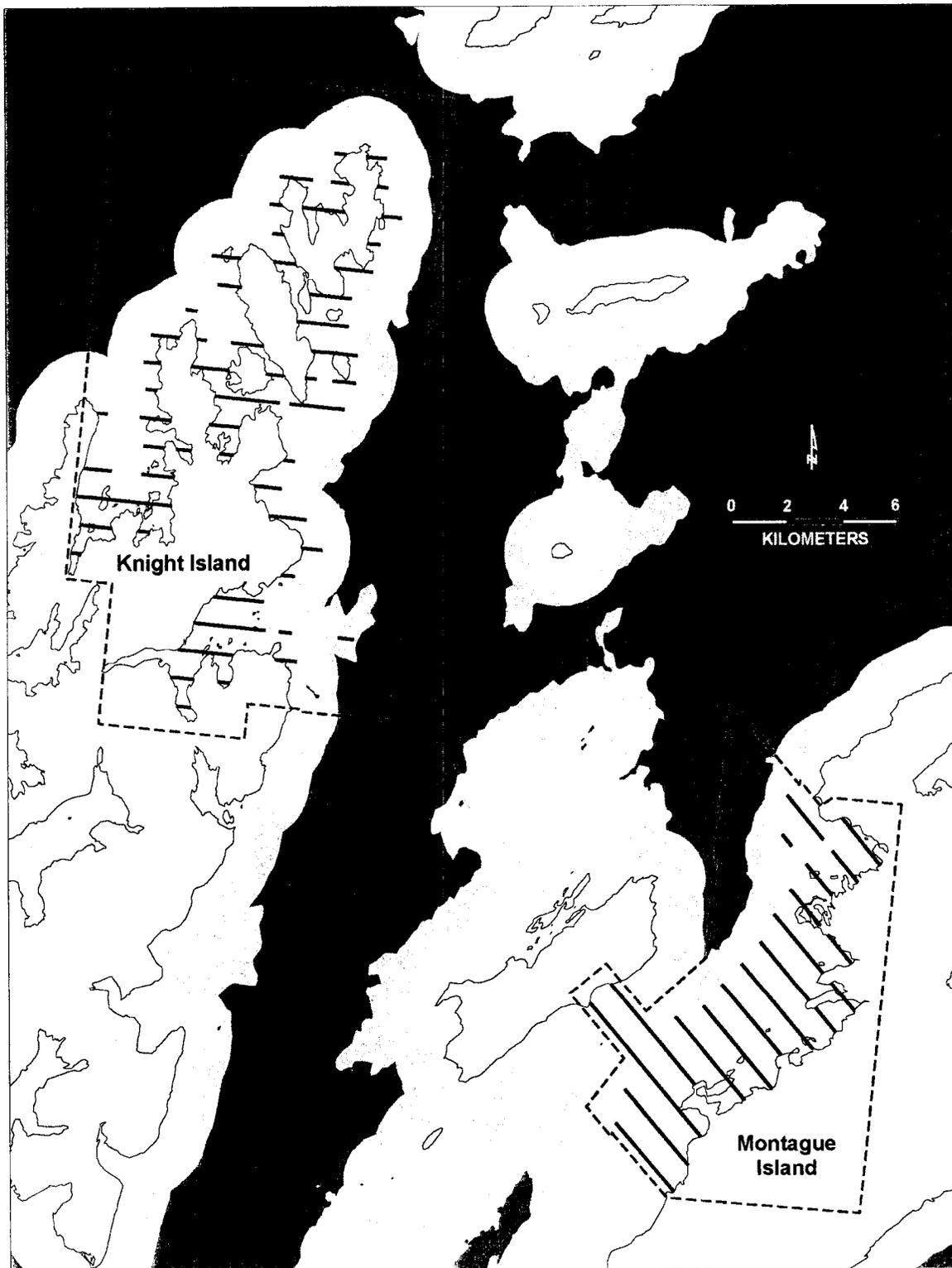


Figure 2. Knight and Montague Island intensive study areas in western Prince William Sound. Dashed black lines delineate the study areas. Solid red and yellow lines depict transects flown for aerial sea otter surveys.

## SECTION 1. SEA OTTERS

### Introduction

In the sea otter (*Enhydra lutris*) component of Project //423, we monitored patterns of population demographics, including abundance and survival, individual metrics of health and oil exposure, and processes underlying change in the nearshore system. We selected sea otters because they (1) were injured by the oil spill and continued to show evidence for lack of a full recovery, at least through 1998, (2) presumably reflect the health and recovery status of the nearshore system generally, and (3) are represented by abundant post-spill information that can be utilized for long-term restoration monitoring. Specific research activities included aerial surveys of sea otter abundance and distribution, collection of beach-cast carcasses to evaluate ages at death, and biological sampling to monitor body condition, blood parameters, and the CYP1A biomarker of oil exposure. In addition, we examined ecological interactions between sea otters and green urchins, a common and preferred invertebrate prey.

Sea otter populations in western PWS (WPWS) were injured as a result of the *Exxon Valdez* oil spill (EVOS). Estimates of sea otter mortality due to the spill range from 750 to 2,650 individuals (Garrott et al. 1993, Garshelis 1997). A population model (Udevitz et al. 1996) predicted recovery of the western Prince William Sound (WPWS) sea otter population in 10 to 23 years, projecting maximum annual growth rates from 0.10-0.14. Results of sea otter population surveys through 2000 have shown a significant increase of nearly 600 animals in WPWS, an average annual increase of 4% per year since 1993 (Bodkin et al. 2002).

Studies conducted in 1996-1998 as part of the NVP program provided evidence that sea otters in WPWS, in at least the area of northern Knight Island, had not fully recovered from oil spill injury (Holland-Bartels 2002, Bodkin et al. 2002). Shortly after the spill, in April 1989, a total of 33 sea otters were captured or recovered from Herring Bay, a heavily oiled embayment on northern Knight Island (Bodkin and Udevitz 1994). Fourteen aerial surveys conducted in 1996 found an average of 3 (maximum of 11) sea otters in this same location. Through 2000, sea otter abundance at northern Knight Island remained at about 50% of the estimated pre-spill abundance (Dean et al. 2000; Bodkin et al. 2002). Constraints to recovery most likely were demographic, either through reduced survival among residents, or higher emigration from the oiled area (Holland-Bartels 2002, Bodkin et al. 2002). Analysis of ages at death of beach cast sea otters found before and after the spill implicate elevated mortality of sea otters that survived the spill, and of those born after 1989, as a factor contributing to delayed recovery (Monson et al. 2000).

Evidence of *Exxon Valdez* oil remaining in nearshore habitats of Prince William Sound and elsewhere became increasingly apparent from the mid 1990's to the present (Hayes and Michel 1999, Irvine et al. 1999, Short et al. 2003), with evidence of oil present in important invertebrate prey species such as clams and mussels (Fukuyama et al. 2000, Carls et al. 2001). Monitoring of CYP1A induction in sea otters at northern Knight Island, from 1996-98, indicated ongoing exposure to hydrocarbons, most likely lingering EVOS oil (Ballachey et al. 2002b, Bodkin et al. 2002). However, there was some suggestion of a decline in exposure, based on declines (non-significant) of mean CYP1A levels over the three years of sampling (Ballachey et al. 2002b). In addition to CYP1A estimates being greater in the oiled area sea otters, other species of nearshore

vertebrate predators including river otters, Barrow's goldeneye, harlequin ducks, pigeon guillemots, and masked greenling all expressed higher CYP1A levels at oiled, compared to unoiled sites (Ballachey et al. 2002b, Trust et al. 2000, Jewett et al. 2002). The greatest differences were noted among species that consume invertebrate prey, including sea otters and harlequin ducks.

Body condition and blood parameters were also monitored in the NVP study, to provide additional insight into overall health of individual animals residing in the oiled area, and alterations that might be associated with oil exposure. We found that condition (measured as the ratio of weight to length) of most sea otters in the oiled area was equivalent to that of their unoiled counterparts, and in the case of young females (ages 1-4 years), condition was superior in the oiled area (Bodkin et al. 2002). This result, combined with other data on prey sizes and foraging effort (Dean et al. 2002) indicated that prey resources in the oiled area were not a limiting factor and should not be constraining recovery of sea otters in the oiled area.

The blood values monitored as part of the NVP research augmented a database of sea otter blood parameters that was initiated in 1989, during the process of rehabilitating sea otters after the spill (Williams et al. 1995). Samples collected from wild otters in 1991-92 showed a general trend of elevations of serum enzymes associated with liver function (Rebar et al. 1996; Ballachey et al. 2002a); of these, gamma glutamyl transferase (GGT) showed the most marked differences. During the NVP study, we continued to observe elevated GGT in sea otters from the oiled area (Ballachey et al. 2002a), whereas for other serum enzymes, the oiled and unoiled areas converged in value. Given these observations, it appears that GGT may be a useful measure to help link exposure (evaluated by the CYP1A biomarker) with an effect on liver function.

#### *Sea urchins.--*

In the NVP study, sea otter prey populations were studied as an ancillary means of assessing recovery. Sea otters are considered keystone predators within coastal marine systems of the North Pacific, as they exert strong top-down control on the structure of the nearshore community (Power et al. 1996). Throughout their range, sea otters reduce densities of large sea urchins, which are a preferred prey. Observations of sea urchins and kelp in areas with and without sea otters (Estes & Palmisano 1974, Estes et al. 1978, Duggins 1980, Breen et al. 1982, Estes & Duggins 1995), and in areas before and after recolonization by sea otters after decades of absence (Lowry & Pearse 1973, Laur et al. 1988, Watson 1993, Estes & Duggins 1995, Kvitek et al. 1998) indicate that large sea urchins are rare where sea otters are abundant but can be locally abundant where sea otters are absent. Fewer studies have examined the transitions during recolonization by sea otters (Laur et al. 1988, Watson 1993, Estes & Duggins 1995, Konar 2000), and only two recent studies (Estes et al. 1998, Konar 2000) have examined community response to a reduction in the abundance of sea otters. The observations made during transitional phases have generally indicated an inverse relationship between densities of sea otters and large sea urchins, but this has not always been the case (Konar 2000).

In the NVP work, we described responses of sea urchin populations to reduction in sea otters following the *Exxon Valdez* oil spill, based on sampling conducted in 1996 and 1997 (Dean et al. 2000). In spite of the approximately 50% or greater reduction in sea otter that persisted for nearly a decade, there was little evidence of a strong response by sea urchins to the reduction in

sea otters (Dean et al. 2000). In the Knight Island region, where sea otter densities were reduced, there were proportionally more large sea urchins, but except in some widely scattered aggregations, both density and biomass of sea urchins were similar in an area of reduced sea otter density compared to Montague Island where sea otters remained about ten times more abundant. We speculated that in oiled areas of PWS, the number of surviving sea otters might have been high enough to suppress sea urchin populations. However, we also speculated that a future strong recruitment year for sea urchins could result in an increase in sea urchin biomass in oiled areas of PWS, and that this may have strong cascading effects on the nearshore system that could lead to a reduction in algae that are grazed by sea urchins.

In Project //423, we extended our earlier work on interactions between sea otters and sea urchins by including observations made in 1998, 1999, and 2000. During this period, there was no increase in sea otter density at northern Knight Island and sea otters remained about ten times more abundant at the Montague Island study area than at Knight Island. Continued prey assessment provided a unique opportunity to complete the testing of an innovative approach for estimating the status of a predator population. When sea otter populations near complete recovery, we predict that differences in prey sizes between areas should diminish.

In summary, the sea otter research in Project //423 builds upon our previous findings from NVP and other restoration studies to provide an understanding of the status of recovery of sea otters, and potential demographic and toxicological constraints to recovery which may improve future recovery models. An additional benefit of these studies will be the provision of long-term population trend and biomarker data that may be used in assessing initial damage and subsequent recovery of sea otter populations in the event of future oil spills.

## **Methods**

### *Aerial surveys.--*

The aerial sea otter survey methodology consists of two components: (1) strip transect counts and (2) intensive search units, which are fully described in Bodkin and Udevitz (1999). Sea otter habitat was sampled in two strata, high density and low density, distinguished by distance from shore and depth contour. Survey effort was allocated proportional to expected sea otter abundance by adjusting the systematic spacing of transects within each stratum. Transects with a 400 meter strip width on one side of a fixed-wing aircraft were surveyed by a single observer at an airspeed of 65 mph (29 m/sec) and altitude of 300 feet (91 m). The observer searched forward as far as conditions allowed and out 400 m, indicated by marks on the aircraft struts, and recorded otter group size and location on a transect map. A group was defined as one or more otters spaced less than three otter lengths apart. Intensive search units (ISU's) were used to estimate the proportion of sea otters not detected on strip transect counts. ISU's were flown at intervals dependant on sampling intensity throughout the survey period, and were initiated by the sighting of a group, then followed by five concentric circles flown within the 400 m strip perpendicular to the group that initiated the ISU.

Replicate surveys in the intensive oiled (northern Knight Island) and unoiled (Montague Island) areas, using the same techniques described in Bodkin and Udevitz (1999) were conducted to gain

precision in estimates for these two areas. Rates of change in population estimates over time were calculated by regressing the log (N) of estimates over years.

#### *Capture.--*

In summer 2001, 2002, and 2003, sea otters were captured in western PWS, at Knight (oiled area) and Montague (unoiled area) islands, in the same areas used in previous studies (NVP, 1996-98, described in Bodkin et al. 2002). Capture and handling methods were similar to those employed previously (Bodkin et al. 2002). Tangle nets and diver-operated traps were used to capture individuals. Sea otters were sedated and body measurements taken, including mass to the nearest 0.5 lb (230 g) and total length (from the tip of the nose to the tip of the tailbone in a supine position) to the nearest mm. A vestigial premolar tooth was removed and used to determine age by counting annuli resulting from cementum depositions (Bodkin et al. 1997). Up to 35 cc of blood was taken by jugular venipuncture for hematology, clinical chemistry, and biomarker analyses. In addition, three liver biopsies were collected by endoscopy from adults and juveniles to quantify levels of the biomarker, cytochrome P4501A and for histological examination (see biomarker methods below). Each individual captured was marked on the rear flippers with numbered colored plastic tags (Temple Tag, Temple, Texas). Following reversal from anesthesia, sea otters were released in the same vicinity as captured. Capture efforts were directed to areas where sea otters were most abundant based on aerial surveys and reconnaissance. Due to relatively small numbers of individuals in each age, we grouped individuals into one of 3 age classes, 0-3, 4-9, and > 9, and tested for differences between areas and periods with the chi-square statistic.

Age-adjusted total length, body mass, and mass to length ratios were compared between sea otters captured at northern Knight and Montague Islands in the periods 1995-1998 and 2000-2001. Analyses were conducted for three groupings: non-palpably pregnant females age 1-4, non-palpably pregnant adult females > 4 years, and adult males > 5 years of age. If significant differences were not detected between periods, samples within areas were combined and the condition variables were compared between periods. We tested the null-hypothesis of no difference between age-adjusted length, mass, and mass to length ratio using analysis of variance (GLM SAS Institute).

#### *Carcass collections.--*

Systematic beach surveys were conducted each year in April or May along shorelines in WPWS soon after snowmelt, prior to the regrowth of beach grasses, that can conceal carcass remains. We collected sea otter carcasses from Green, Naked, Eleanor, Ingot, Knight, Evans, Latouche, and Elrington Islands and numerous smaller islands in the spill area. Beaches were walked by one or usually two observers, who searched 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. The skull was collected when present, and a tooth (preferentially a premolar) removed for age estimation based on a reading of cementum annuli (Bodkin et al. 1997). Matson's Laboratory (Box 308, Milltown, MT 59851) sectioned and aged all teeth, from carcasses and from live-caught animals.

### *Sea urchins.--*

Sea urchin density and size distributions were compared between a heavily oiled area with reduced sea otter densities (northern Knight Island) and an unoiled area (Montague Island) where sea otter densities were unaffected by the spill and remained high. Sampling was conducted yearly in the summers of 1996 through 2000 (1996-98 as part of the NVP project). In 1996 and 1997, densities and size distributions were estimated from approximately 68 km of shoreline in Bay of Isles and Herring Bay, on northern Knight Island, and along approximately 51 km of shoreline on Montague Island, using methods described in Dean et al. (2000). Briefly, 29 to 30 different systematically selected shoreline segments, each 200-m long, were sampled in each year. Sea urchins from within a 50-m long by 0.5-m wide transect, placed parallel to shore, and were counted and measured. All movable rocks were turned to search for sea urchins. Sampling from these systematically selected sites was supplemented by sampling in preferred sea urchin habitat (the lower intertidal zone on gently sloping cobble beaches) where we observed widely scattered aggregations of sea urchins.

In 1998, 15 new systematically selected transects were sampled in each of the Knight Island and Montague areas, and preferred sea urchin habitats identified previously were resampled in 1998, 1999, and 2000. Sampling conducted in 1996 and 1997 included nearshore subtidal as well as intertidal areas and found much higher densities in the intertidal zone. Therefore, we restricted sampling in 1998, 1999, and 2000 to the intertidal, and present only intertidal data here.

### *Biomarkers.--*

Approximately 10-15 ml of blood were drawn from the jugular by venipuncture. Approximately 3 ml were collected in a blood tube with an EDTA additive (Becton-Dickinson Vacutainer™ Systems) and the remainder into tubes without additives and allowed to clot for at least 30 minutes before centrifuging to separate serum. Serum was frozen in 0.5-0.75 ml aliquots in snap-cap serum vials. Two blood smears on glass slides were made from the whole blood. For hematology, 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 fieldwork was complete and then submitted as a batch to Quest Laboratories, Portland for serum chemistry assays. Blood samples were sent to the Portland facility of Quest to maintain consistency with samples that had been submitted to the Portland facility in previous years. An additional 20-25 ml of whole blood were drawn into a 35-ml heparinized syringe for isolation of peripheral blood mononuclear cells (PBMC) that were frozen in liquid nitrogen for later cytochrome P4501A assays. In addition, three liver biopsies (weighing approximately 0.5 gm total) were surgically collected, using endoscopic procedures, from 15 otters per area (liver component conducted as part of Project 01534; Ballachey et al. 2003b). Two biopsies were frozen immediately in liquid nitrogen and a third biopsy fixed in neutral buffered formalin.

In the NVP study, the RT-PCR assay (quantitative reverse transcriptase PCR assay; Vanden Heuvel et al. 1993, 1994; Snyder et al. 2002, Ballachey et al. 2003b) was adapted to measure CYP1A levels in sea otters. This assay quantifies the messenger RNA (m-RNA) that codes for the CYP1A protein, and results are reported as molecules of CYP1A mRNA x 10<sup>6</sup> per 100 ng of total RNA. In 2001, the assay was done on the PBMC samples and also on liver cells from the

same animals. Samples (liver, blood cells, and frozen archived liver) were shipped to Purdue University for analysis in the laboratory of Dr. Paul Snyder. Liver samples in formalin were processed for histology and sections were examined microscopically (the liver component was part of Project 01534).

## Results

### *Aerial Surveys.--*

**Western Prince William Sound:** With the exception of 2001, annual estimates of sea otter abundance have been made since 1993 in WPWS (Figure 1.1). From 1993 through 2000, a significant positive average annual growth rate of 0.04 ( $r^2=0.56$   $p=0.03$ ) was evident. In 2002 it appeared as though there was an overall decline in WPWS sea otter abundance, but the 2003 estimate confirms the long-term trend toward an increasing sea otter population in WPWS. The 2003 sea otter population estimate for WPWS (2,631) represents an increase of nearly 600 animals since we initiated aerial surveys in 1993 (2,054) following the EVOS. This increase represents a non-significant average annual increase of 0.01 in sea otter abundance in western Prince William Sound between 1993 and 2003. If we remove the 1998 and 2002 data points as anomalous, we calculated a significant average annual increase of 0.03 ( $r^2 = 0.88$ ,  $P<0.001$ ) from 1993-2003.

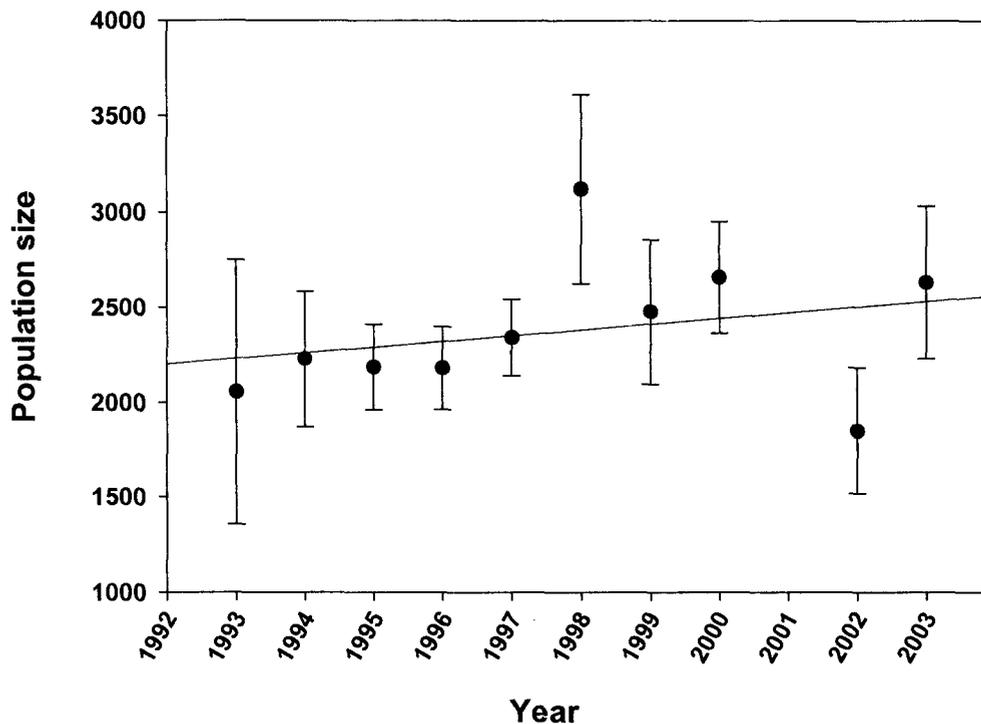


Figure 1.1. Sea otter population trend in western Prince William Sound, 1993-2003. Line is linear regression fitted to all points, bars equal  $\pm$  one SE.

**Northern Knight and Montague Island:** At Montague Island there has been a significant trend toward increasing abundance, with an average annual increase of about 0.06 ( $R^2 = 0.43$ ,  $P < 0.03$ ; Figure 1.2). The trend toward increasing abundance at Montague Island is consistent with the rate of increase observed in the larger spill area of WPWS (Figure 1.1).

Between 1993 and 2001 the sea otter population size at northern Knight Island remained constant, with an average population size of 77 (SE=2) (Figure 1.2). In July 2002, we estimated the population size at northern Knight to be 38 (SE=11) and in July 2003 to be 23 (SE=8). The decline from the long-term average of 77 to 38 represents a decline of 0.50 in a single year. The decline from the long-term average of 77 to 26 in 2003 represents a decline of 0.66 between 2001 and 2003.

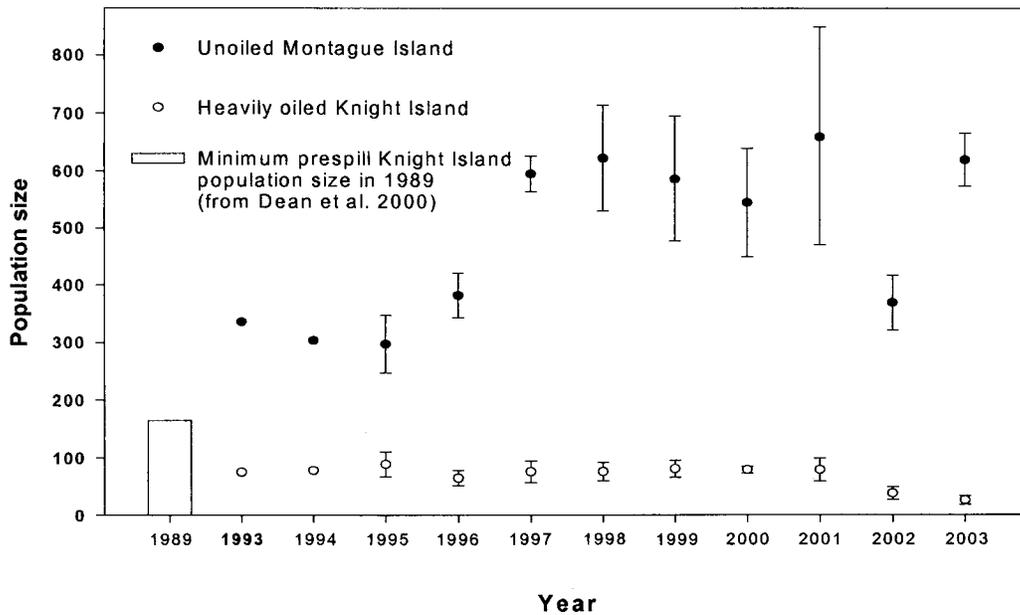


Figure 1.2. Sea otter population trends at northern Knight and Montague Island study areas, 1993-2003.

**Prince William Sound:** In 1994, 1999, 2002, and 2003 we surveyed all of Prince William Sound and estimated sea otter population size (Figure 1.3). We do not include Orca Inlet transects in the population estimates due to a poorly defined boundary. There was no significant trend in sea otter abundance at the geographic scale of the entire Sound over this 10 year time period. The low estimate was 8,317 (SE=1,176) obtained in 2002, and the high estimate was 9,284 (SE=1,579) obtained in 2003. The mean of the four population estimates is 8,762 (SE=249).

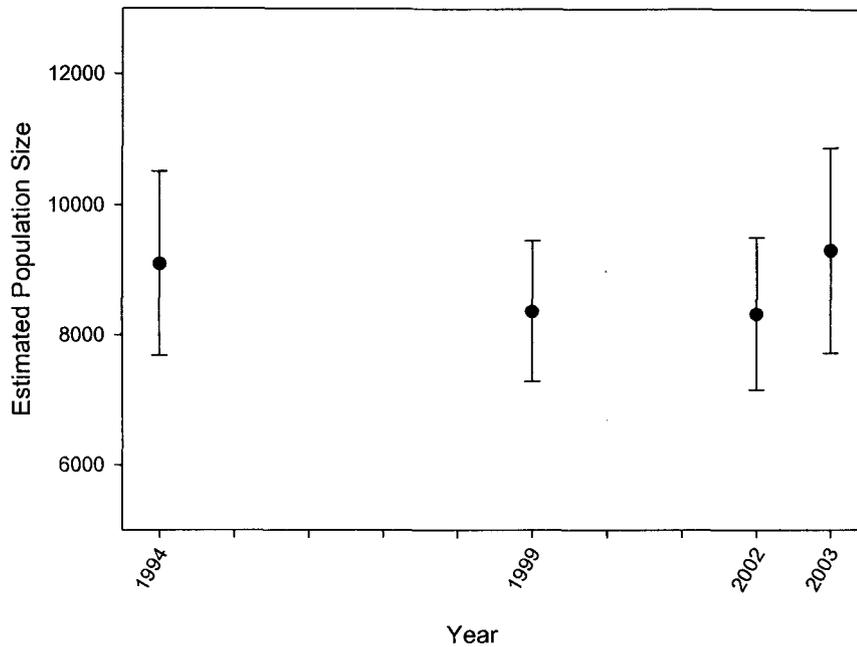


Figure 1.3. Sea otter population trend in Prince William Sound, 1994-2003, excluding Orca Inlet.

*Capture.--*

We captured and sampled 103 sea otters, 74 from Knight Island and 29 from Montague Island, between 2001 and 2003. Twelve of these were dependent pups that are not included in the following capture summaries. We captured 15 sea otters at Knight and 15 at Montague in 2001, 27 at Knight and 11 at Montague in 2002, and 23 at Knight and none at Montague in 2003. The sex ratio of sea otters captured at Knight Island over all years was 41♀ : 10♂ (80% : 20%), and at Montague Island 19♀ : 7♂ (73% : 27%). Age distributions of male and female sea otters captured in 2001-2003 are presented in Figure 1.4b, and the age distributions of sea otters captured at those same locations in 1996-1998 are presented for comparison in Figure 1.4a. Samples used in the age distributions do not include 2003 captures that resulted from targeted captures of previously marked animals (N=10).

Because sample sizes are relatively small when considering up to 20 age categories, we grouped individuals into three age classes, juvenile (0-3 y), adult (4-9 y) and aged adult ( $\geq 10$  y) (Bodkin et al. 2002) to compare age distributions between areas and the time periods 1996-1998 and 2001-2003. The proportion of individuals in each age class did not differ between time periods, within each area (Table 1.1). There was a trend toward proportionally fewer young and more adult (~20% change) in the 2001-2003 period at Knight compared to the earlier period ( $p=.076$ ) (Table 1.1).

Age classes, age-adjusted total length, mass, or mass to length ratio differed significantly between the samples taken in the years 1996-1998 and 2001-2003 for several age/sex classes (Tables 1.1-1.3). In all cases where significant differences were observed, measurements were greater in the later period (2001-2003). We also detected significant differences in measures of condition between Knight and Montague Island, similar to patterns observed during the NVP study, where

young females were heavier and with greater weight length ratios than their counterparts at Montague Island (Table 1.2).

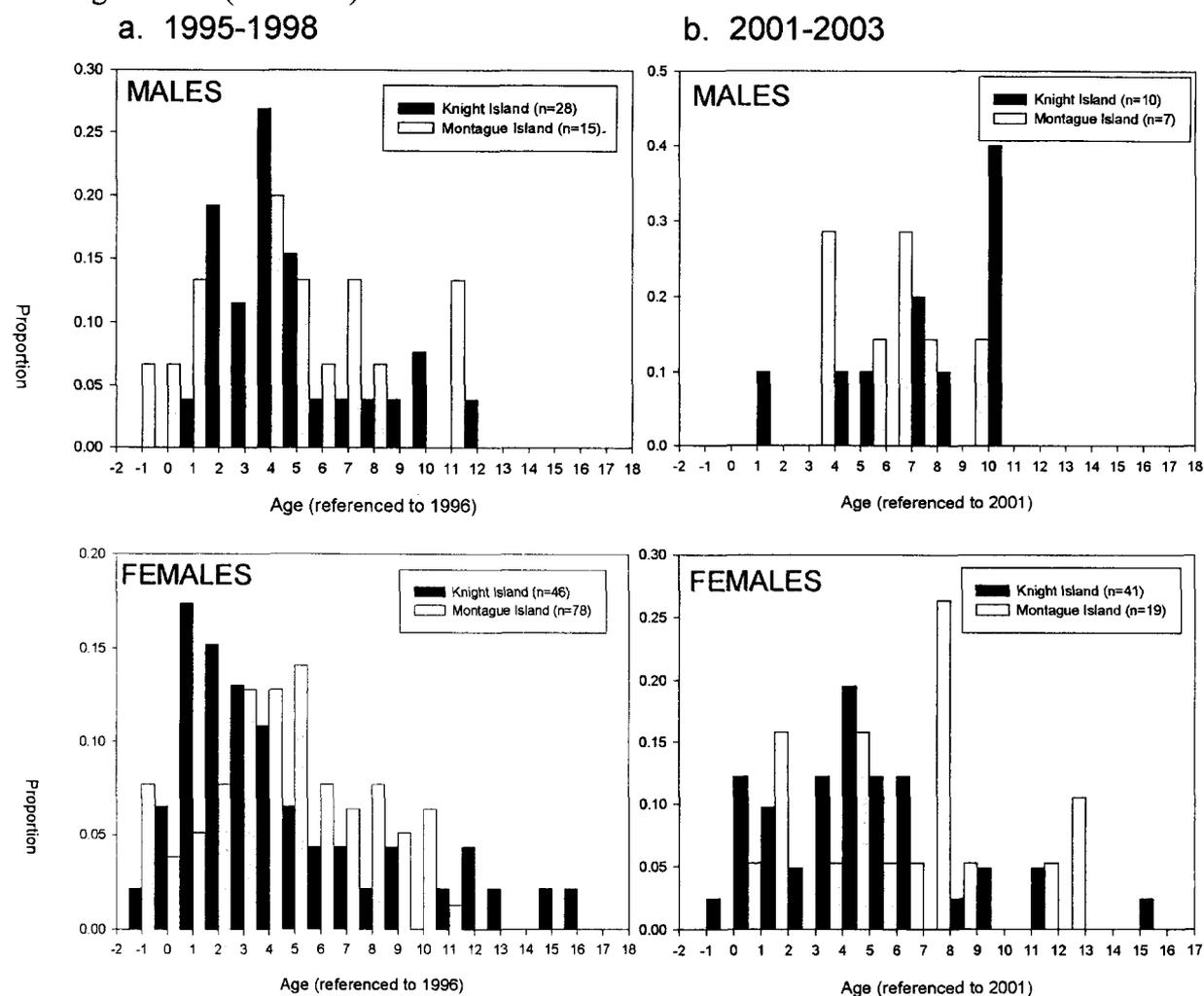


Figure 1.4. Age distributions of non-pup, male and female sea otters captured at Knight and Montague Islands, PWS in 1995-1998 (1.4a) and in 2001-2003 (1.4b).

Table 1.1. Age class composition and sex ratio of non-pup sea otters captured at Knight and Montague Islands, PWS during the periods 2001-2003 and 1996-1998. Table includes only the first capture of any individual within the two time periods. Individuals captured in the 2001-2003 period that were originally captured in 1995-1998 were counted once in each capture period. Of 225 individuals included in this table, 24 were captured more than once; 6 within 1996-1998, 8 within 2001-2003, and 10 were captured at least once in each time period.

Period	Area	Age Classes <sup>a</sup>			Sex Ratios
		0-3	4-9	≥10	%♀ : %♂ (N:N)
2001-2003	Knight	0.27 (14)	0.57 (29)	0.16 (8)	80:20 (41:10)
	Montague	0.15 (4)	0.73 (19)	0.11 (3)	73:27 (19:7)
1996-1998	Knight	0.48 (31)	0.38 (25)	0.14 (9)	66:34 (43:22)
	Montague	0.23 (21)	0.66 (61)	0.12 (11)	83:17 (80:16)

<sup>a</sup> Age class comparison: Knight 1996-1998 vs 2001-2003 Chi-square=5.163, df=2, P=0.076; Montague 1996-1998 vs. 2001-2003 Chi-square=0.672, df=2, P=0.715.

Table 1.2. Age-adjusted mean body length (cm), mass (kg) and mass to length ratio for juvenile and adult female, and male sea otters captured at Knight and Montague Islands in Prince William Sound. Results of analysis of variance (GLM, SAS Institute) and contrasts of mean values are given by area and time period. Significant differences between pairs of values are indicated by shading with associated p values in red.

	Total Length, cm			Mass, kg			Total Length/Mass		
	NVP	01-03	All	NVP	01-03	All	NVP	01-03	All
Juvenile Females									
<b>Knight</b>	113.5 (22)	.01 117.5 (15)	.	19.2 (22)	20.3 (15)	19.7 (37)	0.17 (22)	0.17 (15)	0.17 (37)
<b>Montague</b>	.05 112.4 (27)	110.0 (5)	.	.01 18.3 (27)	18.1 (5)	.001 18.3 (32)	.01 0.16 (27)	0.16 (5)	.006 0.16 (32)
Adult Females									
<b>Knight</b>	122.3 (22)	121.5 (29)	.	22.8 (22)	23.4 (24)	.	0.19 (22)	0.19 (24)	.
<b>Montague</b>	.001 119.5 (47)	.004 121.9 (13)	.	.003 22.6 (47)	24.3 (13)	.	.007 0.19 (46)	0.20 (13)	.
Adult Males									
<b>Knight</b>	129.4 (10)	131.2 (16)	130.5 (26)	31.6 (10)	35.5 (16)	.	0.24 (10)	.04 0.27 (16)	.
<b>Montague</b>	128.5 (11)	130.1 (5)	129.0 (16)	30.9 (11)	33.0 (5)	.	0.24 (11)	0.25 (5)	.

Table 1.3. Ages for juvenile and adult female and male sea otters captured at Knight and Montague Islands in Prince William Sound during the NVP study (1996-1998) and this study (2001-2003).

	Mean Age/St Dev (n)					
	Juvenile Females		Adult Females		Males	
	Knight	Montague	Knight	Montague	Knight	Montague
NVP	2.32/1.0 (22)	2.70/1.3 (27)	8.77/3.7 (22)	7.96/2.8 (47)	7.60/2.2 (10)	7.46/2.2 (11)
01-03	2.33/1.1 (15)	1.60/1.3 (5)	7.41/2.7 (29)	7.85/2.5 (13)	9.19/2.7 (16)	7.00/1.6 (5)
ALL	2.32/1.0 (37)	2.53/1.4 (32)	.	.	8.58/2.6 (26)	7.31/2.0 (16)

*Carcass collections.--*

During spring of 1999-2003, we located and collected the remains of 173 beach cast sea otters in the oil spill area of WPWS (Table 1.4). Annual totals of carcasses found were 27, 53, 32, 19, and 42 in the years 1999-2003, respectively. Age estimates have been obtained for all carcasses

reported and the distribution of those ages are reported in Table 1.4 and Figure 1.5. In addition, we report the ages of all sea otter carcasses collected during the pre-spill period of 1977-1989 and the earlier post-spill period, 1990-1998 (Table 1.4 and Figure 1.5). The proportion of prime age adult sea otters represented in carcass collections since 1990 remains nearly twice the proportion of prime age carcasses recovered prior to the spill (Table 1.4). Rather than finding a decline to pre-spill patterns of age specific mortality, we continue to document an elevated, and apparently increasing, proportion of prime age adults in our annual collections, through 2003 (Figure 1.5). This pattern of elevated proportions of prime age-sea otters found dead following the spill (1989-1998) produced population models that identified elevated mortality as a contributing factor in the protracted recovery of sea otters in PWS (Monson et al. 2000, Figure 1.6).

Table 1.4. Proportion (number) of beach-cast sea otters that were estimated as juvenile (0-1 y), prime-age adult (2-8 y) and aged adult ( $\geq 9$  y), recovered from western Prince William Sound shorelines during spring 1999-2003, 1990-1999 and 1977-1989.

Period	Juvenile	Adult	Aged Adult	# carcasses recovered
1999-2003	0.47 (81)	0.35 (60)	0.18 (32)	173
1990-1998	0.36 (80)	0.32 (70)	0.32 (70)	247
1977-1989	0.44 (90)	0.17 (34)	0.39 (79)	203

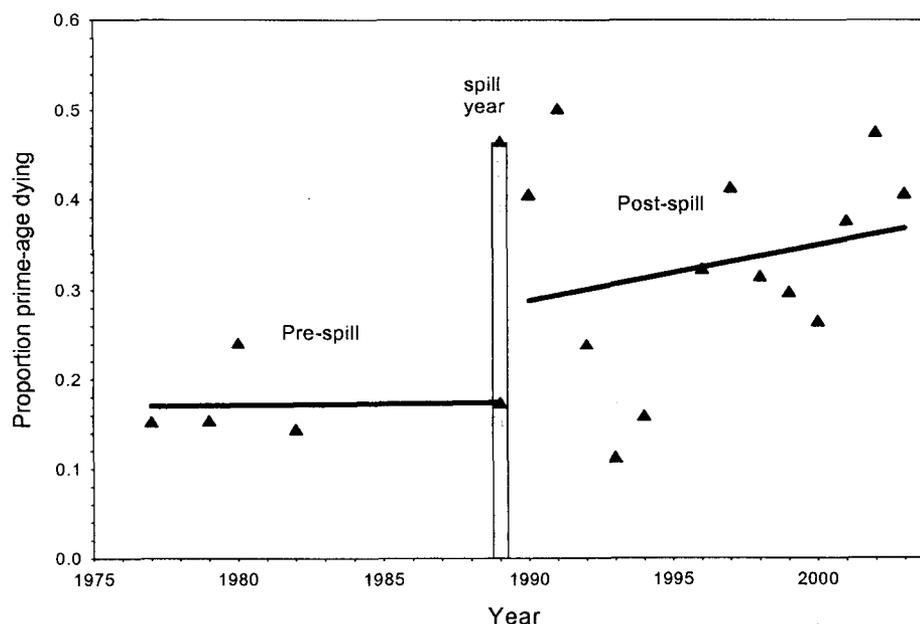


Figure 1.5. Trends in annual sea otter mortality in western Prince William Sound as represented by spring collections of beach-cast carcasses between 1977 and 2003. Prime age adult animals are 2-8 years of age.

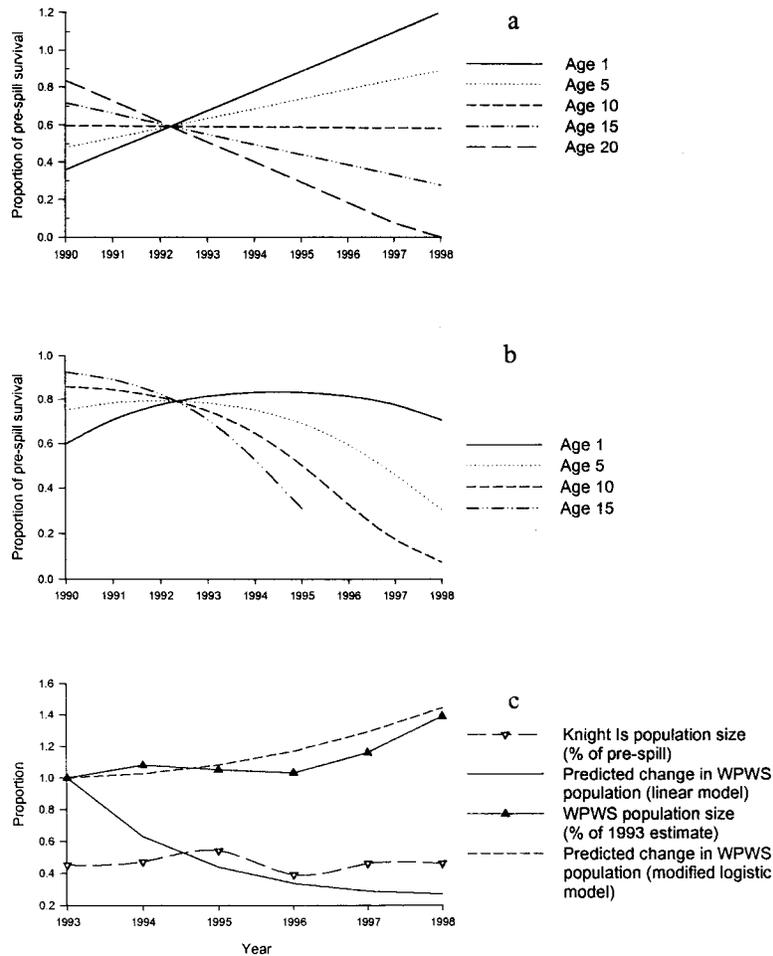


Figure 1.6. Estimated post-spill effects on age-specific survival rates (linear model; 1.6a) and for cohorts of a given age (1.6b) expressed as a proportion of pre-spill survival, and predicted vs. observed population trends (1.6c) in western Prince William Sound (from Monson et al. 2000).

#### *Sea Urchins.--*

In 1996 through 1998, sea urchins were relatively rare at both the northern Knight Island and Montague study areas. On systematically selected transects sampled in 1996 through 1998, the mean density never exceeded 0.5 individuals  $m^{-2}$  (Table 1.5). In almost all cases, sea urchins were found under cobble or boulders, and were not visible unless rocks were overturned. Mean densities differed significantly between years, but not between areas (Knight and Montague). At sites where there were moderate densities of sea urchins in 1997, we found no significant difference between areas and no increase in sea urchin density between 1997 and 2000 (Table 1.6).

Table 1.5. Mean density of sea urchins (number m<sup>-2</sup>) from intertidal sites on northern Knight Island and Montague Islands from 1996 through 1998.

Year	Knight Island			Montague Island		
	N	Mean	SD	N	Mean	SD
1996	30	0.17	0.26	30	0.05	0.17
1997	30	0.19	0.64	29	0.47	1.09
1998	15	0.03	0.05	15	0.04	0.11

ANOVA Results Summary		
Source	F	Probability
Year	3.02	0.03
Area	0.72	0.54

Table 1.6. Changes in mean sea urchin density at sites in Knight and Montague Islands sampled in 1997, 1999, and 2000.

Year	Knight Island			Montague Island		
	N	Mean	SD	N	Mean	SD
1997	7	0.22	0.19	8	1.58	1.68
1999	7	1.13	0.79	8	0.54	0.45
2000	7	1.06	1.76	8	0.29	0.41

ANOVA Results Summary		
Source	F	Probability
Year	0.78	0.33
Area	<0.01	0.99

Relatively few large (greater than 40 mm) sea urchins were found at either Knight or Montague Island in any year (Figure 1.7). Sea urchins were larger on average in areas with few sea otters (Knight Island) in 1996 and 1997, but were on average slightly larger in the area with high densities of sea otters (Montague Island) in 1999 and 2000.

The changes in the relative proportion of smaller vs. larger sea urchins within each area over time appeared related to the timing of recruitment events. We do not have good estimates of growth, but preliminary data from tagged sea urchins suggest that individuals 10 to 20 mm in size grow in the range of 2 to 8 mm per year. Similar estimates have been given for *S. droebachiensis* in Kodiak (Munk & McIntosh 1993) and for *S. pallidus* in the Aleutian Islands (Estes & Duggins 1995). Thus, we suspect that the different modes in size frequency distributions represent different cohorts. Size distributions for the population at Knight Island were unimodal in 1997 and 1998, suggesting dominance by a single cohort of sea urchins that had recruited sometime prior to 1996. A second cohort was evident in 1999. At Montague, the size frequency distribution was strongly dominated by 10 to 14 mm individuals in 1997. A secondary peak (mode = of 11 mm individuals) appeared in 1998 suggesting a second recruitment event, and yet another cohort appeared in 2000.

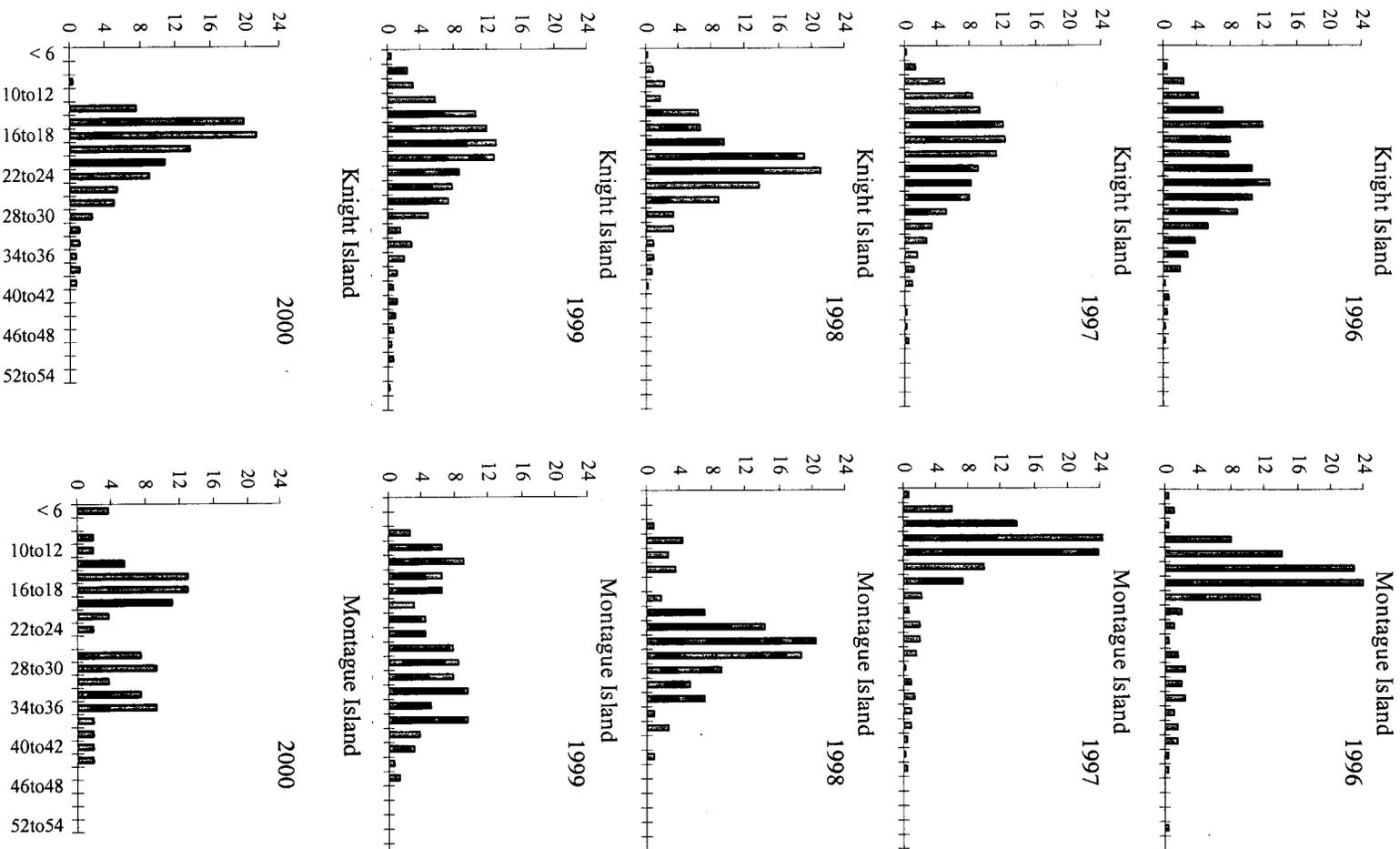


Figure 1.7. Size frequency distributions for sea urchins at Knight and Montague Islands, 1996-2000.

*Biomarkers.--*

In 2001-2003, we collected blood for hematology and serum chemistry assays from 98 sea otters: 27 in the unoiled area (2001: 15, 2002: 12, including 1 pup) and 71 in the oiled area (2001: 15; 2002: 30 including 3 pups; 2003: 26 including 3 pups). We collected PBMC from 97 sea otters: 29 in the unoiled area and 68 in the oiled area (2001: 18 unoiled, 16 oiled; 2002: 11 unoiled, 29 oiled; 2003: 23 oiled). The 2001 PBMC samples were analyzed by quantitative reverse-transcriptase PCR, the same assay used on 1996-98 PBMC samples, and results are directly comparable with the earlier years of data. In 2002 and 2003, CYP1A data were generated by real-time PCR and thus are not directly comparable with earlier years; however, elevated levels were still evident in the oiled area (Ballachey et al. 2003a). Results of CYP1A assays on 2003 samples are still pending.

Levels of CYP1A in PBMC continued to be elevated in the oiled area in 2001, relative to the unoiled area (Table 1.7, Figure 1.8). The 2001 oiled area mean was 7.91 (SE = 1.57; units are molecules of CYP1A mRNA x 10<sup>6</sup> per 100 ng RNA), compared to a mean of 2.44 (SE = 0.69) for the unoiled area. Differences among areas were highly significant (ANOVA on rank values, F = 271.2, P < 0.001) but no year differences were detected (F = 0.62, P < 0.60). As the area x year interaction was marginally significant (F = 2.6, P < 0.053), we compared annual means, and found that only the 1996 and 2001 oiled area means differed significantly from each other (P < 0.012).

Table 1.7. Cytochrome P450 1A statistics for sea otters captured in oiled (northern Knight) and unoiled (Montague) areas of PWS from 1996-98 and in 2001.

		CYP1A <sup>a</sup>				
		n	Mean	SE	Median	Range
1996						
	Unoiled	22	1.18	0.10	1.20	0.4-2.3
	Oiled	22	28.86	7.48	18.6	5.6-155.9
1997						
	Unoiled	29	1.92	0.48	1.00	0.06-10.5
	Oiled	26	38.64	12.12	15.01	0.65-228-85
1998						
	Unoiled	35	1.36	0.40	0.94	0.32-14.81
	Oiled	23	13.07	1.70	10.45	5.54-39.66
2001						
	Unoiled	18	2.44	0.69	1.28	0.44-9.70
	Oiled	16	7.91	1.57	6.46	2.18-25.1

<sup>a</sup>Data were obtained with a quantitative reverse-transcriptase assay and are expressed as the number of molecules of mRNA for CYP1A x 10<sup>6</sup> per 100 ng of total RNA.

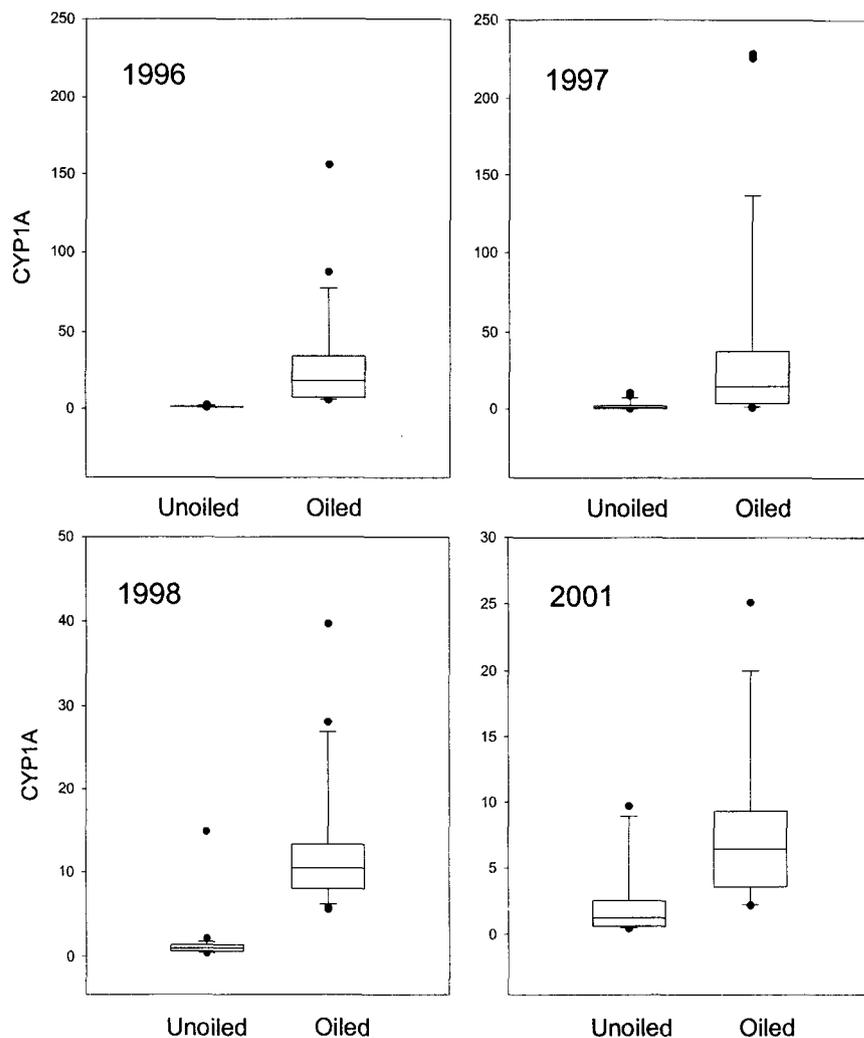


Figure 1.8. Cytochrome P450 1A (CYP1A) values for sea otters from unoiled (Montague) and oiled (northern Knight) study areas, in 1996-98 and 2001. Values are expressed as the number of molecules of mRNA for CYP1A  $\times 10^6$  per 100 ng of total RNA. (Note difference in Y axis scales among years).

Endoscopic procedures were done on captured adult otters (2001, 2002 and 2003) to biopsy livers for CYP1A assays and histopathology (see Ballachey et al. 2003a, 2003b). During the endoscopies, livers were viewed on a TV monitor, and any variations or lesions present could be observed. The gross appearance of livers of sea otters in the unoiled area was largely normal. However, in 2001, two sea otters from the oiled area (of 15 examined) showed marked liver abnormalities, including swollen margins, irregular surfaces, and an unusual dark, mottled color. Gross abnormalities have also been observed in 2002 and 2003 in oiled area sea otters, although there have been no individuals with abnormalities as severe as noted in 2001. Liver pathology has been addressed in the reports for Restoration Project 01534 and //585 (Ballachey et al. 2003a, 2003b); further data on livers will be collected in 2004 (Restoration Project //620).

Over the last decade of post-spill studies, one of the most notable and consistent differences observed between areas in hematology and serum chemistry results has been for gamma glutamyl transferase (GGT), a serum enzyme generally thought to be indicative of liver function. In 2001, the mean GGT values were 18.3 IU/L (SD = 10.5) for the oiled area, and 14.6 IU/L (SD = 3.3) for

the unoiled area. Although for 2001 the area difference in GGT was not significant ( $P < 0.11$ ), it nevertheless reflects a pattern of elevated GGT values in oiled areas that has been observed in other years (Table 1.8). Area differences in proportion of sea otters with elevated GGT values have persisted through 2002 and 2003 (Table 1.8), and will be examined again in 2004 (Restoration Project //620). Other hematology and chemistry values for the 2001-2004 period will be included with the //620 final report.

Table 1.8. GGT values (IU/l) for sea otters captured in oiled and unoiled areas<sup>a</sup> of PWS from 1992 through 2003.

Year	GGT Mean, IU/l		Percentage of values >20IU/l <sup>b</sup>	
	Unoiled (SE) n	Oiled (SE) n	Unoiled	Oiled
1992 Adult	15.7 (0.5) 53	29.5 (4.1) 36	11.3%	48.6%
1992 Pup	15.9 (1.2) 41	51.7 (12.6) 35	7.3%	50%
1996-1998	14.0 (0.9) 89	17.8 (1.2) 80	9%	22.5%
2001	14.6 (3.3) 15	18.3 (10.5) 15	0%	20%
2002	13.2 (3.7) 11	22.2 (27.2) 30	0%	23%
2003	-- (--) 0	21.7 (16.1) 25	--	36%

<sup>a</sup> 1992 captures: Unoiled in eastern PWS; oiled in wider area of western PWS; 1996-1998, 2001, and 2002 captures: unoiled at Montague Island in western PWS, oiled at northern Knight Island in western PWS; 2003 captures: oiled at northern and western Knight, no unoiled.

<sup>b</sup> 20 IU/l chosen as cutoff point as it approximates the upper 95% CI of GGT values from sea otters in the unoiled area.

## Discussion

### *Aerial Surveys.--*

A remnant PWS sea otter population survived the commercial fur harvest of sea otters that ended early in the 20th century. The remnant population probably numbered less than 50 animals and was centered in southwest PWS, and the long-term average annual growth rate of the population was 0.099 (Bodkin et al. 1999). Recolonization of PWS apparently was complete by 1980, although our recent survey data indicate very low densities in the far northwest portions of the Sound. Over the period 1994-2003, four estimates of the entire PWS sea otter population were similar, averaging 8,762 (Fig 1.3). The results of these surveys suggest a relatively stable population of sea otters within the entire PWS region during this period.

It has generally been accepted that the WPWS sea otter population was at or near equilibrium density at the time of the spill (Bodkin et al. 2000). Within WPWS, including primarily oiled areas, we have observed a significant trend of increasing sea otter abundance between 1993 and 2003 (Fig. 1.1). The lowest estimate was obtained in 2002 (1,849) and the highest in 1998 (3,119), and the most recent estimate in 2003 was 2,631. The average annual rate of growth from 1993-2000 was 0.04, less than ½ the long-term rate of sea otter recovery in PWS during the

20<sup>th</sup> century (Bodkin et al. 1999). This trend is consistent with a population recovering from the population decline that resulted from the 1989 oil spill. The reduced growth rate following the spill may reflect residual density dependent effects on food (Fukuyama et al. 2000) or space availability, or possibly residual spill effects such as continued oil exposure to sea otters and/or their prey. Since 2000 there has been no significant trend in sea otter abundance in WPWS, a pattern similar to the PWS wide survey results.

At Montague Island, we have seen the mean estimated population size significantly increase from about 300 in 1993 to nearly 650 in 2003 (average annual increase = 0.06, adj.  $R^2$  = 0.43,  $P < 0.03$ ). During much of this same period, 1993-2001, at northern Knight Island, sea otter abundance remained unchanged (mean = 77, se = 2), and was about half the estimated pre-spill abundance of about 150 (Dean et al. 2000). In 2002 and 2003, we began to see a further reduction in sea otter abundance at northern Knight Island. This result suggests that recovery of sea otters at northern Knight remains delayed, relative to the remainder of the spill affected areas. Causes for the delayed recovery at northern Knight likely include increased mortality and/or emigration rates. Sea otters captured and sampled at Knight have exhibited elevated levels of the CYP1A biomarker, compared to Montague Island (Ballachey et al. 2002b, 2003b; see below), and unanticipated volumes of *Exxon Valdez* oil remain in intertidal sediments, particularly on northern Knight Island shores (Short et al. 2003), implicating oil exposure in the delayed recovery. It also appears that residual oil may be adversely affecting some of the sea otters' prey by increasing mortality and decreasing growth rates in some clam species (Fukuyama et al. 2000). However, the pattern of decline in sea otters at northern Knight Island may be inconsistent with oil exposure as the primary factor constraining recovery, given the potential decline in oil exposure as measured through CYP1A and GGT values in sea otters. We cannot exclude the possibility that other factors acting in concert with, or independent of oil exposure, are contributing to the recent declines in sea otter abundance at northern Knight Island.

#### *Capture.--*

The results of comparisons of length, mass and weight to length ratios (condition) between our study areas at Knight and Montague Islands and between the time periods 1996-1998 and 2001-2003 periods are suggestive of processes that are occurring at both relatively small (northern Knight Island) and larger (WPWS) spatial scales. In 1996-1998, we found mass and condition to be significantly greater in young female sea otters at Knight Island compared to Montague Island (Dean et al. 2002). This difference reflected relatively greater prey availability at Knight Island, presumably from the reduced effects of sea otter predation as a consequence of spill related reductions in sea otter density. We had no area effect between Knight and Montague Island for any age or sex class tested, although sample sizes of young females and adult males at Montague Island were small in 2001-2003, likely limiting the power of between area tests (Table 1.2). However, the area effect remained significant, with young female sea otters at Knight Island heavier, and in better condition than their counterparts at Montague Island during the combined period of 1995-2003. This finding remains consistent with the data and conclusions of Dean et al. (2002) and Bodkin et al. (2002), and supports the population survey data illustrating a lack of recovery at Knight Island. The observation of area differences in the young, pre-reproductive females likely reflects a greater flexibility on their part in terms of increasing body size in response to increasing prey availability. Juvenile males were rare in either study area, precluding comparisons of their weights and lengths.

Our finding of significant increases in length (juvenile and adult females), mass (adult females), and condition (adult males and females) of sea otters sampled between 1996-1998 and 2001-2003 are indicative of processes that are occurring at scales equal to or larger than WPWS. Of the 18 between period contrasts by age and sex the mean values of 12 increased, while three did not change and three decreased slightly (Table 1.2). These results are suggestive of processes that resulted in increased food availability across both study areas in WPWS late in the 20<sup>th</sup> century. Our finding that three of the four significant increases in condition were at Montague Island likely reflects the generally better condition of Knight Island animals described in prior work (Dean et al. 2002) and thus the greater opportunity for animals at Montague to respond to improved environmental conditions. Our finding of improved overall condition of sea otters between periods, and our conclusion of improved food availability, are inconsistent with food limitation as the cause of the reduced growth rates observed in WPWS and declines in abundance at northern Knight during the 2001-2003 period.

#### *Carcass Collections.--*

Elevated sea otter mortality in the years following the *Exxon Valdez* oil spill was a contributing factor to the limited rate of recovery in WPWS (Bodkin et al. 2002). Based on ages at death of beach cast sea otter carcasses, Monson et al. (2000) 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, through 1998, 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, and survival declined rather than increased after the spill, particularly for older animals. Further, 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 over time as those animals affected by the spill eventually die. The divergent population trends at heavily oiled Knight Island, compared to the larger WPWS (Fig. 1.5) suggest that effects of the spill on survival reported by Monson et al. (2000) may persist longest where initial oil impacts were greatest. The modeling techniques used by Monson et al. (2000) require relatively large sample sizes (e.g. 203 pre- and 384 post-spill ages, based on carcasses collected through 1998). The sample size we have accumulated since 1998 (N = 173 ages at death) will allow incorporating these data into the Monson et al. (2000) model to evaluate the continuing role of age-specific sea otter mortality as a factor contributing to the delayed sea otter recovery, particularly at northern Knight Island. Because carcass collections are ongoing, we will report the results of this survival modeling with the final report for EVOS restoration project //620. Our finding of continued elevated proportions of prime age sea otter dying through 2003 suggest that mortality remains an important factor contributing to the observed trends in population abundance and the protracted period of sea otter recovery since 1989. Incorporation of the age at death data obtained through 2003 into the previous mortality modeling (Monson et al. 2000) will provide added insight into the demographic factors driving population change in WPWS.

#### *Sea Urchins.--*

Based on the relative lack of large sea urchins at both Knight and Montague Islands, it appears that sea otters in both areas continue to structure these preferred prey by consuming larger individuals. Thus, in spite of the reduction in the number of sea otters in heavily oiled portions

of Knight Island, and the continued lack of recovery of sea otters, predation by remaining sea otters is apparently sufficient to suppress sea urchin population growth. Even though sea urchin densities within our study areas were low, sea otters continued to prey on sea urchins. In a collection of 102 sea otter scats from WPWS in winter 1998, 29% had sea urchin remains (J.L. Bodkin, unpublished data).

The lack of response by sea urchins to a reduction in sea otters is similar to the lack of a response noted following a similar reduction in sea otters in the Semichi Islands (Konar 2000), but in contrast to the boom in sea urchin biomass following a nearly 90% reduction in sea otters in the Western Aleutian Islands (Estes et al. 1998). Thus, it appears that community response to changes in predator abundance may relate in part to the magnitude of the change and the non-linear nature of the response by prey.

The differences in size distributions of sea urchins, both between areas and between times within an area, were due mostly to fluctuations in sizes of sea urchins that were smaller than those generally consumed by sea otters. These fluctuations were apparently due to recruitment that did not occur in equal strength in the same years at both Montague and Knight Island. A particularly strong cohort of small individuals (approximately 11 mm test diameter) dominated the size frequency distribution of sea urchins at Montague Island in 1997, and continued to have a strong influence on the population structure through 1999. Also, a second relatively strong recruitment at Montague was evidenced by a second cohort in 1998. In contrast, there was little evidence of small (less than 14 mm test diameter) individuals at Knight Island in 1997 or 1998 suggesting a relative lack of recruitment there in the recent past. While these data suggest potentially interesting comparisons with respect to factors regulating recruitment, the overriding influence on sea urchin community structure appears to be predation by sea otters that prevents survival of larger individuals.

We cannot dismiss the possibility that the lack of a stronger response by sea urchins in WPWS was related, in part, to impacts associated with the oil spill. Although Dean et al. (1996) found no evidence of an impact of oil on sea urchin populations, very few sea urchins were found at either oiled or unoiled sites and the power to detect differences was low. For several more abundant intertidal and subtidal animals, for which differences were more easily detected, higher densities were observed in unoiled vs. heavily oiled areas (Dean *et al.* 1996, Highsmith et al. 1996, Jewett et al. 1999).

While we observed no substantial increase in sea urchins following the reduction in sea otters in 1989, future changes are possible. The number of sea otters at northern Knight Island has remained low, and a strong recruitment year for sea urchins could result in an eventual increase in sea urchin biomass and a reduction in algae. However, given that there were relatively few small sea urchins in the Knight Island population in 2000, and that it takes several years for sea urchins to reach a size large enough to substantially affect sea urchin biomass; it is unlikely that such an increase will occur in the next several years.

#### *Biomarkers.--*

Elevated levels of the CYP1A biomarker were found in 6 predator species during the NVP study (Ballachey et al. 2002b, Trust et al. 2000, Jewett et al. 2002), and almost certainly appeared to be due to residual EVOS oil. For sea otters, there was some indication that in the third year of the NVP

study (1998), CYP1A levels had declined somewhat, although the decline was not statistically significant. One objective in resampling the oiled area population in 2001 was to see if there had been further decline. Although we did note a slightly decreased mean and lower variation in 2001, supporting the previous suggestion that exposure is decreasing over time, the difference between 2001 and 1998 or 1997 was not statistically significant. The only significant difference among years was between 1996 and 2001, in the oiled area. Thus, although we do have an indication of a decline over time, the evidence for this is not conclusive. For harlequin ducks (see Section 2) CYP1A values in oiled and unoiled areas have converged by 2003. Further CYP1A data on sea otters captured in 2002-2004 should improve our understanding of changes in CYP1A induction over time, as amounts of lingering oil presumably decrease. Data from 2002 were obtained using an alternate assay (real-time PCR) and thus are not directly comparable with previous years; however, for sea otters, an area difference in CYP1A persisted through 2002 (Ballachey et al. 2003b). Data on CYP1A in 2003 are pending, as is a direct comparison of the two assays. Further findings will be reported with results from upcoming sea otter capture and sample collection in 2004 under restoration project //620.

Based on the data collected over the past decade, in conjunction with liver observations from 2001, it appears that GGT is a marker of exposure to toxic compounds in sea otter liver tissues. Although the difference in GGT between areas in 2001 was not statistically significant, the data nevertheless conform to a pattern observed in earlier studies (Ballachey et al. 2002a, 2003a), where a proportion of otters from the oiled area had high GGT values. In the 1992 study, that proportion approached 0.5, and some of the individual values were 2-3 fold higher than observed in 2001. By 1996-98, and in 2001-2003, the proportion of otters with high GGT values is about 20-25% (2003 value is actually 36%), with few extremely high GGT values (> 50 IU/L) detected. This decline in GGT values is consistent with declines for CYP1A, and further suggests that exposure to oil is diminishing, although not absent, by 2003.

Our finding of elevated exposure to oil in sea otters residing at northern Knight Island is consistent with the findings of Short et al. (2003), and other earlier findings (Hayes and Michel 1999, Carls 2001), that lingering oil from the 1989 spill persists in nearshore WPWS habitats. However, the question remains as to how lingering oil in the habitat results in exposure to sea otters, as well as other nearshore consumers. At least two potential pathways of exposure of lingering oil to sea otters exist. One results from consumption of prey that retain elevated concentrations of oil, and the other through excavations of sediments that retain oil. For some species of prey it is likely that these two pathways of exposure are not exclusive. Sea otter diets at northern Knight Island are dominated by clams (>0.70) (Dean et al. 2002), at least some of which are known to occur in oiled sediments and contained elevated levels of EVOS hydrocarbons at least as recently as 1996 (Fukuyama et al. 2000). Sea otters are known to excavate large quantities of sediments to recover clams (Kvitek et al. 1988), and allocate large proportions of their foraging effort to the intertidal zone, where lingering oil has been quantified (Short et al. 2003). Bodkin et al. (2004) found that nearly 0.20 of sea otter foraging in southeast Alaska occurred in water depths from 0-5 m in depth, and that similar to PWS, sea otter diet was comprised principally of infaunal clams (USGS unpub. data). As the range of the intertidal zone in PWS extends nearly 7 m below the high, high water mark, and assuming foraging depths in southeast Alaska are similar to those in PWS, sea otters are likely foraging where potential exposure to lingering oil exists, during a significant portion of their feeding.

## SECTION 2. HARLEQUIN DUCKS

### Introduction

Harlequin ducks were particularly vulnerable to effects of the *Exxon Valdez* oil spill. Much of the spilled oil was deposited in the nearshore intertidal and shallow subtidal zones (Galt et al. 1991), the coastal habitats where harlequin ducks occur. Also, Goudie and Ankney (1986) suggested that harlequins were near the lower limit of body size for sea ducks occurring in environments similar to Prince William Sound in winter. Because harlequin ducks exist close to an energetic threshold, anything that either affects health or condition directly (via toxic effects or increased metabolic costs) or indirectly (via food abundance) could have significant consequences for the population.

Vulnerability to oil spill effects was exacerbated by the harlequin duck's diet, which consists of a variety of intertidal and shallow subtidal benthic invertebrates (Goudie and Ankney 1986). Oil constituents can accumulate in bottom sediments and subsequently, benthic invertebrates (Peterson 2000), suggesting that food could be a route of oil contamination of harlequin ducks. Studies have documented hydrocarbons in harlequin duck prey and intertidal sediments from immediately post-spill through 2002 (Babcock et al. 1996, Boehm et al. 1995, Short and Babcock 1996, Wolfe et al. 1996, Short et al. 2003).

Also, among ducks, sea ducks have life histories that are particularly K-selected. Harlequin ducks typically defer reproduction for 3 years, have relatively low annual investment in reproduction, and are long-lived (Goudie et al. 1994). Species with these characteristics have relatively low potential rates of population change and, thus, following a perturbation such as an oil spill, require many years in the absence of continued adverse effects to recover to previous population levels. Further, population dynamics of animals with this life history strategy are particularly sensitive to variation in adult survival (Goudie et al. 1994).

Sea ducks have a general pattern of high philopatry throughout their annual cycle and harlequin ducks seem to follow this pattern, with some evidence of high fidelity to molting and wintering sites (Robertson 1997). High site fidelity could result in vulnerability to population effects because, 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). High site fidelity is an adaptive behavioral strategy in natural situations and predictable environments (Robertson 1997), but does not accommodate movement to undisturbed sites in the face of human-caused perturbations.

Evidence from Nearshore Vertebrate Predator (NVP) studies suggests that, as might be predicted from their vulnerability, harlequin duck populations had not fully recovered from the oil spill by 1998. Over the course of 3 winters, survival probabilities were lower in oiled areas than unoiled (Esler et al. 2000a). Analyses revealed that history of oil contamination was a more likely explanation for the survival difference than intrinsic differences between oiled and unoiled study areas. Further, projections of population trends using models incorporating these survival probabilities predicted declining populations on oiled areas and increasing populations on unoiled areas. This pattern was observed during Alaska Department of Fish and Game surveys

(EVOSTC Project /427), suggesting that differences in survival were a likely mechanism for observed differences in population trends. Also, harlequin duck densities were lower on oiled Knight Island than on unoiled Montague Island, after accounting for intrinsic habitat differences; this is the pattern that would be predicted given poorer survival on oiled areas. Finally, differences in CYP1A induction were detected between populations from oiled and unoiled areas, indicating continued exposure to residual oil (Trust et al. 2000); also, a negative relationship was detected between winter body mass and CYP1A level (Esler et al. 2002).

One can speculate on mechanisms by which continued exposure to oil could be related to differences in survival probabilities. Most lab studies have shown that mallards 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, similarly, be unaffected by residual Exxon Valdez oil (Stubblefield et al. 1995, Boehm et al. 1996). However, other studies have found that, with addition of other stressors such as cold temperatures, oiled ducks in the lab suffered considerably higher mortality than unoiled (Holmes et al. 1978, 1979). This seems to be a much more appropriate analog for wild harlequin ducks. Particularly given their vulnerability to spill effects and hypothesized existence near an energetic threshold, harlequin ducks may not be able to handle additive effects of the oil spill, even if relatively small.

To fully understand the process of harlequin duck population recovery from the oil spill, it is important to address putative links between oil exposure, survival probabilities, and resulting population trends. This project was designed to explore the aforementioned potential mechanisms constraining population recovery through captive studies of behavioral and CYP1A responses to controlled oil exposure and field studies of winter survival and CYP1A induction. Additionally, because harlequin ducks use nearshore intertidal and shallow subtidal zones for foraging, they are an ideal species for monitoring the recovery process in these environments.

Another important attribute to consider in terms of population recovery is how demographic rates are linked by movements between oiled and unoiled areas of Prince William Sound. Rates of movement determine the extent to which population segments are maintained by internal production, and what extent by immigration (Nichols and Pollock 1990, Connor et al. 1983). The exchange of individuals can link demographic rates across areas, whereas site fidelity produces subpopulations having independent demographic processes (Ranta et al. 1997, Bjørnstad et al. 1998). By estimating the frequency of exchange between population segments, both from one year to the next and over the course of the winter period, inferences can be drawn about the degree to which individuals in different locations are demographically connected. This knowledge is critical for delineating subpopulations and managing them for sustainability (Dizon et al. 1992, Moritz 1994). For harlequin ducks in PWS, these kinds of data will lead to an understanding of the relative contribution of dispersal to population recovery, as well as the temporal and spatial scales over which this may occur. To derive dispersal probabilities and delineate demographically independent subpopulations, direct measurements of philopatry, site fidelity, and movement rates are extremely useful (Nichols and Kaiser 1999, Bennetts et al. 2001); the extensive banding and radio telemetry that have been part of harlequin duck studies lends itself perfectly to that objective.

The specific questions addressed by the harlequin duck components of this study are: (1) what is the relationship between levels of oil exposure and CYP1A induction, and what levels of oil exposure result in CYP1A values similar to those measured in PWS? (2) are there behavioral consequences of oil exposure that compromise harlequin duck survival? (3) is oil exposure (as indicated by CYP1A induction) related to survival of harlequin ducks in the wild? (4) is contaminant exposure declining over time and, similarly, are survival rates on the oiled area improving through time? and (5) at what scale are harlequin duck subpopulations demographically independent, and how does that influence recovery time?

Questions 1 and 2 were addressed using captive birds at the Alaska SeaLife Center during winters 2000-01 and 2001-02. Questions 3, 4, and 5 have been addressed by biosampling and radio telemetry work during winters 2000-01, 2001-02, and 2002-03. This work examines both the process of recovery (through understanding of the mechanisms constraining population demography) and monitors the progress of recovery by sampling survival and CYP1A induction of wild birds starting 3 years subsequent to the work done as part of NVP (winter 1997-98).

## Methods

### *Captive Studies – Behavior Responses to Oil Exposure.--*

#### *Capture and Captive Husbandry*

Twenty-five adult female harlequin ducks were captured during wing molt from the northwest coast of unoiled Montague Island (60.2°N, 147.2°W), Prince William Sound, Alaska in early September in each of 2 years (2000 and 2001). Flightless birds were herded with sea kayaks into a corral trap placed along the shoreline. Captured birds were sexed by plumage characteristics and cloacal anatomy, and age class was determined by bursal depth (Mather and Esler 1999). Adult female harlequin ducks captured in each year were transported by float plane to the Alaska SeaLife Center (ASLC), in Seward, Alaska (60.1°N, 149.4°W), where they were held in captivity for the duration of that winter and used as subjects in the experiments described below.

Captive harlequin ducks were housed in a mesh-enclosed outdoor pen exposed to local climate and photoperiod conditions. The enclosure was partitioned into three 3.7 x 3.7 m sections, each with a flowing seawater pool (2000-01: diameter 1.8 m, depth 0.9 m; 2001-02: diameter 2.4 m depth 0.6 m). The captive diet consisted of *ad libitum* commercially obtained Atlantic silversides (*Menidia menidia*) presented in trays on land during winter 2000-01, and an *ad libitum* mixed diet of Atlantic silversides and Antarctic krill (*Euphausia superba*) placed in pools during winter 2001-02. The changes in diet composition and presentation made in the second year of the study were intended to increase the foraging effort, which was low under the conditions of the first year of the study. Freshwater was offered in each pen in heated plastic bowls to prevent freezing, along with a mixture of sand and crushed oyster shell for grit.

#### *Oil ingestion experiments*

In both years of the oil ingestion experiment, birds were given 4 weeks to acclimate to the captive environment and diet before the start of oil-dosing. After acclimation, the 21 birds that had adjusted to captivity best, based on maintenance of body mass, were randomly assigned to 1 of 3 treatments (control, low-dose, high-dose), each with 7 birds. The birds assigned to each treatment group were kept together in one of the 3 partitions of the outdoor enclosure. Each bird was banded with a pair of colored plastic tarsus bands to allow identification of individuals.

Different dosing regimes were used in the 2 years of the oil ingestion experiment (Table 2.1). In 2000, dosing was conducted twice weekly. Low-dose birds were fed 1 ml of oil per kg body mass at each dosing, high-dose birds were fed 10 ml of oil per kg body mass at each dosing, and controls were fed no oil, although they were handled identically to the dosed birds. The average body mass ( $\pm 1$  SD) of the harlequin ducks used in the study was  $523.0 \pm 25.8$  g and was similar in both years. Doses were administered via gavage with a syringe and feeding tube; control birds were gavaged with an empty feeding tube. Dosing was done in the morning before birds had fed.

Table 2.1. Oil dosing regimes administered to captive harlequin ducks in each of two winters of oil-dosing experiments at the Alaska SeaLife Center, Seward, Alaska.

Year	Oil-dosing Treatment	n	Crude Oil Dose (ml kg <sup>-1</sup> )	Dosing Frequency	Cumulative dose per week (ml kg <sup>-1</sup> )
2000-01	Control	7	0.00	Twice-weekly	0.0
	Low Dose	7	1.00	Twice-weekly	2.0
	High Dose	7	10.00	Twice-weekly	20.0
2001-02	Control	7	0.00	Every-other-day	0.0
	Low Dose	7	0.57	Every-other-day	2.0
	High Dose	7	5.71	Every-other-day	20.0

We changed the dosing regime in the second year of the study to better simulate low-level, intermittent ingestion of weathered crude oil. During winter 2001-02, dosing was conducted every other day in the evening, after birds were allowed to feed. Low-dose birds were fed 0.57 ml of oil per kg body mass, high-dose birds were fed 5.71 ml of oil per kg body mass, and control birds were handled identically, but were fed no oil. The cumulative dose over a 2-week period for each treatment was equivalent in both years of the study (low-dose 4 ml kg<sup>-1</sup>, high-dose 40 ml kg<sup>-1</sup>), but doses were smaller and more frequent in the second year. In both years, birds were held for 60-90 minutes after dosing in an indoor, mesh-bottomed pen before being returned to the outdoor enclosure to prevent contamination from oily excreta.

We dosed birds with weathered crude oil from Prudhoe Bay, Alaska. To simulate weathering, crude oil was mixed with seawater and stirred continuously for 10 days at 25°C, after which it was separated from water and stored in air tight containers until dosing (Ben-David et al. 1999). This process was meant to remove the more volatile, acutely toxic, low molecular weight aromatic hydrocarbons, as would occur when oil is released into the environment during an oil spill (Ben-David et al. 1999). We measured doses on a weight per volume basis using an electronic balance ( $\pm 0.01$  g) and the specific gravity (g mL<sup>-1</sup>) of the weathered oil. Doses were based on body masses measured at the previous handling event. Dosing occurred between 15 October 2000 and 20 February 2001 during the first year (total dosings = 37), and between 15 October 2001 and 10 February 2002 in the second year (total dosings = 60). Birds that became sick during the course of the experiment were treated by ASLC veterinary staff. If possible, oil-dosing was continued through the course of treatment; however no data were collected until sick

birds returned to good health as judged by the veterinary staff. If the illness was severe, the sick bird was removed from the experiment. Mortalities unrelated to oil-dosing occurred in all 3 treatments during both winters of the experiment; the sample size at the end of dosing in both winters was 17.

### *Behavioral observations*

Time-activity budgets were quantified by following a focal individual continuously and recording the duration of time spent in all activities during a 30-minute observation period (Altmann 1974). Activities were categorized as feeding, maintenance (preening, bathing, stretching, comfort movements), locomotion (walking, swimming, flying), resting (sleeping, loafing), social (agonistic interactions), or drinking. Feeding activity in winter 2000-01 when birds fed from trays consisted of handling food items, and in winter 2001-02 when feeding required diving for food, feeding activity consisted of time spent searching, diving, and handling food items. Time spent in activities was recorded using *The Observer* software (Noldus Information and Technology 1991) and a notebook computer. One observer conducted all behavioral observations using binoculars from an elevated blind located 10 m from the outdoor enclosure.

Birds were observed in a random sequence; no resampling occurred until all birds had been sampled in the prior round of observations. Start times were randomly determined within 3 equal diurnal periods (morning, afternoon, evening) beginning 30-minutes prior to sunrise and continuing until 30-minutes after sunset. On each sampling day, 1 bird from each treatment during each of the 3 diurnal periods was sampled, for a total of 9 observations on each sampling day. Temperature (°C), estimated wind speed (Beaufort scale), and day length (minutes) were recorded at the start of each observation session.

During the second year of the study, the feeding protocol was modified during the first 6 weeks of dosing, therefore only behavioral observations conducted after these modifications were complete (06 December 2001) were included in analyses. Three birds died from causes unrelated to oil-dosing between the start of dosing in winter 2001-02 and the start of behavioral observations, and therefore time-activity budget data were collected from only 18 birds during winter 2001-02.

### *Plumage-oiling Experiment*

We conducted a plumage-oiling experiment between 21 February and 18 March 2002, drawing subjects from the group of birds used in the oil ingestion experiment conducted earlier that winter. Ten days after the conclusion of the ingestion experiment, we randomly assigned birds to 3 plumage-oiling treatments such that each of the former ingestion treatments was represented in each new plumage-oiling treatment. Plumage-oiling treatments consisted of a lightly oiled group exposed to 1 ml of crude oil for 40 minutes ( $n = 6$  birds), a moderately oiled group exposed to 2.5 ml of crude oil for 50 minutes ( $n = 4$  birds), and a heavily oiled group exposed to 5.0 ml of oil for 60 minutes ( $n = 6$  birds). Birds were individually exposed to weathered Prudhoe Bay crude oil in the laboratory while at rest in a tub containing 14 L of seawater. After a 10 minute acclimation period, weathered crude oil was injected through a port using a syringe. The experiment was stratified over a 3-week period. Birds from 2 treatments were oiled on day 1 of each oiling period and remained oiled for 7 days.

After oil exposure, birds were housed in the outdoor enclosure previously described. Behavioral observations were conducted over each 7-day experimental period using the same protocol as described for the oil ingestion experiment. The behavior of 1 to 4 randomly selected, non-oiled birds was sampled during each observation session during the first 2 weeks of the experiment as controls; birds that had been oiled and washed during a previous week of the experiment were not included in the pool of control birds so that by the final week of the experiment no birds remained that had not been previously oiled (i.e., no control observations were conducted during the final week).

### *Data Analysis*

We focused our analyses on the 2 activities most important to the winter survival of harlequin ducks: feeding and maintenance. Time-activity budgets can be adjusted at multiple scales including the frequency of occurrence of each activity, as well as duration of time spent in each activity. Because feeding and maintenance activities did not occur during all observation periods, the data were analyzed in 2 stages: (1) the presence or absence of a behavior during an observation period was treated as a binary response and analyzed using logistic regression models; and (2) for the subset of observation periods during which the activity of interest occurred, the proportion of time spent in the activity was analyzed using general linear mixed models. Thus, this analytical approach addressed both the factors affecting the probability that the activity would occur during a given 30-minute observation period, and the factors affecting the time spent in the activity when it was observed.

We used an information theoretic approach (Akaike Information Criterion, AIC; Burnham and Anderson 2002) to select the best-fitting model(s) from among a set of *a priori* candidate models. AIC evaluates the strength of evidence for each model using the principle of parsimony to balance model fit (log likelihood) with model complexity (the number of parameters). The model having the lowest AIC value was interpreted as being best supported by the data from among the models considered. We used AIC adjusted for small sample sizes ( $AIC_c$ ) for all data other than binary responses exhibiting overdispersion, for which we used  $QAIC_c$ , the quasi-likelihood adjustment of  $AIC_c$  (Burnham and Anderson 2002). Models in a given candidate set were ranked according to their AIC values, and the difference between the top-ranked model and each subsequent model,  $\Delta AIC$ , was calculated. Models with  $\Delta AIC = 2$  were considered to be strongly supported by the data (Burnham and Anderson 2002).

We calculated Akaike weights to evaluate the strength of evidence for each model and to estimate model-selection uncertainty. Akaike weights within a set of candidate models are standardized to sum to 1 and therefore an Akaike model weight near 1.0 indicates a high probability of being the best model given repeated samples (Burnham and Anderson 2002). Model-selection uncertainty was incorporated into parameter estimates and measures of precision using model averaging based on Akaike weights (eqs. 4.5 and 4.11, respectively; Burnham and Anderson 2002). Model-averaged estimates are less biased than estimates from a single, top-ranked model when the top-ranked model is not strongly supported (i.e., an Akaike weight  $< 0.90$ ). The relative support for each explanatory variable included in a candidate model set was evaluated by summing the Akaike weights from all models that included each explanatory variable (Burnham and Anderson 2002). Thus, explanatory variables that occur in

most or all of the models most supported by the data will have high summed Akaike weights (close to 1.0), and therefore are well supported for drawing inference (Burnham and Anderson 2002).

Behavior of wintering waterfowl has been demonstrated to be associated with a number of time and weather variables (Brodsky and Weatherhead 1985, Goudie and Ankney 1986, Fischer and Griffin 2000). However, the behavioral response to these variables may differ under the conditions of captivity. Therefore, the sets of candidate models we evaluated were necessarily broad. None of the explanatory variables considered in any model set were highly correlated, and all models were judged to be biologically plausible prior to inclusion in the candidate set. Because candidate model sets were large, we based our inference primarily on the relative support for explanatory variables across models (summed Akaike weights for explanatory variables) and unconditional 95% confidence interval coverage around model-averaged adjusted mean estimates of response variables for each oil treatment group, rather than basing inference on a particular model or set of models.

Explanatory variables considered for the oil-dosing experiment included: oil-dosing treatment (TREAT: control, low-dose, high-dose; categorical), time of day (DIURN: morning, afternoon, evening; categorical), wind speed (WIND: low 0-6 knots, moderate 7-21 knots, high 22<sup>+</sup> knots; categorical), temperature (temp: °C; continuous), day length (dayl: minutes of photoperiod; continuous), and year (YEAR: winter 2000-01, winter 2001-02; categorical). Categorical variables were included as indicator variables, with the first level of each designated as a reference level (Ramsey and Schafer 2002). Models including interactions always included the main effects of interactive terms (Ramsey and Schafer 2002).

Candidate models, both logistic regression and general linear mixed, considered for the analysis of the feeding and maintenance activities by oil-dosed harlequin ducks consisted of all possible combinations of main effects of the explanatory variables temp, dayl, DIURN, TREAT, YEAR, and the TREAT × YEAR interaction, as well as an intercept-only null model, for a total of 41 candidate models. The effects of oil ingestion on behavior were considered *a priori* to be physiologically based and additive to the extrinsic effects of the environment (Holmes et al. 1978, 1979). We constrained all models to include YEAR to account for expected changes in behavior related to feeding protocol differences between years. The TREAT × YEAR interaction was considered to account for the potential influence of differences in feeding protocol and (or) dosing regime on the behavioral response to oil-dosing. Changes in response with time were confounded with changes in day length through the season, and therefore only day length was included as an explanatory variable in the models.

Behavior of externally-oiled birds was evaluated in relation to the same set of explanatory variables as considered for the oil-dosing experiment; however, because day length did not vary substantially over the 7-day experimental periods, it was not included. The effect of external-oiling on feeding activity was expected to operate through increased thermoregulatory costs, which could vary with temperature and wind speed. Interactions between external-oiling treatment and weather variables (TREAT × temp, TREAT × WIND) were included in the set of logistic regression models for the probability of feeding. For these data, wind speed was included as a 2-level categorical variable (WIND: low 0-6 knots, moderate-high 7<sup>+</sup> knots) to

allow evaluation of the TREAT  $\times$  WIND interaction with the limited data available (i.e., not all oiling treatments were observed under the original wind speed categories). Time of day was included as a 2-level categorical variable because observations were made only during afternoon and evening periods. Linear and quadratic trends in feeding as a function of days post-oiling (day; continuous) were considered as interactions with TREAT to allow estimation of a separate slope parameter for each treatment (TREAT  $\times$  day, TREAT  $\times$  day<sup>2</sup>), because non-oiled birds were not expected to respond to days post-oiling. The candidate model set of logistic regression models for feeding by externally-oiled birds consisted of all possible combinations of these main effects and interactions, as well as an intercept-only null model, leading to a set of 62 candidate models.

The small sample of observations during which feeding occurred in the external-oiling experiment ( $n = 76$  observations) precluded consideration of complex models. The set of candidate general linear mixed models for time spent feeding consisted of all possible combinations of the main effects of TREAT, WIND, DIURN, and temp, as well as an intercept-only null model, leading to a set of 16 candidate models.

The effect of external-oiling treatment on maintenance activity was expected to operate through decreased plumage quality and to be additive to the effects of weather and time. Linear and quadratic trends in the response of maintenance activity to external-oiling over days post-oiling were incorporated in models as interactions with TREAT. The set of candidate models for both logistic regression and general linear mixed models was composed of all possible combination of main effects, TREAT  $\times$  day, and TREAT  $\times$  day<sup>2</sup> interactions, and an intercept-only null model, leading to a set of 30 candidate models.

Logistic regression models were fit using PROC GENMOD of program SAS (SAS Institute 1999). Violation of the assumption of independent outcomes caused by repeated observations on the same subjects can lead to overdispersion from the binomial distribution and underestimation of variance (McCullagh and Nelder 1989). To assess overdispersion in the data, a variance inflation factor,  $c$ , was estimated from the global model from each candidate set, where  $c$  was the ratio of the deviance statistic to its degrees of freedom. When  $c > 1$ , quasi-likelihood estimation (McCullagh and Nelder 1989) and QAIC <sub>$c$</sub> , the quasi-likelihood adjusted calculation of AIC <sub>$c$</sub> , were used; when  $c = 1$  no adjustments were made and model-selection was based on AIC <sub>$c$</sub>  (Burnham and Anderson 2002). The goodness-of-fit of the global model in each set of logistic regression models was assessed using a deviance chi-square goodness-of-fit test to test the null hypothesis that the model was adequate (i.e.,  $P > 0.05$  indicated adequate fit; Ramsey and Schafer 2002). Model-derived estimates of response values for treatment groups were adjusted for average values of the other explanatory variables included in each model and averaged across all models based on Akaike weights. Model-averaged, adjusted mean estimates were back-transformed from the logit scale to estimates of probability.

General linear mixed models were fit using PROC MIXED of program SAS (SAS Institute 1999) and were used to account for correlation among residuals from repeated observations. General linear mixed models structure the correlation among residuals from each subject into a parameterized correlation matrix (Wolfinger 1993, Littell et al. 2000). A parameter for a random between-subject effect was also considered in the covariance structure of the model. For general

linear mixed model sets the best-fitting covariance structure was selected from a set of candidate structures using  $AIC_c$  model-selection criteria (Wolfinger 1993, Littell et al 2000). The spatial powers law structure to model decreasing correlation with increasing time between repeated observations, and the compound symmetric structure to model constant variance-covariance were the candidate residual structures considered (Wolfinger 1993, Littell et al. 2000). The best-fitting covariance structure was incorporated into all fixed effects model structures in the candidate set. The fit of the global general linear mixed model in each candidate set was assessed using a null model likelihood ratio test to test the null hypothesis that the global model did not improve fit over the null model (i.e.,  $P < 0.05$  indicated adequate fit; Ramsey and Schafer 2002).

Prior to analysis, measurements of the proportion of time spent in a given activity ( $p$ ) were logit transformed to meet model requirements of constant variance and normal distribution, where:

$$\text{logit } p = \ln[p/(1-p)].$$

Residual plots from the global model and top-ranked models from each candidate model set were used to confirm that model assumptions of normal distribution and constant variance were met; case influence statistics were calculated and examined for influential data points (Ramsey and Schafer 2002). Model-derived estimates of the mean response for each treatment were adjusted for average values of all other explanatory variables included in the model and averaged across all models based on Akaike weights. Model-averaged adjusted mean estimates were back-transformed to yield the proportion of time spent in the activity.

Variables included in the top-ranked model(s) for each candidate set ( $\Delta AIC = 2.0$ ) were interpreted as being important to the process being examined (either probability of occurrence or duration of occurrence of an activity). Inclusion of oil-dosing treatment in the top model(s) of a candidate set was interpreted as evidence of an oil treatment effect. Summed Akaike weights for explanatory variables were used to judge the importance of the TREAT main effect and the TREAT  $\times$  YEAR interaction. Unconditional 95% confidence intervals around model-averaged adjusted mean estimates of response variables for oil exposure treatments were used to identify differences among treatments.

#### *Captive Studies – CYP1A Induction.--*

Following the captive bird oil ingestion experiments in both years, we surgically obtained small (< 0.1 g) liver biopsies for CYP1A analysis. 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 (EROD), 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<sup>0</sup> C using a Cytofluor® fluorescent plate reader (Millipore, Bedford, MA). Each well contained 200  $\mu$ l consisting of 1  $\mu$ l of microsomes (4-15  $\mu$ g protein), 2  $\mu$ M 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.

We compared average ( $\pm$  SE) EROD activity across oil ingestion treatments. We detected dramatic interannual differences in EROD activity for both captive and field samples. We determined that these differences were a laboratory artifact and that, while comparisons among treatments were appropriate in any given year, between year comparisons were not. Therefore, we developed an index to EROD activity, based on the assumption that EROD activity would be similar in controls in each year. We set controls in each year to 1, and adjusted other treatment estimates according to their multiplicative relationship with the reported measurements of controls. We calculated estimates of variance on original data and adjusted those using the same correction factor.

#### *Field Studies – CYP1A Induction.--*

Harlequin ducks were captured during November of 2000, 2001 and 2002, using a modified floating mist net trap (Kaiser et al., 1995), at Montague Island (unoiled) and Green Island, Bay of Isles, Lower Passage, Crafton Island, and Foul Bay study sites. Captured females were placed under Isoflourane® anesthesia and livers were surgically biopsied to obtain a small (approximately 0.10 g) sample for CYP1A analysis. Immediately following biopsy, liver samples were placed in a cryogenic vial and frozen in liquid nitrogen. Following recovery from surgery (at least 1 hour), ducks were released near their capture site. The individuals from which liver biopsies were obtained were the same as those receiving radios (see below). Laboratory and statistical analyses followed those described for CYP1A analysis of captive birds (above). Correction factors for field CYP1A data followed the same procedure as described above, based on the assumption that EROD activity was similar between years on the unoiled, Montague Island site.

#### *Field Studies – Female Winter Survival.--*

This research required capture of flighted harlequin ducks during early winter, after they had been on wintering sites long enough to be potentially exposed to residual oil, yet before the mid-winter period when survival probabilities diverged during NVP studies (Esler 2000). The interval between capture and the critical mid-winter period must allow for at least a 2-week censor period to ensure that survival data are not biased by effects of capture, handling, or surgery (Esler et al. 2000c; Mulcahy and Esler 1999). Thus, we captured birds during 3-week periods in November 2000, 2001, and 2002 to generate both survival data and exposure data from the same individuals.

We used floating mist nets (Kaiser et al. 1995) to catch flying birds in oiled (Knight Island, Green Island, Crafton Island, Main Bay, Foul Bay) and unoiled (Montague Island) study areas. Use of the same study areas as the NVP project allows for direct comparisons of results. Captured birds were banded with uniquely coded USFWS bands, aged by bursal probing (Mather and Esler 1999), and sexed by plumage characteristics. We radioed females of all age classes, using abdominally implanted VHF radio transmitters with external antennas (Korschgen et al.

1996). 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, 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), especially for diving ducks (Korschgen et al. 1984). NVP studies (Esler et al. 2000c) demonstrated that recapture probabilities of radio-marked harlequin ducks were not lower than unradioed individuals. Surgeries were conducted by certified veterinarians experienced in avian implant surgeries, following procedures outlined in Alaska Biological Science Center, USGS Biological Resources Division standard protocol. Transmitters weighed approximately 18g, which is < 3% of the body mass of the smallest wintering female harlequin duck captured. Transmitters were equipped with mortality sensors; the pulse rate changed from 45 to 90 beats per minute when mortality was indicated. Mortality status was confirmed by either carcass recovery or detection of signals from upland habitats, which are not used by harlequin ducks during nonbreeding periods.

Radio signals were monitored approximately every 2 weeks from the time of marking through the end of March. Each bird's status (alive, dead, or missing) was recorded, as well as a general location. Unbiased survival estimation using telemetry requires that several critical assumptions are met (Pollock et al. 1989a, Tsai et al. 1999), 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 explicitly tested assumptions 3 and 4 (Esler et al. 2000c) and found that these were met under the conditions of this project.

For each week's sample of relocations, we counted mortalities and numbers of harlequin ducks at risk of mortality (i.e., numbers of detected radios), following procedures outlined in Pollock et al. (1989a,b) and Bunck et al. (1995). We calculated survival probabilities by month, accounting for the history of each individual within each month, and calculated cumulative survival probability as the product of the monthly survival, with variance estimates calculated using Greenwood's formula (Pollock 1989a).

#### *Field Studies – Dispersal and Population Structure.--*

For this portion of the project, we used band recovery and radio telemetry data collected during the NVP and 423 projects to estimate dispersal, with implications for population structure.

#### *Band recovery data*

Harlequin ducks were captured during wing molt by herding flightless birds into funnel traps using sea kayaks. Capture sessions were conducted during five molt seasons: 1995, 1996, 1997, 2000, and 2001, with capture dates ranging from 1 August to 17 September in 1995-1997 and 6 to 12 September in 2000-2001. Unlike most waterfowl, harlequin ducks undergo wing molt on the same marine areas where they winter (Robertson and Goudie 1999, but see Brodeur et al. 2002). Therefore, inferences drawn from molting birds are applicable to both molting and wintering stages. Capture-mark-recapture analyses were performed on birds captured at three

different trapping locations on Montague Island: Stockdale Harbor, Port Chalmers, and West Montague). Distances between sites ranged 8.4 to 16.1 km. These capture dates encompassed the periods of peak wing molt by adult females. Adult males typically undergo wing molt before females, and were not targeted during 2000-2001. All captured individuals were marked with U.S. Fish and Wildlife Service aluminum bands. Sex determinations were made based on plumage characteristics and cloacal examination and age class was determined by probing bursal depth (Mather and Esler 1999). Our analyses were restricted to after hatch year birds because waterfowl do not undergo a wing molt during their first autumn and, as a result, hatch year birds were not susceptible to our trapping methodology.

Given our moderate sample size, we made an *a priori* decision to estimate philopatry and survival in separate analyses. Although models which simultaneously estimate survival, movement, and recapture probabilities have been developed (Hestbeck et al. 1991, Brownie et al. 1993), these “multi-strata” models tend to be highly parameterized and therefore extremely data hungry. In order to estimate parameter values with the greatest confidence and improve model selection capabilities, our approach was to infer philopatry directly from homing rates, and obtain annual survival estimates using a Cormack-Jolly-Seber (CJS) modeling approach (Cormack 1964, Jolly 1965, Seber 1965). An *a posteriori* analysis also was used to evaluate survival and movement probabilities concurrently using a multi-strata model. This analysis was performed to check consistency in our results across analysis approaches. Methodologies for the three analyses are presented below:

#### A. Philopatry—homing rate

In studies that use multiple capture areas, homing rate can be estimated directly as the ratio of birds recaptured on their original capture site to those recaptured elsewhere (Robertson and Cooke 1999). This ratio considers only individuals alive at both time  $t$  and time  $t+1$ , thus eliminating survival as a confounding variable. The method assumes that recapture probabilities are similar at all study sites, and will tend to overestimate the true homing rate if recapture effort outside the study area is low.

We estimated homing rates and 95% confidence intervals (95% CI), for harlequin ducks originally banded and then recaptured at the three capture sites on Montague Island. Two-tailed Fisher’s exact tests were used to assess variation in homing rate with respect to sex, trapping location, and year of original capture. To complement this fine scale analysis, we used the supplemental dataset for recaptured birds from Green Island, Knight Island, and the Kenai Peninsula to estimate the numbers of birds that had moved outside the Montague Island study area (a requirement of unbiased homing rate estimation), as well as to quantify the range of distances moved.

#### B. Annual survival—Cormack-Jolly-Seber model.

Individual CMR histories were analyzed using the CJS model procedure in program MARK (White and Burnham 1999). Our data set included: four encounter occasions with time intervals 1,1,3,1, set to match capture years; and two attribute groups, after hatch year males and after hatch year females (hereafter males and females). Model selection was based on Akaike’s Information Criterion (AIC) (Burnham and Anderson 2002), with notation described by Leberton et al. (1992). Model parameters were survival probability ( $\phi$ ), which is the probability an

animal alive at the time of sampling in year  $t$  is alive at the time of sampling in year  $t+1$ ; and recapture probability ( $\rho$ ), which is the probability that an animal in the sampling area during time  $t$  is captured. It should be noted that in CJS models permanent emigration cannot be distinguished from mortality, therefore  $\phi$  is a measure of “apparent” rather than “true” survival. Subscript notation, which denotes the sources of variation within each parameter were: sex ( $s$ ) and time ( $t$ ). A dot in the subscript indicates that a model parameter was constant. A logit link function was used to bound parameter estimates within their logical parameter space of 0-1.

The most general model we considered,  $\phi_{(s^*t)}\rho_{(s^*t)}$ , allowed for variation in survival and recapture probability according to sex and among years. We evaluated overall goodness of fit for our general model by applying the parametric bootstrap procedure in program MARK. The proportion of simulations out of 1000 that had a greater deviance than our actual model provided a test of model fit. Simulation results also were used to compute a variance inflation factor,  $\hat{c}$ , which is the ratio of deviance from the actual data to the mean deviance from the bootstrap replicates. This adjusted  $\hat{c}$  estimate was used to compute quasi-likelihood AIC values adjusted for sample size, QAIC<sub>c</sub>. In addition to the general model, 15 candidate models, with reduced numbers of parameters also were considered. Akaike weights were used as indications of support for each model and parameter estimates were calculated using model averaging across all candidate models (Burnham and Anderson 2002).

### C. Philopatry and Survival—Multi-strata model.

To check consistency in our results individual CMR histories also were analyzed using the multi-strata, live recapture procedure in program MARK (White and Burnham 1999). Multi-strata models permit estimation of movement rate ( $\psi$ ), by separating the joint probability of surviving and making a transition between two states. Model notation denotes  $\rho_i^s$  as the probability that a marked animal alive in stratum  $s$  at time  $i$  is recaptured at time  $i+1$  and  $\phi_i^{rs}$  as the joint probability that an animal alive in stratum  $r$  at time  $i$  is alive and in stratum  $s$  at time  $i+1$ . The joint probability  $\phi_i^{rs}$ , is then divided into survival and movement components, such that:  $\phi_i^{rs} = S_i^r \psi_i^{rs}$ , where  $S_i^r$  is the probability that an animal survives in stratum  $r$  from time  $i$  to time  $i+1$ , and  $\psi_i^{rs}$  is the conditional probability that a marked animal alive in stratum  $r$  at time  $i$  is in stratum  $s$  at time  $i+1$ , given that the animal is alive at time  $i+1$ .

Attribute groups were identical to those used in the CJS analysis; however, the individual encounter history files were modified such that the 1's and 0's used in the CJS model (where 1 denoted a capture event and 0 a year when the bird in question was not captured) were replaced with: H (for home), denoting the first time a bird was captured or any recapture event on the same site as the previous capture event; M (for move), denoting that a bird had been recaptured at a site other than the one it had previously been captured; and 0, for a year when the bird in question was not captured.

A total of 32 candidate models were considered, with the most general,  $\phi_{(s^*t)}\rho_{(s^*t)}\psi_{(s)}$ , allowing for variation in survival and recapture probability according to sex and among years, as well as sex-related variation in the probability of transition from one strata to another (H to M or vice

versa). Differences among any of the three parameters were, however, constrained to be equal among strata. This meant, for example, that while survival could differ between the sexes and over time, it could not differ between individuals which had moved and those which had not. As in the CJS analysis a logit link was used to bound parameter estimates between 0-1.

Parametric goodness of fit tests have not been developed for multi-strata models in program MARK (White and Burnham 1999), so non-parametric bootstrap goodness of fit tests were conducted to ensure that models adequately fit the data (Barry Smith, pers. comm.). The variance inflation factor,  $\hat{c}$ , was estimated using bootstraps of the data, and observed frequencies of encounter histories were compared to simulated values generated by model parameters and the binomial distribution. Thus, the proportion of simulations out of 1000 that had a greater deviance than our actual model provided a test of model fit.

#### *Telemetry data*

Movement data were collected during six different winters (1995-1996 through 1997-1998 and 2000-2001 through 2002-2003). Radio marked females were monitored at approximately 2 week intervals from an airplane to determine mortality status and location. All frequencies were monitored throughout the winter season, including those for which signals were lost. During monitoring flights individual birds were located by *site*, which we defined as a bay, inlet, group of islets, or continuous section of coastline bounded by a prominent geographic feature. Because we were interested in defining the spatial scale of movements, we created two additional geographic strata to evaluate movement probabilities. These were: *zones*, which were defined as groupings of adjacent sites, separated from other zones by >3 km of habitat in which harlequin duck densities approached zero; and *areas*, which were islands or mainland regions separated from other regions by >5 km of open water. Thus, each bird would be assigned a three level geographic strata location (e.g. *site*: Stockdale Harbor; *zone*: Northwestern Montague Island, *area*: Montague Island) each survey.

In addition to geographic strata locations, exact positions were recorded for a subset of 35 adult females in 1996 and 1997. These records were taken only when weather conditions allowed identification of the transmitting bird, and therefore, are as accurate as our pilot's ability to map them on a 1:80,000 marine chart. Birds selected for this dataset were balanced among oiled and unoiled portions of Prince William Sound, with 1-3 birds selected at random from each capture site each year.

Two different analytical approaches were used to evaluate the spatial scale and demographic implications of harlequin duck winter movements. The first was designed simply to estimate the probability that an individual would emigrate from the location in which it had been trapped, and then compare movement probabilities at the *site*, *zone*, and *area* scales. The second quantified individual movements by calculating home ranges and distances traveled between successive observations. The two analyses are described below:

#### A. Probability of emigration at different spatial scales—Kaplan Meier analysis.

We used a modified Kaplan-Meier procedure to estimate the probability that an individual would remain site faithful during the winter period. The Kaplan-Meier estimator is commonly used in telemetry studies to estimate survival rates (Pollock et al. 1989a, b, Bunck et al. 1995). In its

familiar usage, the survival function ( $S[t]$ ) describes the probability that an arbitrary animal will survive from the beginning of the study to time  $t$ . In our analysis we replaced the survival function with a fidelity function ( $F[t]$ ) (Bennetts et al. 2001). Our flight surveys occurred at 1-3 week intervals, therefore, movement events could be detected only at discrete time points  $a_1, a_2, \dots, a_g$ . Thus, we defined  $r_1, r_2, \dots, r_g$  to be the number of previously site faithful individuals at risk of moving at these points and  $e_1, e_2, \dots, e_g$  to be the number that emigrated at the same points. The probability of remaining site faithful from time zero to time  $a_1$  was estimated by  $\hat{F}(a_1) = 1 - e_1 / r_1$ , where  $e_1 / r_1$  was the proportion emigrating during that interval. The probability of remaining site faithful from  $a_1$  to  $a_2$  was  $1 - e_2 / r_2$ , and  $\hat{F}(a_2)$  was calculated as the product  $(1 - e_1 / r_1)(1 - e_2 / r_2)$ . Similarly, the cumulative fidelity function  $\hat{F}(a_g)$  was calculated as  $(1 - e_1 / r_1)(1 - e_2 / r_2) \dots (1 - e_g / r_g)$ , and the probability of emigration for the entire winter period was defined as  $1 - \hat{F}(a_g)$ .

In our analysis we defined the geographic stratum in which an individual was originally trapped as its reference location, and estimated fidelity functions at each of the three scales (*site, zone, and area*). During each survey individuals were categorized as *alive, dead, or missing*. Live individuals with functioning radios were assigned to one of three categories based on their location histories: (1) *home*—an individual detected at its reference location; (2) *foray*—an individual detected away from its reference location for a single interval, but detected back at its reference location the next interval; or (3) *emigrant*—an individual detected away from its reference location for consecutive intervals. Because we wanted our estimates of winter emigration probabilities to be conservative, we stipulated that individuals categorized as on a *foray* would be considered in the *site faithful (r)* pool rather than the *emigrant (e)* pool. Data from all six years were pooled, and encounter histories were coded by monthly intervals. Separate calculations were made to determine the frequency individuals returned to their original site after having been categorized as *emigrant*. Statistical variation in movement probability by month, and according to age classes (for birds captured in 2000-2002) were assessed using contingency analyses (Fisher's exact test).

The assumptions for unbiased movement rate estimation are similar to those for survival in the Kaplan-Meier framework (Pollock et al 1989a, b, Tsai et al 1999, Esler et al. 2000c). They include: (1) radioed animals are representative of the population of interest, (2) movement probability is independent among individuals, (3) radio-marking does not affect movement during the study period, and (4) censoring of animals for which signal is lost is independent of the fate of those individuals. We expected little systematic bias based on susceptibility to capture and felt the first two assumptions were met on the basis of our capture technique and marking regime. The third assumption was explicitly tested with respect to survival by Esler et al. (2000c), and no effect was detected. The fourth assumption required validation for which calculations are described below.

Most signals that went undetected during a given interval were subsequently relocated. We assumed these birds remained in the study area and allowed staggered entry and re-entry of *temporarily missing* signals. When mortality signals were recorded individuals were categorized as *dead* and censored from the dataset. Birds whose signals went *permanently missing* at some

time during the winter also were censored; however it was important to know the proportions of these signals that went undetected due to radio failure versus movement outside the detection area, as undetected long distance movement would lead to an overestimation of the fidelity function.

To determine the proportion of birds that left the detection area we made use of a sample of harlequin ducks that had been implanted with radios in 1995 and 1996, which were monitored over the course of the winter, and were then recaptured during molt drives the following year (Esler et al. 2000b). We considered four conditions of interest for inferring the fate of the transmitters attached to these birds: (A) the transmitter remained in the study area and functioned normally, (B) the transmitter remained in the study area, but ceased functioning, (C) the transmitter moved outside the study area, and continued to function normally, and (D) the transmitter moved outside the study area and ceased functioning. By assuming that all recaptured birds remained in the study area ( $C_{recaptured}$  and  $D_{recaptured} = 0$ ) and that radio failure rates were similar among birds regardless of recapture status

$\left( \frac{A_{recaptured}}{A_{recaptured} + B_{recaptured}} = \frac{A_{tracked}}{A_{tracked} + B_{tracked}} \right)$ , we were able to estimate the proportion of radios

which moved outside the detection area using the following equation:  $P_{OutsideArea} =$

$$\frac{A_{tracked}}{A_{tracked} + B_{tracked} + C_{tracked} + D_{tracked}} - \frac{A_{recaptured}}{A_{recaptured} + B_{recaptured}}$$

B. (2) Spatial scale of individual movements-- kernel home range estimates.

Individual home ranges and movement patterns were estimated for the subsample of birds for which exact locations data were collected using geographic information system software (GIS; ArcView, ESRI Inc., Redlands, California). Bird locations were plotted on a digital base map of Prince William Sound and analyzed using the Animal Movements extension for ArcView 3.2 (Hooge and Eichenlaub. 2000). We considered a number of home range methods, including fixed kernel, minimum convex polygons, and bivariate ellipses. Based on recommendations by Seaman et al. (1999), sample size considerations, and distributions of the data, we determined use of fixed kernels, with smoothing selected by least squares cross validation (LSCV) Silverman (1986), was the most appropriate method for our dataset. Home ranges (95% fixed kernels), core areas (50% fixed kernels), and successive distances traveled between observations were calculated for each adult female. An *a priori* decision was made to pool data between the two years (1996 and 1997).

Kernohan et al. (2001) identified several potential sources of error that can bias home range estimates which we considered prior to our analysis. They included: (1) uneven sampling over distance and truncated dispersal distances through failure to detect long distance movements—which we believe our aerial survey methodology and previous analyses addressed; (2) induced behavioral changes—which were explicitly test with respect to survival by Esler et al. (2000c) and shown to have no effect; (3) serial correlation of successive observations—which we feel was not an issue based on the frequency of our surveys, and (4) sample size—which we evaluated by regressing the number of locations obtained per individual against its kernel home

range size to determine if home range sizes were biased low when fewer detections were made per individual.

## Results

### *Captive Studies – Behavior Responses to Oil Exposure.-- Effects of Oil Ingestion on Feeding Behavior*

A total of 820 behavior observations were conducted on 39 birds during the 2 winters of the oil ingestion experiment, 482 during winter 2000-01 and 338 during winter 2001-02. Feeding activity occurred during 68% of all observation periods. We found no evidence that feeding activity was affected by oil-dosing treatment. The fit of the global logistic regression model to the data was adequate (deviance goodness-of-fit  $\chi^2_{807} = 807.9, P = 0.4845$ ) after adjusting for slight overdispersion ( $c = 1.25$ ), however the intercept-only null model was selected as best-fitting (Table 2.2). Summed Akaike weights for each of the explanatory variables considered in the models were low ( $< 0.40$ ) indicating that no explanatory variable considered in the *a priori* logistic regression models was important for explaining variation in the probability of feeding (Table 2.3). Thus the probability of feeding activity by harlequin ducks during an observation period was unrelated to the weather, time, or oil treatment variables considered in our *a priori* models.

Table 2.2. Model-selection statistics from logistic regression and general linear mixed models for feeding and maintenance activities by harlequin ducks in an oil-dosing experiment at the Alaska SeaLife Center, Seward, Alaska during winters 2000-01 and 2001-02. The models best fit by our data ( $\Delta AIC_c \leq 2$ ) are presented.

Response variable	Explanatory variables	$k^b$	Log-likelihood	$\Delta AIC^c$	Weight
Probability of Feeding	Intercept	2	-513.70	0.00	0.20
	YEAR	3	-513.30	1.35	0.10
	TREAT + YEAR + TREAT x YEAR	7	-508.40	1.52	0.09
	DIURN + YEAR	5	-511.20	1.95	0.08
Proportion of Time Feeding	temp + dayl + WIND + DIURN + YEAR	11	-605.80	0.00	0.30
	dayl + WIND + DIURN + YEAR	10	-606.80	0.01	0.29
	dayl + DIURN + YEAR	8	-609.80	1.88	0.12
Probability of Maintenance	temp + TREAT + YEAR + TREAT x YEAR	7	-246.76	0.00	0.38
Proportion of Time Maintenance	TREAT + DIURN + YEAR	9	-1532.82	0.00	0.16
	DIURN + YEAR	7	-1535.01	0.29	0.14
	temp + TREAT + DIURN + YEAR	10	-1532.69	1.80	0.07
	TREAT + DIURN + YEAR + TREAT x YEAR	11	-1531.72	1.92	0.06

<sup>a</sup>See Methods for explanatory variable abbreviations.

<sup>b</sup>Number of estimated parameters includes +1 for intercept for all models, +1 for the estimate of the variance inflation factor for QAIC<sub>c</sub> models, and +3 for parameters in the covariance structure of general linear mixed models (spatial powers law covariance structure, random between-subject effect, and variance estimate).

<sup>c</sup>See Results for form of AIC model selection criteria used for each response.

Table 2.3. Variable weights for explanatory variables considered in models describing variation in feeding and maintenance activities by harlequin ducks in an oil-dosing experiment at the Alaska SeaLife Center, Seward, Alaska during winters 2000-01 and 2001-02. Variable weights are Akaike weights summed across all models that included a given variable, and indicate the relative support for that variable. Variables with the greatest relative support are those with variable weights close to 1.0.

Response variable	Variable weight					
	Time of Day	Day Length	Treatment	Temperature	Wind	Treatment x Year
Probability of Feeding	0.288	0.186	0.375	0.247	0.097	0.310
Proportion of Time Feeding	1.000	0.999	0.294	0.417	0.832	0.203
Probability of Maintenance	0.255	0.185	0.890	0.844	0.199	0.609
Proportion of Time Maintenance	0.723	0.233	0.604	0.233	0.149	0.134

When feeding activity occurred during an observation period ( $n = 558$  observations from 39 birds), modeling results did not support an oil-dosing effect on the proportion of time spent feeding. The oil-dosing treatment explanatory variable (TREAT) was not included among the 3 models with  $\Delta AIC_c = 2$  (Table 2.2). Summed Akaike weights for TREAT and the TREAT  $\times$  YEAR interaction were low (Table 2.3) and indicated that these variables did not account for important variation in time spent in feeding activity. Summed Akaike weights indicated the importance of day length, time of day, wind speed, as well as year (Table 2.3). Model-averaged parameter estimates ( $\pm$  SE) indicated a negative association between time spent feeding and day length (dayl:  $-0.002 \pm 4.0E-4$ ), as well as greater feeding activity during the second year of the study (YEAR:  $0.821 \pm 0.18$ ), and increased feeding through the day (afternoon:  $0.22 \pm 0.07$ , evening:  $0.46 \pm 0.07$ ), and during moderate wind conditions ( $0.16 \pm 0.07$ ). However, time spent feeding was not affected by oil-dosing (Figure 2.1).

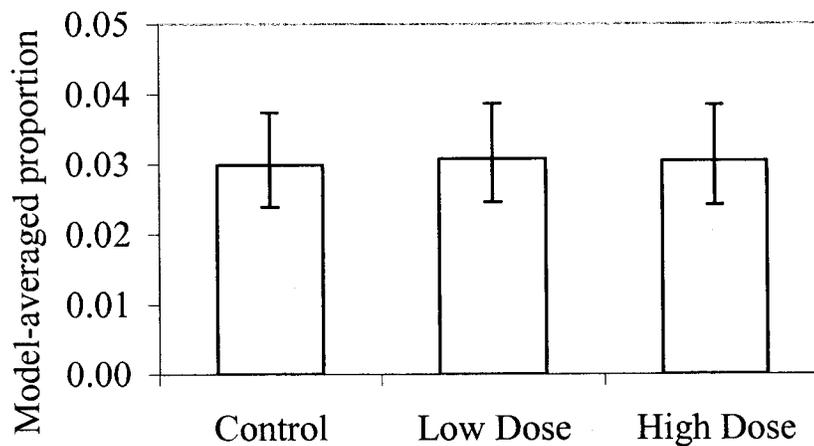


Figure 2.1. Estimates of the proportion of time spent feeding by harlequin ducks in an oil-dosing experiment at the Alaska SeaLife Center, Seward, Alaska during winters 2000-01 and 2001-02. Estimates are adjusted for the effects of covariates and averaged across all considered models based on Akaike model weights. Error bars represent 95% confidence levels.

### *Effects of Oil Ingestion on Maintenance Behavior*

Maintenance behavior occurred during 90% of behavior observation periods. The probability that maintenance behavior would occur during an observation period was related to oil-dosing treatment during the first year of the study. The global logistic regression model adequately fit the data (deviance goodness-of-fit  $\chi^2_{808} = 487.53, p = 1.00$ ) and no evidence of overdispersion was found ( $c = 0.60$ ). The TREAT main effect and TREAT  $\times$  YEAR interaction were included in the model selected as best-fitting the data (Table 2.2) and both were supported by summed Akaike weights (Table 2.3) indicating that the effect of oil-dosing treatment differed across years. Modeling results also indicated the importance of ambient temperature in the model (Table 2.3), which had a model-averaged parameter estimate ( $\pm$  SE) of  $0.07 \pm 0.03$ , indicating increased occurrence of maintenance activity at higher ambient temperatures. Probability estimates for the high and low dose treatments during winter 2000-01 were 0.84 (95% CI: 0.78 to 0.90) and 0.86 (95% CI: 0.79 to 0.91) respectively, 10% less than the estimated probability for birds in the control treatment (0.95, 95% CI: 0.90 to 0.98). Estimates for each treatment during the second winter had highly over-lapping 95% confidence intervals (Figure 2.2). Thus, after accounting for the effect of ambient temperature oil-dosed birds had a lower probability of engaging in maintenance activity than controls during the first year of the study, while no oil-dosing effect was evident during the second year.

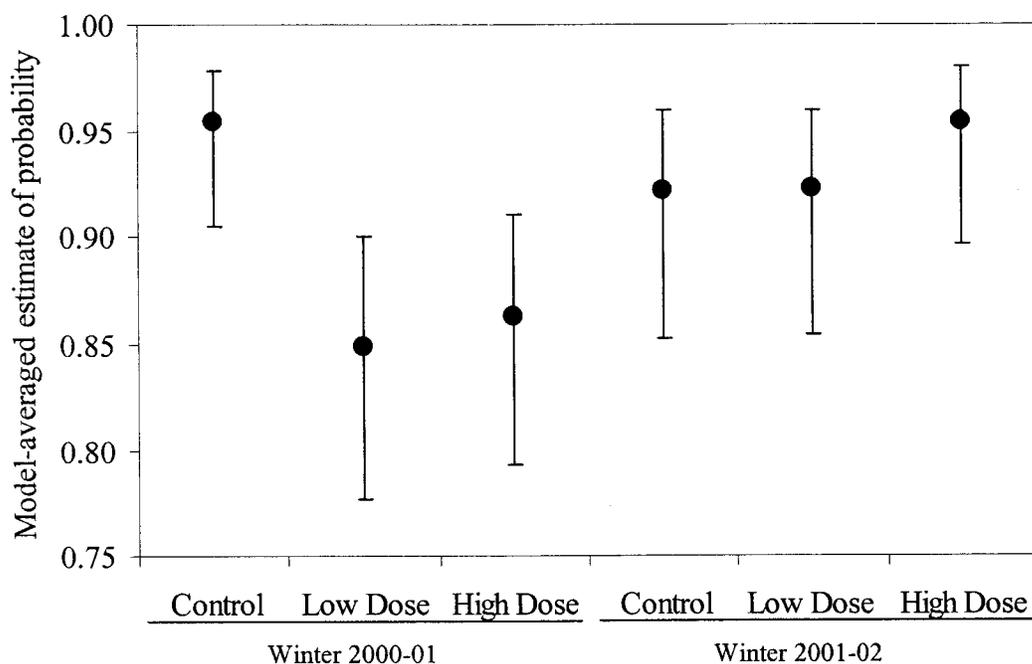


Figure 2.2. Estimates of the probability of engaging in maintenance activity during a 30-minute observation period by harlequin ducks in an oil-dosing experiment at the Alaska SeaLife Center, Seward, Alaska during winters 2000-01 and 2001-02. Estimates are adjusted for the effects of covariates and averaged across all considered models based Akaike weights. Error bars represent 95% confidence levels.

Modeling results supported an oil-dosing treatment effect on proportion of time spent in maintenance activity, however only for birds in the low-dose treatment. The global general

linear mixed model for the proportion of time spent in maintenance activity showed adequate fit ( $\chi^2_{14} = 57.0, P < 0.0001$ ). Three of the 4 models with  $\Delta AIC_c = 2$  included TREAT and one model included the TREAT  $\times$  YEAR interaction (Table 2.2). Summed Akaike weights, however showed little support for the TREAT  $\times$  YEAR interaction, while moderate support was indicated for the TREAT main effect (Table 2.3). The model-averaged parameter estimate ( $\pm$  SE) for the effect of YEAR indicated greater time spent in maintenance behavior during the second year of the study ( $0.73 \pm 0.22$ ), most likely associated with increased time spent in the water for dive-feeding; no other parameters estimates for covariates indicated an effect of time spent in maintenance activity. Because the TREAT  $\times$  YEAR interaction was included among models with  $\Delta AIC_c = 2$ , the proportion of time spent in maintenance activity was estimated for oil-dosing treatments separately in each year. Estimates of the mean proportion of time spent in maintenance activity were similar in the second year of the study, while estimates for 2000-01 indicated the mean time spent in maintenance activity by birds in the low dose treatment was 48% less than the estimates for birds in the control and high dose treatments (Figure 2.3).

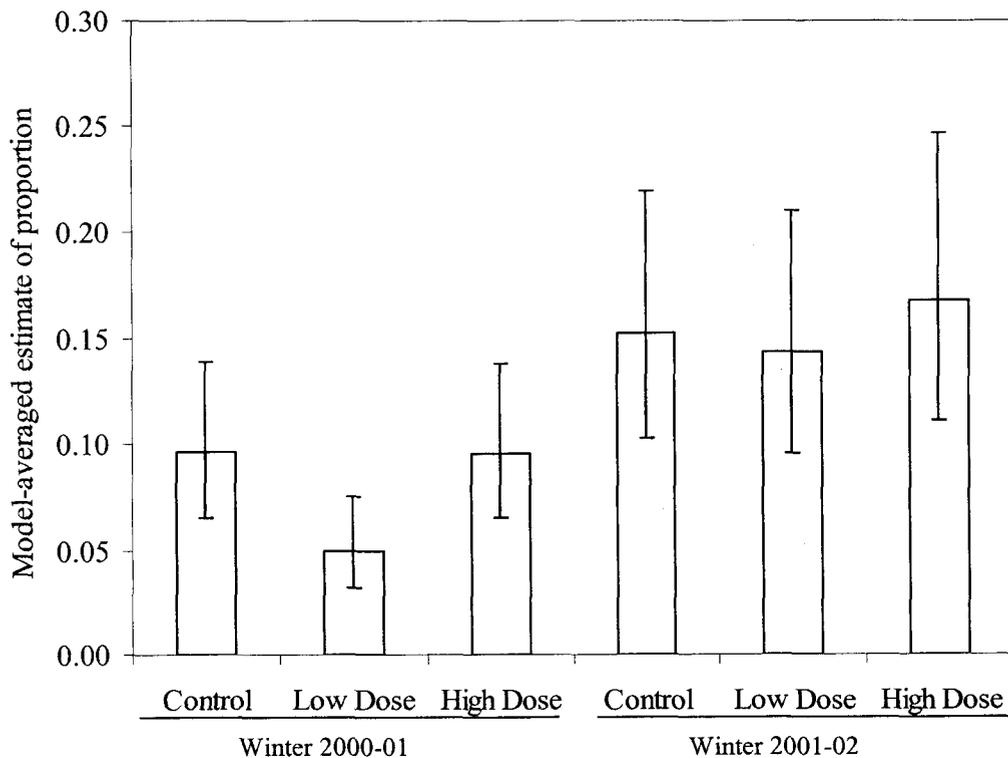


Figure 2.3. Estimates of the proportion of time spent in maintenance activity by harlequin ducks in an oil-dosing experiment at the Alaska SeaLife Center, Seward, Alaska during winters 2000-01 and 2001-02. Estimates are adjusted for the effects of covariates and averaged across all considered models based on Akaike model weights. Error bars represent 95% confidence levels.

#### *Effects of Plumage-oiling on Feeding Behavior*

We conducted 160 behavioral observations from 16 birds during three one-week long periods of the plumage-oiling experiment between 21 February and 18 March 2002. Feeding activity occurred during 47.5 % of all observation periods. The global logistic regression model for the

probability of feeding adequately fit the data (deviance goodness-of-fit  $\chi^2_{141} = 141.9$ ,  $p = 0.4608$ ) after adjusting for slight overdispersion ( $c = 1.36$ ). Modeling results indicated that the heavy-oiling treatment reduced the probability of feeding activity by birds during an observation period. The TREAT main effect was included among the 2 logistic regression models with  $\Delta\text{QAIC}_c = 2$  (Table 2.4). Summed Akaike weights for explanatory variables showed strong support for the TREAT main effect (Table 2.5). Estimates of the probability of feeding for each plumage-oiling treatment indicated a 43% reduction in the probability of feeding by birds associated with the heavy plumage-oiling treatment, but no apparent effects associated with light- or moderate-oiling (Figure 2.4). The probability of feeding by control birds was estimated to be 0.45 (95% CI: 0.29 to 0.84), while the probability of feeding by birds in the heavy-oiling treatment was only 0.21 (95% CI: 0.10 to 0.31); the 95% confidence intervals around these estimates showed only slight overlap providing strong evidence of a negative heavy-oiling treatment effect on the probability of feeding.

Table 2.4. Model-selection statistics from logistic regression and general linear mixed models for feeding and maintenance activities by harlequin ducks in a plumage-oiling experiment at the Alaska SeaLife Center, Seward, Alaska during February - March 2002. The models best fit by our data ( $\Delta\text{AIC}_c \leq 2$ ) are presented.

Response variable	Explanatory variables	$k^b$	Log-likelihood	$\Delta\text{AIC}_c$	Weight
Probability of Feeding	TREAT	5	-101.29	0.00 <sup>c</sup>	0.28
	TREAT + WIND	6	-100.70	1.30	0.15
Proportion of Time Feeding	TREAT	6	-72.88	0.00 <sup>d</sup>	0.16
	WIND	5	-74.15	0.17	0.15
	temp	4	-75.30	0.19	0.14
	DIURN	4	-75.57	0.72	0.11
	TREAT + DIURN	7	-72.44	1.55	0.07
	DIURN + WIND	6	-73.73	1.70	0.07
	Intercept	1	-60.28	0.00 <sup>d</sup>	0.21
Probability of Maintenance	TREAT	4	-57.39	0.44	0.17
	temp	2	-60.18	1.84	0.09
	DIURN	2	-60.25	1.99	0.79
	temp	4	-295.42	0.00 <sup>d</sup>	0.19
Proportion of Time Maintenance	temp + DIURN	5	-294.73	0.77	0.13
	temp + WIND	6	-293.78	1.05	0.11
	temp + TREAT	7	-292.90	1.51	0.09

<sup>a</sup>See Methods for explanatory variable abbreviations.

<sup>b</sup>Number of estimated parameters includes +1 for intercept for all models, +1 for the estimate of the variance inflation factor for  $\text{QAIC}_c$  models, and +2 for parameters in the covariance structure of general linear mixed models (compound symmetric residual structure, and variance estimate).

<sup>c</sup>See Results for form of AIC model selection criteria used for each response.

Table 2.5. Variable weights for explanatory variables considered in models describing variation in feeding and maintenance activities by harlequin ducks in a plumage-oiling experiment at the Alaska SeaLife Center, Seward, Alaska during February – March 2002. Variable weights are Akaike weights summed across all models that included a given variable, and indicate the relative support for that variable. Variables with the greatest relative support are those with variable weights close to 1.0.

Response variable	Variable weight							
	Diurnal	Treatment	Temperature	Wind	Treatment x Temperature	Treatment x Wind	Treatment x Days Post Oiling	Treatment x Days Post Oiling <sup>2</sup>
Probability of Feeding	0.149	0.978	0.346	0.394	0.069	0.036	0.089	0.039
Proportion of Time Feeding	0.365	0.388	0.359	0.368	NA	NA	NA	NA
Probability of Maintenance	0.261	0.486	0.248	0.171	NA	NA	0.070	0.046
Proportion of Time Maintenance	0.451	0.361	0.764	0.325	NA	NA	0.006	0.006

When feeding activity was observed during observation periods ( $n = 76$  observations from 16 birds), we found evidence of decreased time spent feeding with increased quantity of plumage-oiling. The global general linear mixed model for the proportion of time spent feeding by birds in the external-oiling experiment showed adequate fit to the data (null model likelihood ratio test  $\chi^2_6 = 16.2$ ,  $P = 0.0127$ ). The model that included only the TREAT explanatory variable was selected as best-fitting; however this model received little support (Table 2.4). Five additional models had  $\Delta AIC_c = 2$ , indicating considerable uncertainty in model-selection for this response variable. Summed Akaike weights for the plumage-oiling treatment variable received the greatest amount of support relative to the other explanatory variables considered, although support for all variables was low (Table 2.5). However, a clear trend of decreased average proportion of time spent feeding with increased quantity of external-oiling was found in estimates of the mean proportion of time spent feeding for each of the plumage-oiling treatments (Figure. 2.5). The plumage-oiling treatment effect was most evident in birds in the heavy-oiling treatment which had an estimated mean proportion of time spent feeding of 0.042 (95% CI: 0.026 to 0.066), 43% less than the estimated mean proportion of time spent feeding by birds in the control treatment (0.074, 95% CI: 0.053 to 0.102; Figure. 2.5).

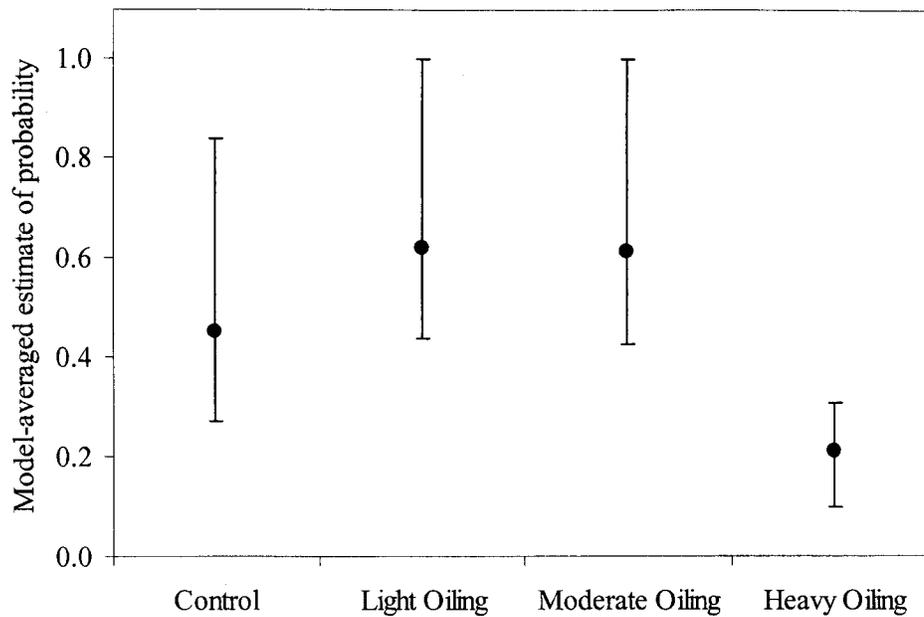


Figure 2.4. Estimates of the probability of engaging in feeding activity during a 30-minute observation period by harlequin ducks in a plumage-oiling experiment at the Alaska SeaLife Center, Seward, Alaska during February – March 2002. Estimates are adjusted for the effects of covariates and averaged across all considered models based Akaike weights. Error bars represent 95% confidence levels.

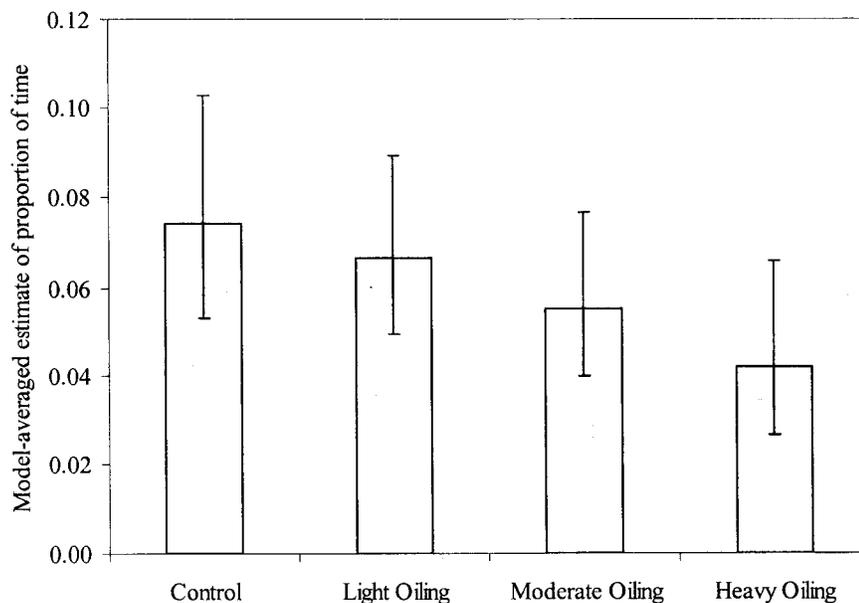


Figure. 2.5. Estimates of the proportion of time spent in feeding activity by harlequin ducks in a plumage-oiling experiment at the Alaska SeaLife Center, Seward, Alaska during February – March 2002. Estimates are adjusted for the effects of covariates and averaged across all considered models based on Akaike model weights. Error bars represent 95% confidence levels.

### *Effects of Plumage-oiling on Maintenance Behavior*

Maintenance behavior occurred during 87% of behavior observation periods. The global logistic regression model for the probability of maintenance activity indicated adequate fit (deviance goodness-of-fit  $\chi^2_{147} = 107.0, p = 0.9946$ ) with no evidence of overdispersion in the data ( $c = 0.73$ ). Modeling results indicated little support for a plumage-oiling effect on the probability of birds engaging in maintenance activity during an observation period. The intercept-only null model and the model including only the TREAT explanatory variable had  $\Delta AIC_c = 2$  (Table 2.4), indicating that the data did not convincingly support a plumage-oiling treatment effect. Summed Akaike weights for explanatory variables showed moderate support for the plumage-oiling treatment variable, and little support for the other explanatory variables considered (Table 2.5). Estimates of the probability of maintenance by lightly oiled (0.896, 95% CI: 0.718 to 0.967), and moderately oiled birds (0.882, 95% CI: 0.735 to 0.953) were greater than the estimate for control birds (0.778, 95% CI: 0.600 to 0.891); however 95% confidence intervals around these estimates were highly over-lapping (Figure 2.6). The estimated probability for heavily oiled birds (0.953, 95% CI: 0.822 to 1.00) was suggestive of an increased incidence of maintenance activity compared to control birds.

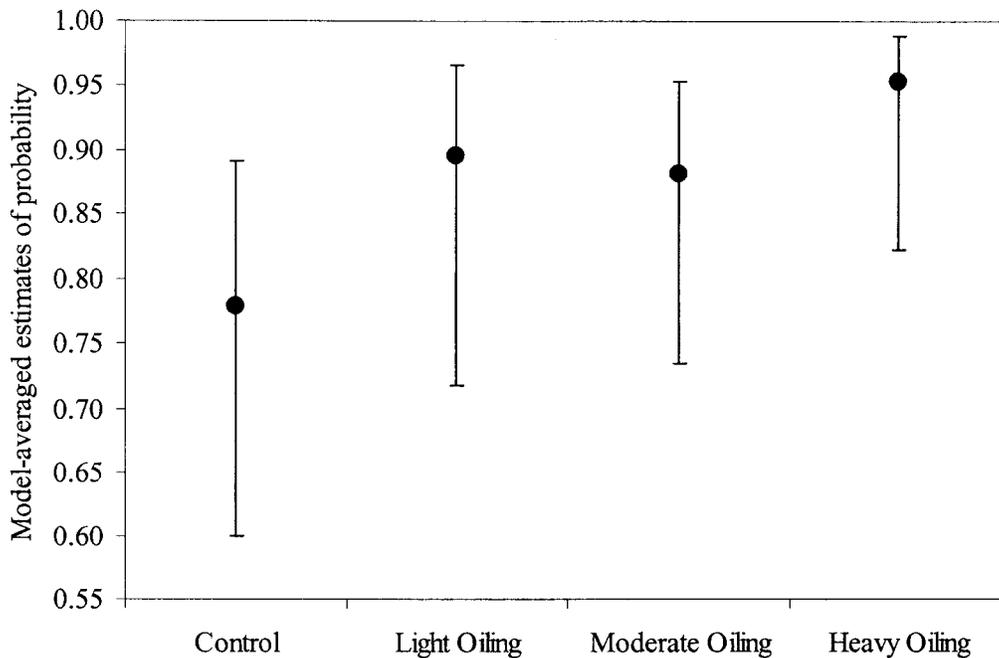


Figure 2.6. Estimates of the probability of engaging in maintenance activity during a 30-minute observation period by harlequin ducks in a plumage-oiling experiment at the Alaska SeaLife Center, Seward, Alaska during February – March 2002. Estimates are adjusted for the effects of covariates and averaged across all considered models based Akaike weights. Error bars represent 95% confidence levels.

Modeling results provided little support for the hypothesis that the plumage-oiling treatment affected the proportion of time spent in maintenance activity. The global general linear mixed model for proportion of time spent in maintenance activity indicated adequate fit to the data (null

model likelihood-ratio-test  $\chi^2_{12} = 21.5$ ,  $P = 0.0387$ ). Of the 4 models with  $\Delta AIC_c = 2$ , only one included the explanatory variable for plumage-oiling treatment (Table 2.4). Summed Akaike weights indicated little support for the plumage-oiling treatment (Table 2.5). The model-averaged estimate of the mean proportion of time spent in maintenance activities by non-oiled control birds (0.142, 95% CI: 0.07 to 0.27) was similar to that of the lightly oiled birds (0.121, 95% CI: 0.065 to 0.213). While the mean proportion of time spent in maintenance activities for moderately oiled birds (0.276, 95% CI 0.162 to 0.427) and heavily oiled birds (0.227, 95% CI: 0.131 to 0.365) were 48% and 37% greater than the estimate for non-oiled control birds respectively, although the large amount of variation around these estimates prevents strong inference from being drawn (Figure 2.7).

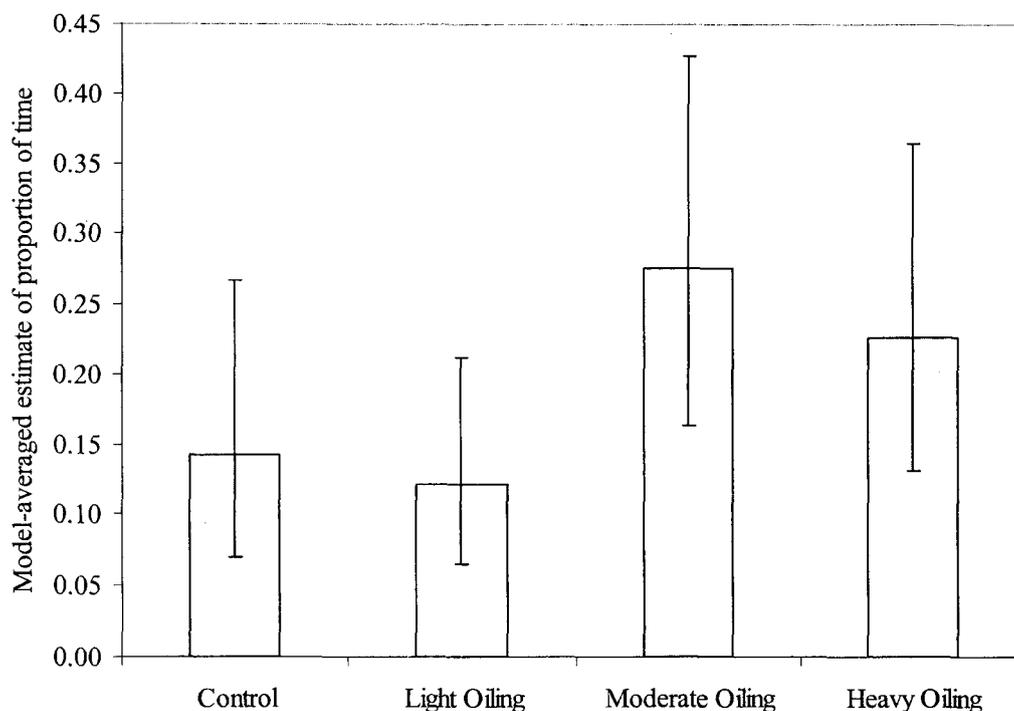


Figure 2.7. Estimates of the proportion of time spent in maintenance activity by harlequin ducks in an plumage-oiling experiment at the Alaska SeaLife Center, Seward, Alaska during February – March 2002. Estimates are adjusted for the effects of covariates and averaged across all considered models based on Akaike model weights. Error bars represent 95% confidence levels.

#### *Captive Studies – CYP1A Induction.--*

As expected, CYP1A induction, as measured by EROD activity, was higher in captive harlequin ducks in the oiled treatments than for controls (Figure 2.8). Not only did EROD activity average higher in oil-dosed birds, there was considerably higher variation among oil-dosed individuals relative to variation among control birds. This led to broadly overlapping confidence intervals for the low and high dose treatments in both years (Figure 2.8). When low and high dose treatments were combined, these combined data did not differ between years, but were higher than controls.

The variation among individuals, and lack of distinct differences between oil dosing treatments, may have been the result of individual-specific levels of CYP1A response to dosing, or differential passing of part of the administered doses. However, these data clearly indicate that crude oil induces CYP1A in harlequin ducks, and levels of induction were similar, or somewhat higher, to induction observed in wild harlequin ducks (see below).

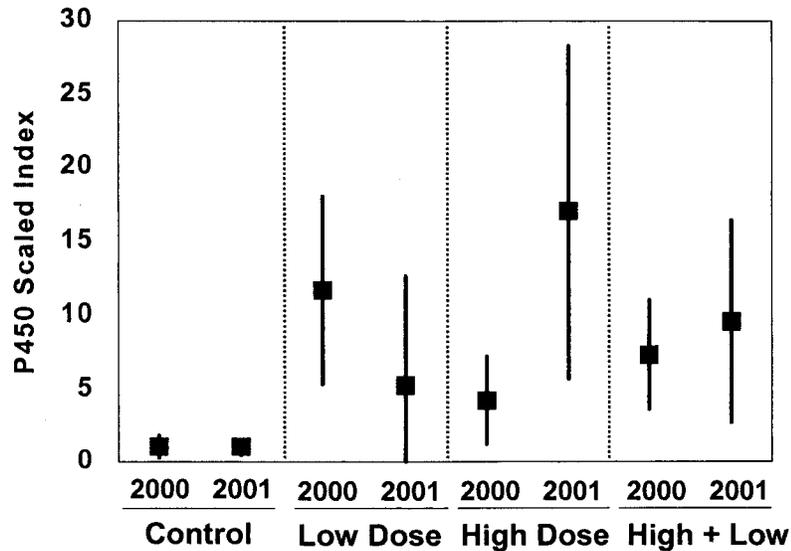


Figure 2.8. Comparisons of scaled (+/- 95% confidence intervals) hepatic EROD activity of harlequin ducks under different oil dosing regimes at the Alaska SeaLife Center, Seward, Alaska.

*Field Studies – CYP1A Induction.--*

In 1998, during NVP studies, we found that average CYP1A induction in harlequin ducks was considerably higher in oiled areas than unoiled areas (Figure 2.9). In this project, we found a similar pattern in 2000, but that average CYP1A induction did not differ between oiled and unoiled areas during November 2001 or 2002. The data suggest that oil exposure had diminished in the final 2 years of the study.

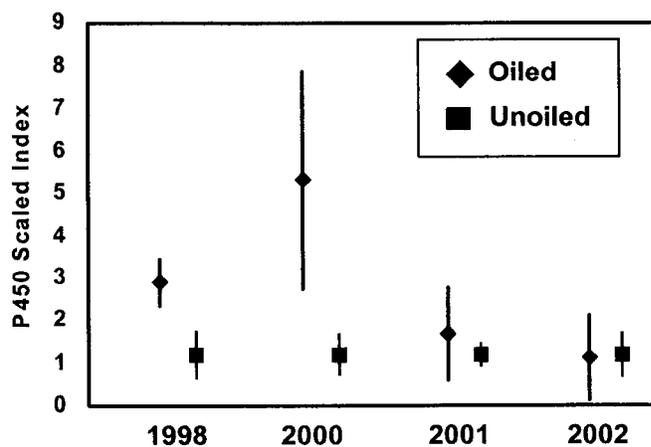


Figure 2.9. Comparisons of scaled (+/- 95% confidence intervals) hepatic EROD activity of harlequin ducks from oiled and unoiled areas of Prince William Sound, Alaska.

*Field Studies – Female Winter Survival.--*

Female survival during winters 2000-01, 2001-02, and 2002-03 followed some patterns (Figure 2.10) that were evident in data from NVP studies (Esler 2000). Mortality on oiled areas was highest during mid-winter (December to January interval), although the effect was not nearly as strong as in NVP studies (Esler 2000). Point estimates of cumulative survival over the 3 winters combined did not differ between unoiled (84.5%, 95% CI: 75.2–93.8%) and oiled (79.9%, 95% CI: 69.9-89.8%) areas. Most of the lower point estimate for oiled areas was due to the winter 2000-01 data, in which oiled and unoiled areas differed by 6.9%, whereas differences in the subsequent winters were 2.2% and 2.8% for winters 2001-02 and 2002-03, respectively.

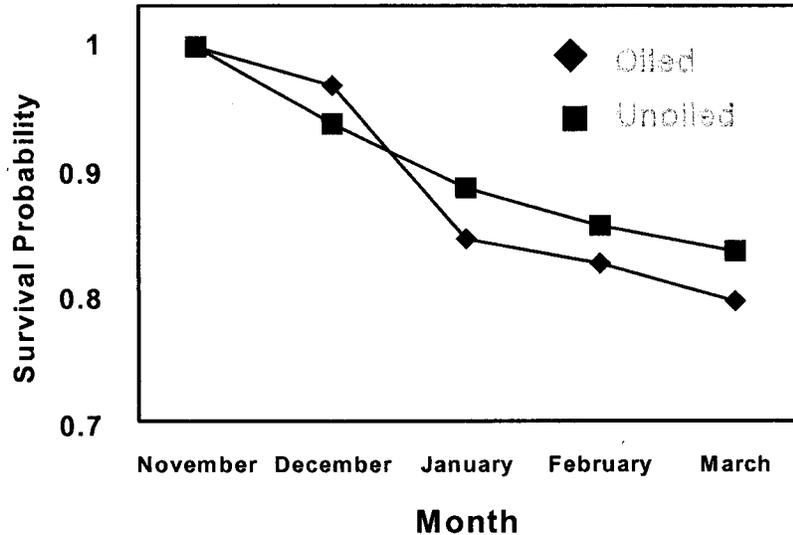


Figure 2.10. Survival probabilities of radio-marked, female harlequin ducks in areas of Prince William Sound, Alaska oiled during the *Exxon Valdez* oil spill compared to unoiled areas for the winters 2000-01, 2001-02, and 2002-03.

*Field Studies – Dispersal and Population Structure.--*

*Banding data*

A. Philopatry—homing rates.

In total, 799 captures were made during the five years of trapping on Montague Island, of which 348 were males and 451 were females. Among these, 102 were recaptures (28 male and 74 female). Homing rates to the three Montague Island capture locations were high. In total, 94.6% ( $n=74$ ; 95% CI: 86.7-98.5%) of recaptured females were trapped on their original site, as were 100% of recaptured males ( $n=28$ ; 95% CI: 92.9-100.0%). Sex related differences in homing rate were not statistically significant (Table 2.6; *Fisher exact test*,  $P=0.57$ ). Variation in homing rate among the three trapping locations was minimal (Table 2.6; *Fisher exact test*,  $P=0.32$ ), as were annual differences (*Fisher exact test*,  $P=0.29$ ). An additional 676 after hatch year birds, 334 male and 342 female, were captured at the eight trapping locations along Green Island, Knight Island, and the Kenai Peninsula during 1995-1997. Among these were 73 recaptures. Female homing rate in this sample was 91.8% ( $n=49$ ; 95% CI: 80.4-97.7%) and male homing rate was 95.8% ( $n=24$ ; 95% CI: 78.8-99.9%). None of the birds originally banded on Montague Island during 1995 or 1996 was recaptured in outlying areas during 1996 or 1997.

In the combined datasets, a grand total of 175 after hatch year harlequin ducks were recaptured, nine of which were at sites other than their original location. The maximum dispersal distance for any bird was 51.9 km, by a female dispersing from Foul Bay on the Kenai Peninsula to the north end of Green Island. The remaining eight recapture events in which movement was detected included one male and seven females. These ranged in distance from 3.1 to 8.9 km.

Table 2.6. Homing rates of adult harlequin ducks to three trapping locations on Montague Island, Prince William Sound, Alaska (A: Stockdale Harbor, B: Port Chalmers, C: West Montague). Homing rate is calculated as the number of individuals recaptured at their original capture site divided by the sum of the number captured at their original capture site plus those captured elsewhere.

Location	Number captured	Number recaptured	Homing rate	95% Confidence Interval
<b>Females</b>				
A	213	39	0.974	0.87-1.00
B	136	21	0.952	0.76-1.00
C	103	14	0.857	0.57-1.00
ALL	451	74	0.946	0.87-0.99
<b>Males</b>				
A	207	20	1.00	0.87-1.00
B	64	6	1.00	0.66-1.00
C	77	2	1.00	0.41-1.00
ALL	348	28	1.00	0.92-1.00

#### B. Annual survival—Cormak-Jolly-Seber Model.

Model fit was acceptable for the most general model ( $\phi_{(s^*t)}\rho_{(s^*t)}$ ), with 201 of 1,000 simulated datasets having greater deviance than our actual data ( $P = 0.20$ ). There was evidence of overdispersion in the data, therefore an adjusted  $\hat{c} = 1.66$ , calculated as the ratio of deviance from the actual data to the mean deviance from the bootstrap replicates, was used to compute QAIC<sub>c</sub>.

The best supported model, ( $\phi_{(s)}\rho_{(s^*t)}$ ), contained nine estimable parameters, suggesting a constant survival probability, but variable recapture probabilities, among sexes and across years (Table 2.7). This model had a weight of 0.41 and was approximately 2.8x better than the two next best supported models: ( $\phi_{(s)}\rho_{(s^*t)}$ ) and ( $\phi_{(t)}\rho_{(s^*t)}$ ). Model averaged parameter estimates for apparent survival were 0.661 (95% CI: 0.402-0.849) and 0.678 (95% CI: 0.522-0.802) for males and females respectively, with annual estimates ranging between 0.534-0.734 for males and 0.537-0.738 for females. Recapture probability for females was much less variable than for males, with female recapture probabilities ranging 0.199 to 0.330 and male probabilities ranging from 0.040 to 0.386.

Table 2.7. Cormack-Jolly-Seber models used to assess differences in annual survival ( $\phi$ ) and recapture probability ( $\rho$ ) for adult harlequin ducks captured in PWS, Alaska, during 95-97, 2000-01. Subscript notation denotes variation related to sex (s) and time (t). The best supported model has the lowest quasi Akaike's Information Criterion (QAICc)<sup>c</sup>. Of 16 candidate models, only those with  $\Delta\text{QAICc} < 5$  are shown. The effective sample size was 727 observations.

Model	Description	np <sup>b</sup>	$\Delta\text{QAICc}^c$	Weight <sup>d</sup>
$\phi_{(\bullet)}\rho_{(s*t)}$	Constant $\phi$ ; sex and time related variation in $\rho$	9	0.00	0.41
$\phi_{(s)}\rho_{(s*t)}$	Sex related variation in $\phi$ and $\rho$ ; time related variation in $\rho$	10	2.03	0.15
$\phi_{(t)}\rho_{(s*t)}$	Time related variation in $\phi$ and $\rho$ ; sex related variation in $\rho$	11	2.04	0.15
$\phi_{(s*t)}\rho_{(t)}$	Sex related variation in $\phi$ and $\rho$ ; time related variation in $\rho$	10	2.46	0.12
$\phi_{(s*t)}\rho_{(s*t)}$	General model; sex and time related variation in $\phi$ and $\rho$	12	3.10	0.08

<sup>a</sup> The variance inflation factor,  $\hat{c}$ , was 1.66

<sup>b</sup> np = number of estimable parameters in the model.

<sup>c</sup>  $\Delta\text{QAICc}$  is the difference between QAICc of the current model versus the best supported model.

<sup>d</sup> Relative likelihood of a model among the 16 tested.

### C. Philopatry and survival—multi-strata model.

Results of the multi-strata model were very similar to those obtained in the separate analyses of philopatry and survival. Fit for the most general model,  $\phi_{(s*t)}\rho_{(s*t)}\psi_{(s)}$ , was acceptable, with bootstrap  $\hat{c} = 1.81$ , and model deviance ranked 479<sup>th</sup> of the 1000 simulated values ( $p = 0.52$ ). The three best supported candidate models all indicated sex-related differences in movement probability. These models had AICc weights that were similar in magnitude and identical in rank to the QAICc values obtained using the CJS analysis with respect to survival and recapture probability. Model averaged parameter estimates for philopatry in the multi-strata analysis were nearly identical to the homing rate analysis [male: 0.996 (95% CI: 0.980-1.000); female: 0.948 (95% CI: 0.876-0.979)], but annual survival estimates were slightly lower than in the CJS model [male: 0.634 (95% CI: 0.451-0.785); female: 0.636 (95% CI: 0.518-0.740)].

### Telemetry data

A total of 434 female harlequin ducks were outfitted with VHF transmitters over the six years of data collection. Among these, 31 were SY females, 31 were HY females, and 372 were ASY females. Sample sizes, survey date ranges, and the frequency and number of detections per individual are summarized in Table 2.8.

Table 2.8. Sample sizes, survey dates, and the frequency female harlequin ducks were detected in aerial surveys.

Year	Date range	# ♀ radioed	Age class			# surveys conducted	Days between surveys ( $\pm\text{SE}$ )	Detections per individual (SE)
			ASY	SY	HY			
1995	4 Oct 95 – 30 Mar 96	87	87	.	.	21	7.7 ( $\pm 0.8$ )	14.9 (0.6)
1996	1 Oct 96 – 29 Mar 97	92	92	.	.	24	7.8 ( $\pm 0.4$ )	20.1 (0.7)
1997	2 Oct 97 – 18 Mar 98	109	109	.	.	23	7.6 ( $\pm 0.6$ )	18.2 (0.6)
2000	8 Nov 00 – 2 Apr 01	54	32	15	7	13	12.1 ( $\pm 1.9$ )	9.6 (0.5)
2001	19 Nov 01 – 31 Mar 02	49	29	9	11	15	10.3 ( $\pm 0.9$ )	11.2 (0.6)
2002	5 Dec 02 – 2 Apr 03	43	22	7	13	13	9.8 ( $\pm 2.2$ )	10.2 (0.7)

A. Probability of emigration at different spatial scales—Kaplan-Meier analysis.

At the *site* scale, which we defined as bays, inlets, groupings of islets, or continuous sections of coastline bounded by a prominent geographic feature, monthly emigration probabilities ranged between 0.02 and 0.08, with a cumulative fidelity function estimate of  $F[t] = 0.74$  (95% CI: 0.72-0.77), for the entire winter period. Movement at the *zone* scale occurred roughly half as frequently, with monthly emigration probabilities ranging between 0.01 and 0.04, and a cumulative estimate of  $F[t] = 0.88$  (95% CI: 0.87-0.89). At the *area* scale, which we defined as large islands or mainland regions separated from other regions by >5 km of open water, movement was infrequent, with monthly emigration probability estimates never exceeding 0.02, and a cumulative estimate for  $F[t] = 0.96$  (95% CI: 0.96-0.97) (Table 2.9).

Table 2.9. Monthly emigration probability ( $e/r$ ) and cumulative fidelity function ( $F[t]$ ) estimates for female harlequin ducks at the *site*, *zone*, and *area* scale in Prince William Sound, Alaska.

	October	November	December	January	February	March
<b>SITE</b>						
Site Faithful ( $r$ )	269	323	319	279	250	230
(Home: Foray)	(234:35)	(302:21)	(285:34)	(256:23)	(238:12)	(206:24)
Emigrant ( $e$ )	15	30	25	9	7	4
Censored	150	81	90	146	177	200
Period $e/r$	0.05	0.08	0.07	0.03	0.03	0.02
Cumulative $F[t]$	0.95	0.87	0.80	0.78	0.76	0.74
95% CI $F[t]$	0.94-0.95	0.85-0.88	0.79-0.82	0.76-0.80	0.73-0.78	0.72-0.77
<b>ZONE</b>						
Site Faithful ( $r$ )	275	352	356	316	286	265
(Home: Foray)	(255:20)	(333:19)	(331:25)	(306:10)	(283:3)	(253:12)
Emigrant ( $e$ )	9	6	16	6	4	2
Censored	150	76	62	112	144	167
Period $e/r$	0.03	0.02	0.04	0.02	0.01	0.01
Cumulative $F[t]$	0.97	0.95	0.91	0.90	0.88	0.88
95% CI $F[t]$	0.96-0.97	0.95-0.96	0.90-0.92	0.88-0.91	0.87-0.89	0.87-0.89
<b>AREA</b>						
Site Faithful ( $r$ )	282	362	356	316	286	265
(Home: Foray)	(277:5)	(358:4)	(331:25)	(306:10)	(283:3)	(253:12)
Emigrant ( $e$ )	2	3	16	6	4	2
Censored	150	76	62	112	144	167
Period $e/r$	0.01	0.01	0.02	<0.01	<0.01	<0.01
Cumulative $F[t]$	0.99	0.98	0.97	0.97	0.6	0.96
95% CI $F[t]$	0.99-1.00	0.98-0.99	0.96-0.97	0.96-0.97	0.96-0.97	0.96-0.97

Age related variation in the fidelity function was non-significant at every scale (Fisher's exact test:  $N=145$ ,  $df=2$ ,  $P=0.17$ , 0.19, 0.37, at the *site*, *zone*, and *area* scales respectively). Cumulative  $F[t]$  estimates for ASY females were 0.82 (95 CI: 0.77-0.84), 0.92 (95 CI: 0.91-0.94), and 0.96 (95 CI: 0.94-0.97) at the *site*, *zone*, or *area* scales. This compared to estimates of 0.88 (95 CI: 0.82-0.94), 0.95 (95 CI: 0.93-0.97), and 0.95 (95 CI: 0.93-0.97) for SY females, and 0.94 (95 CI: 0.92-0.97), 1.00, and 1.00 for HY females at the same three scales. Seasonal variation in emigration probabilities was evident at the *site* and *zone* scale, but not at the *area*

scale (Fisher’s exact test: *site* N=1660, df =5, P<0.001; *zone* N=1894, df =5, P=0.03; *area* N=1955, df =5, P=0.12). Movement rates at the smaller scales were highest during November (*site*  $F[t] = 0.91$  (95% CI: 0.90-0.92) and December (*site*  $F[t] = 0.93$  (95% CI: 0.92-0.93), and lowest in February (*site*  $F[t] = 0.97$  (95% CI: 0.97-0.98) and March (*site*  $F[t] = 0.98$  (95% CI: 0.98-0.99).

Few individuals moved back to their reference location after having been categorized as *emigrants*. At the *site* scale, 90 of the 434 radio marked females were detected away from their reference location for consecutive observations at some point during the winter. Among these 14 subsequently moved back, and therefore led to an overestimation of  $F[t]$  of approximately 3%. At the *zone* scale movement back to the reference location occurred 8 times, which accounted for <2% of all cases, and at the *area* scale only a single event occurred. Using calculations based on our subset of birds that were implanted, tracked for one winter, and subsequently recaptured, we estimated that 1.6% of all radio-marked females emigrated outside the detection area (Table 2.10). After accounting for the underestimation of  $F[t]$  that resulted from movements back to the reference location after coding as an *emigrant* (*site*: +0.032; *zone*: +0.018; *area*: +0.002), and subtracting for the overestimation of  $F[t]$  that resulted from movement outside the study area (*site*, *zone*, and *area*: -0.016), corrected estimates of cumulative for  $F[t]$  at the three scales were 0.76, 0.88, and 0.95, respectively.

Table 2.10. Estimate of the proportion of female harlequin ducks outfitted with radios that moved outside the study area ( $P_{OutsideArea}$ ).  $P_{OutsideArea}$  inferred from radio failure rates in a subsample of birds recaptured one year after having been implanted.

<u>Radio-tracked birds</u>		<u>Recaptured birds</u>	
<i>Total</i> <sub>tracked</sub>	185	<i>Total</i> <sub>recaptured</sub>	40
Number of tracked radios detected in study area ( <i>A</i> <sub>tracked</sub> )	122	Number of functioning radios recaptured ( <i>A</i> <sub>recaptured</sub> )	27
Number of tracked radios not detected in study area ( <i>B</i> <sub>tracked</sub> + <i>C</i> <sub>tracked</sub> + <i>D</i> <sub>tracked</sub> )	63	Number of failed radios recaptured ( <i>B</i> <sub>recaptured</sub> )	13
$\frac{A_{recaptured}}{A_{recaptured} + B_{recaptured}} = \frac{13}{40} = 0.325$			
$\frac{A_{tracked}}{A_{tracked} + B_{tracked} + C_{tracked} + D_{tracked}} = \frac{63}{185} = 0.341$			
$P_{OutsideArea} = 0.016$			

B. Spatial scale of individual movements—kernel home range estimates. Home range (95% fixed kernels), core area (50% fixed kernels), and estimates for the mean distance traveled between successive observations by adult female harlequin ducks were low during the winter period (1 October – 30 March). We restricted estimation of home range and

core area size to 26 individuals for which =15 precise locations were obtained. Regression analysis suggested that at that threshold home range size was independent of the number of samples obtained. We estimated mean home range size and core area sizes of  $11.5 (\pm 2.2 \text{ SE}) \text{ km}^2$  and  $1.7 (\pm 0.3 \text{ SE}) \text{ km}^2$ , respectively. For these estimates an average of  $18.9 (\pm 0.4 \text{ SE})$  locations was obtained per individual, with surveys conducted  $8.9 (\pm 0.2 \text{ SE})$  days apart.

All 35 radio-marked adult females for which precise location data was collected were used to calculate the range and mean of distances traveled between successive observations. Two of the 35 adult females (6%) made moves which we considered dispersal events. These ranged from 30 to 40 km in distance, and their frequency of occurrence was similar to the movement function calculated at the *area* scale in the Kaplan-Meier analysis. The remainder of detected movements ranged from  $<0.1$  to 9.2 km, averaging  $1.6 (\pm 0.1 \text{ SE}) \text{ km}$ . There were no differences in average distance moved between early (October/November), mid (December/January), or late (February/March) winter (ANOVA:  $F = 1.06$ ,  $df = 2$ ,  $P = 0.35$ ).

## Discussion

### *Captive Studies – Behavior Responses to Oil Exposure.-- Effects of Oil Ingestion on Behavior*

No overt signs of intoxication were observed in harlequin ducks dosed with weathered Prudhoe Bay crude oil under the dosing regimes and conditions of captivity in this study. Other species of waterfowl have shown similar tolerance of ingested oil in captivity (Coon and Dieter 1981, Rattner 1981, Stubblefield et al. 1995, Boehm et al. 1996), although the toxicity of ingested oil may vary among species, crude oils, and extent of weathering (Leighton 1993). Behavioral differences among birds in oil-dosed and control treatments were detected, but these differences were not consistent between the 2 years of the experiment, nor was there evidence of a dose-response effect; therefore, the hypothesis that oil ingestion causes changes in the feeding and maintenance components of the time-activity budget of captive harlequin ducks is not supported by our results.

Oil ingestion has been hypothesized to cause increased feeding by reducing digestive efficiency (Easton and Murray 1981, Ormseth and Ben-David 2000); increasing basal energy requirements through the chronic induction of detoxification, excretion and repair mechanisms (Calow 1991); or by triggering a generalized physiological stress response (Holmes et al. 1978, 1979). All of these mechanisms would be expected to result in increased food intake; however neither feeding activity nor food consumption rate were affected by oil-dosing treatment in this study. Ben-David et al. (2000) found that oil-dosing in captive river otters caused anemia and reduced dive times. The proportion of total feeding time spent diving during the second year of the study did not differ across oil-dosing treatments, indicating that foraging behavior was not altered at the scale of time spent diving. Additionally, a related study of blood parameters, including packed cell volume, during the first year of dosing found no oil dose-related effects (K. Trust, U.S. Fish and Wildlife Service, unpublished data).

Effects of oil-dosing on maintenance behavior would most likely be indirect. For example, if oil ingestion caused increased lethargy, as was observed in oil-dosed sandhill cranes (Flemming et al. 1982), reallocation of time to resting activity might be at the expense of maintenance activity.

Direct effects of oil ingestion on feather quality are unlikely, although effects on feather molt are not known. Birds used in this study had completed wing molt prior to the start of dosing. The effects of oil-dosing treatment on maintenance activity detected in this study were not consistent across years, and no dose-response was found. Our results indicate that, in the first year of the study, both the low-dose and the high-dose engaged in maintenance activity less often than controls, however when birds did engage in maintenance activity only birds in the low-dose treatment were found to reduce the amount of time spent in maintenance, while no effects were elicited in the second year. Cumulative doses were similar among the 2 years of the study (2000-01: low dose 37 ml kg<sup>-1</sup>, high dose 370 ml kg<sup>-1</sup>; 2001-02: low dose 34 ml kg<sup>-1</sup>, high dose 343 ml kg<sup>-1</sup>), while the frequency and quantity of dosing were greater during the second year when no oil-dosing effects were found. Also, dive-feeding during the second year of the study likely made maintaining the water repellency of the plumage more important and was associated with increased time spent in maintenance activity during the second year. The oil-dosing effect on maintenance activity may be related to either lower frequency, higher quantity oil ingestion, singly or in combination with the low maintenance activity requirements during the first year. The inconsistency of the treatment effect between years, the lack of a dose-response on time spent in maintenance activity, and the absence of observable differences in plumage quality among treatment groups do not support the hypothesis that oil-dosing negatively affects maintenance activity.

The absence of a dose-response effect on proportion of time spent in maintenance activity may be related to the cloacal excretion of ingested oil before it could be absorbed from the digestive tract and physiologically detoxified. Birds and mammals fed doses of crude oil are known to excrete portions of the doses in feces ranging from 45 to 80%, which may confound a dose-response effect (McEwan and Whitehead 1980, Easton and Murray 1981, Ormseth and Ben-David 2000). Cloacal excretion of ingested oil was observed soon after dosing during the 60-90 minute holding period in both winters of this study, and in both oil-dosed treatments. Rapid excretion may serve as an effective mechanism for mitigating the toxicity of oil ingestion (McEwan and Whitehead 1980, Ormseth and Ben-David 2000). However, levels of hepatic CYP1A in birds from this study at the end of the dosing periods were elevated for both oil-dosed treatments, relative to controls (see above). Induction of hepatic CYP1A in birds dosed at both the low- and high-dose levels indicated that some oil was absorbed and detoxified at both dosages and not completely excreted.

The environmental conditions experienced by wild harlequin ducks wintering in Alaska differ greatly from those experienced in captivity, where food was high quality and required minimal foraging effort. This distinction was obvious in the difference between time spent feeding by harlequin ducks wintering in the Aleutian Islands (> 80% of the diurnal period in females during mid-winter; Fischer and Griffin 2000), and time spent feeding by harlequin ducks in captivity (< 10%). This difference in ecological context has major implications for detecting effects of contaminants on time-activity budgets in captivity given that environmental conditions may play an important role in toxicity (Gordon 2003). For example, if oil ingestion induced detectable effects on behavior only under conditions of additive stress (Holmes et al. 1978, 1979), such effects may not have been induced in captivity, which was clearly a less demanding environment than the wild.

In addition to differences in environmental conditions, oil exposure also differed between the dosing regime administered in captivity and the oil exposure likely experienced by harlequin ducks in oil-contaminated areas of PWS. Chronic contamination of benthic invertebrates (Fukuyama et al. 2000, Carls et al. 2001) makes ingestion of contaminated prey a likely source of exposure in the wild. Such exposure would occur in the form of PAHs incorporated in animal tissue, which by nature may lead to effects different from those caused by ingestion of whole, weathered crude oil. Additionally, given the high winter site fidelity of harlequin ducks, birds wintering on oiled areas are likely to experience exposure not only within a given winter but also across multiple winters (Esler et al. 2002). Such chronic exposure creates the potential for cumulative damage over multiple years (Calow 1991), effects which may not be produced by one season of oil ingestion in birds previously naïve to oil exposure.

The low-dose administered in this study was meant to simulate exposure as may be occurring in oiled areas of PWS many years after the oil spill, while the high-dose the level of exposure would only occur in the immediate wake of an oil spill and therefore be most likely to cause effects on behavior. This range of exposure induced hepatic CYP1A to levels similar to those documented in wild harlequin ducks (see above). Despite induction of hepatic CYP1A no consistent behavioral effects were found, suggesting that induction of detoxification mechanisms does not carry corresponding behavioral effects, such as increased feeding (Jenssen 1989, Calow 1991). Thus, CYP1A may be a more sensitive indicator of exposure to PAHs than behavior, but may not be indicative of negative effects, unless negative effects result from a mechanism not induced under the conditions of captivity.

#### *Effects of Plumage-oiling on Behavior*

There was a clear dose-response trend in the proportion of time spent feeding by harlequin ducks in the plumage-oiling experiment, with less feeding by more heavily oiled birds. The effect of plumage-oiling was most evident in the heavy-oiling treatment group, whose estimated proportion of time spent feeding was 43% less than controls. The probability of feeding during an observation period was also 53% lower in the heavy-oiling treatment relative to controls, but no effect on feeding probability was apparent at the other plumage-oiling levels. The decrease in proportion of time spent feeding likely resulted from reduced time spent in the water because dive-feeding with oiled plumage causes high rates of heat loss (Jenssen and Ekker 1990, Jenssen and Ekker 1991a, b). Effects of plumage-oiling operate primarily through increased thermal conductance of wet plumage, which raises the lower critical temperature at which birds must increase their metabolic rate in order to thermoregulate (Hartung 1967, McEwan and Koelink 1973, Jenssen and Ekker 1990, 1991a,b). The effects of plumage-oiling on waterfowl can be dramatic; heat loss in common eiders exposed to 12.5 ml of crude oil was increased by 360% while residing in water (Jenssen and Ekker 1990).

To maintain body temperature, externally-oiled birds with wet plumage must increase their resting metabolic rate, and this increase is proportional to the degree of plumage-oiling (Hartung 1967, McEwan and Koelink 1973, Jenssen and Ekker 1991a). Common eiders resting on land with oiled but dry plumage, however showed no evidence of higher heat loss (Jenssen and Ekker 1990). It seems likely that externally-oiled harlequin ducks spent less time feeding to reduce time in the water and the wetness of their plumage. Common eiders exposed to a 2.5 ml mixture

of crude oil and oil dispersant showed a similar increase in time spent on shore compared to prior to exposure (Ekker et al. 1989).

Increased resting metabolic rate would carry an associated increase in energy requirement, yet externally-oiled harlequin ducks in captivity reduced their feeding time. Despite reduced feeding time, however, no differences in food consumption rate were found among treatment groups of externally-oiled birds, indicating that externally-oiled harlequin ducks increased their foraging efficiency (i.e., increased the quantity of food consumed per unit feeding time). Externally-oiled harlequin ducks lost body mass over the experimental oiling period, indicating that behavioral adaptations to mitigate the costs of thermoregulation, in the absence of increased food consumption, were not sufficient to compensate for the greater heat loss associated with plumage-oiling.

Although variation in the maintenance activity of birds within plumage-oiling treatment groups was large, estimates of both probability of occurrence and proportion of time taken together suggest effects at the moderately- and heavily-oiled levels. Estimates of the proportion of time spent in maintenance activities, suggest a threshold for effect at the moderately-oiled level. Plumage-oiling at the lightly-oiled treatment level did not cause feathers to become matted with oil, whereas at the moderately- and heavily-oiled treatment levels oil did penetrate below the surface of the feathers and caused matting and loss of water repellency. Moderately- and heavily-oiled treatments showed similar increases in the proportion of time spent in maintenance activities, although the differences in exposure did lead to differences in the extent of external-oiling based on visual estimates.

Time spent preening by oiled shorebirds at the site of an oil spill increased with the percentage of plumage-oiling (Burger 1997). Heavily oiled harlequin ducks did not show an increase in maintenance activities above that of moderately-oiled birds. This may reflect some upper limit on maintenance activities above which the cost of heat loss associated with increased exposure while preening exceeds the benefits of preening to remove oil and to dry feathers. Preening of oiled plumage may increase the negative effects of oil on feathers immediately following exposure. Heat production by oiled common eiders was higher 1 day after exposure compared to immediately following exposure, and was attributed to preening of oil deeper into plumage (Jenssen and Ekker 1991*b*). Oiled sanderlings were found to spread oil by preening, causing a 10% increase in the percentage of the plumage covered with oil following exposure (Burger and Tshipoura 1998).

Increased time spent in maintenance activity caused by plumage-oiling, even at low levels and in the absence of acute mortality, would reduce the time available for other activities such as feeding. Reduction of time spent in critical activities may have negative consequences on fitness during energetically demanding periods of the annual cycle (Burger 1997). The winter time-activity budget of harlequin ducks may not accommodate increased maintenance activity, which would likely be much greater than those observed in captivity given the greater amount of time harlequin ducks must spend in the water to feed under natural winter conditions. Indeed, their reliance on dive-feeding in intertidal habitats not only increases the risk of exposure to oil for harlequin ducks, but increases the severity of the effects of plumage-oiling which are greater in birds that must spend time in the water to feed (Jenssen and Ekker 1991*b*). In addition, the small

body size, high surface area to volume ratio, and low thermal conductance of harlequin ducks compared to other waterfowl makes the species especially vulnerable to the effects of external-oiling (Jenssen and Ekker 1991b). The volume of oil applied in the heavy-oiling treatment for this study, 5 ml, is lower than the 10 ml minimal level of exposure that resulted in metabolic effects in common eiders (Jenssen and Ekker 1991b). Although externally-oiled harlequin ducks were apparently able to behaviorally compensate for much of the effect of plumage-oiling in captivity, similar compensation would likely be ineffective in the wild where foraging requires considerable time in the water.

#### *Behavioral Constraints on Population Recovery*

Animal behavior is highly adaptable (Cuthill and Houston 1997, Grue et al. 2002). Because behavior can be adjusted to compensate for underlying biochemical and physiological effects of contaminants, and (or) affected directly by neurotoxic contaminants it can be a useful index of toxicity (Weiss et al 2001, Grue et al. 2002). Additionally, if optimal behavioral responses to environmental or physiological change are impaired by contaminant exposure, particularly during periods when time and (or) energy is limited, then individual fitness may be compromised (Cuthill and Houston 1997). Thus behavioral adjustments to contaminant exposure link biochemical and physiological effects of contaminants with changes in individual fitness and population-level effects (Weiss et al. 2001). However, the context dependent nature of behavior makes quantifying behavioral changes related to contaminants difficult (Peakall 1996).

Additionally, detecting subtle toxic effects on behavior with ecological relevance is challenging (Peakall 1996). This study focused on activities important to wintering harlequin ducks and therefore effects on these behaviors were relevant to survival outside of captivity. Time-activity budgets, however, are a coarse-scale estimate of behavior and may not detect fine-scale changes (Heath et al. 2004). The sampling method and analytical approach used in this study were successful at detecting changes in behavior, for example the increase in feeding time in relation to time of day and an increase in feeding time between winters of the oil-dosing experiment. The effects of plumage-oiling on behavior were also apparent, despite the relatively small sample of repeated observations and the large range of variation in behavioral responses.

Toxicological studies conducted on captive animals are limited in the inferences that may be extended to wild populations (Hienz 1989). In this study, we attempted to find effects on the behavior of harlequin ducks in captivity that may underlie population-level effects documented in the wild (Esler et al. 2002). Based on our results, external exposure to residual oil could contribute to reduced survival in harlequin ducks wintering in contaminated areas of Prince William Sound if residual oil is being released into the water column where it can be encountered by wildlife. Residual EVOS oil documented in 2001 was found in the mid- and lower-intertidal zones buried below the sediment surface where it remained in a largely unweathered state (Short et al. 2003). Additionally, elevated hepatic CYP1A levels in harlequin ducks from oiled areas are a reliable indicator that continued exposure to PAHs has been occurring as many as 9 years after the EVOS (Trust et al. 2000). However, the route of chronic exposure to residual oil in harlequin ducks is not known and may be occurring through ingestion of contaminated prey, external exposure, or a combination of both.

The extent to which residual oil trapped in sediments is released into the water column is not known. External exposure of harlequin ducks to residual oil in PWS has not been documented, although oil has been detected on the pelage of river otters from oiled areas as many as 8 years after the EVOS (Duffy et al. 1999). Future research evaluating the prevalence of residual EVOS oil in the water column, as well as on the plumage of harlequin ducks from oiled areas of PWS would be exceedingly useful. If external exposure is occurring, then the behavioral responses to external exposure documented in captive harlequin ducks would most likely be untenable under natural conditions that require harlequin ducks to spend a high proportion of time in the water to feed resulting in excessive heat loss and hypothermia, or force birds to roost on shore and forego feeding, resulting in starvation. Therefore we conclude that external exposure to oil at levels high enough to penetrate the plumage (= 2.5 ml oil) may contribute to reduce winter survival of harlequin ducks in PWS by reducing feeding activity.

#### *Captive Studies – CYP1A Induction.--*

Our results from captive studies confirm, not surprisingly, that ingestion of weathered crude oil results in induction of CYP1A. These also indicate that the levels of induction, and thus presumably exposure, were similar or higher in captivity than in Prince William Sound. Therefore, behavior data should reflect the kinds of responses that might be expected under wild conditions, although, as noted above there may be conditions experienced in the wild that can not be simulated under captive conditions.

#### *Field Studies – CYP1A Induction.--*

We reported that, in 1998, hepatic CYP1A activity was significantly higher in harlequin ducks from areas of PWS originally impacted with Exxon *Valdez* oil than in birds from unoiled areas (Trust et al., 2000). Here, we report that differences in CYP1A activity diminished over time between the two areas from 2000 to 2002. In fact, by November 2002, point estimates were almost identical.

A large body of research indicates that, more than nine years after the spill, polycyclic aromatic hydrocarbons (PAHs) from residual Exxon *Valdez* oil were likely responsible for elevated CYP1A activities in sea ducks and several other vertebrates in oiled areas of PWS (Marty et al., 1997; Woodin et al., 1997; Trust et al., 2000; Bodkin et al., 2002; Jewett et al., 2002). In birds, correlations between CYP1A induction and direct, toxic biological effects include: interference in heme biosynthesis (Lorenzen et al., 1997), swelling of liver cells and the pericardium around the heart (Rifkin et al., 1984), immune dysfunction (Trust et al., 1994) and somatic chromosomal damage (Custer et al., 2000). In other vertebrates (e.g., mice), toxic compounds which induce CYP IA enzymes are also immunotoxic and teratogenic. These physiological consequences of oil exposure to an individual could have population-level effects on harlequin ducks (Esler et al., 2002).

The CYP1A data reported here are critical for determining recovery status of harlequin duck populations. These provide strong evidence that exposure to lingering Exxon *Valdez* oil was diminishing by 2002, suggesting that direct effects of the spill may be ending. These findings are corroborated by convergence of demographic data from oiled and unoiled areas (see below).

### *Field Studies – Female Winter Survival.--*

During NVP studies oil exposure, as indicated by CYP1A induction, was higher in oiled areas than unoiled areas, which in turn corresponded to differences in survival between areas. Could exposure to residual *Exxon Valdez* oil result in lower survival and concomitant population declines? Some lab studies have shown that mallards are tolerant of ingestion of oil, with acute 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 may occur in wild harlequin ducks, which exist under relatively harsh winter conditions with little flexibility for accommodating additive stresses (Goudie and Ankney 1986). The NVP 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 NVP finding of divergence of survival probabilities between oiled and unoiled areas during mid-winter is consistent with a hypothesis of additive effects of oil in the presence of other stressors. Harlequin ducks are visual foragers, and during mid-winter when day length is shortest, they spend most of their time feeding (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 daylight available for foraging may be particularly limited. Because harlequin ducks have little flexibility for meeting increased energy demands during winter (Goudie and Ankney 1986), which could result from either ingestion of hydrocarbons or plumage oiling (Jenssen 1994), they may be unable to accommodate additive effects of the oil spill, even if relatively small. We speculated that differences in survival and population trends are related to documented differences in contaminant exposure (Trust et al. 2000).

The data from this study shed light on the relationship between oil exposure and survival. First, both levels of exposure and overall survival are more similar between areas during this study than during NVP studies. This corroborates the findings of NVP, which suggested that oil exposure was responsible for increased mortality on oiled areas. Second, on a finer scale, year to year variation in exposure corresponded to variation in survival in a way that would be predicted. For example, differences in CYP1A induction during November 2000 (Fig. 2.9) were associated with a 6.8% lower survival probability on oiled areas. However, during the following 2 years, CYP1A induction was similar, as was survival. This close correspondence of exposure and demography over time and space suggests that exposure to residual oil had direct population-level effects and, also, that those effects have decreased over time to the point where they are roughly equal.

These data contradict the conventional wisdom that effects of major spills on wildlife populations are limited to a year or 2 following the perturbation. We suggest that this is due to several factors. First, persistence of oil was much greater than anticipated (Short et al. 2003). Second, convincing evidence from a range of vertebrates indicates that exposure to oil persisted for at least a decade (e.g., Trust et al. 2000), which was much longer than had been assumed. Third, this study and the companion sea otter work, indicate that this exposure has population

level effects, which was not anticipated. Finally, previous considerations did not take into account variation in life history and natural history attributes that might lead some species, like harlequin ducks, to be particularly vulnerable to longer-term effects.

*Field Studies – Dispersal and Population Structure.--*

Harlequin ducks wintering in Prince William Sound exhibited a high degree of interannual philopatry to specific molting/wintering areas. Homing rates calculated in this study were >90% for both sexes, making them among the highest documented of any waterfowl species (Anderson et al. 1992, Robertson and Cooke 1999). Our results corroborate those of Robertson and Cooke (1999) in coastal British Columbia, who, using a mark-resight approach at a single molting/wintering site, reported return rates of 77% for males and 62% for females. Return rate does not account for survival, suggesting that after mortality is considered homing rates in the two studies would be roughly equivalent.

In contrast to the high degree of winter philopatry, harlequin duck breeding philopatry is much less pronounced. Estimates from several North American studies, which are summarized by Robertson and Goudie (1999), suggest a mean breeding area homing rate of approximately 55% for males (4 studies,  $n = 88$  individuals) and 67% for females (5 studies,  $n = 93$ ). Given that— (1) most of the annual cycle is spent on wintering areas; (2) winter philopatry is significantly greater than breeding philopatry; and (3) pair bonds are formed during winter—it is evident that population genetic and demographic structure are determined primarily during winter for harlequin duck populations.

Our estimate of adult female mortality rate for harlequin ducks on Montague Island met the expectation of high annual survival for a sea duck species. Apparent survival was estimated at 0.678 for females, and if homing rate is used as a surrogate for calculating permanent emigration, then our estimate of true survival would increase by roughly 5.4% ( $1 - \text{homing rate}$ ) to 0.732. While female annual survival rates were high compared to most dabbling duck species (Johnson et al. 1992), the annual survival rates estimated in this study were slightly lower than those of other, larger bodied, sea ducks such as white-winged scoters (*Melanitta fusca*) (Krementz et al. 1997), common eiders (*Somateria mollissima*) (Coulson 1984, Krementz et al. 1996), Steller's eiders (Flint et al. 2000), and spectacled eiders (*Somateria fischeri*) (Grand et al. 1998). Our estimates were also lower than estimates obtained for harlequin duck populations in more southerly parts of their range. In the Strait of Georgia, British Columbia, Cooke et al. (2000) estimated annual survival rates ranging from 0.70 to 0.91 for males (unpaired and paired, respectively), and 0.73 to 0.76 for females (unknown status and paired females respectively). Subsequent researchers in the same area have obtained similar results (Regehr 2003).

Harlequin ducks are smaller than most sea duck species, and Prince William Sound is near the northern extent of their wintering range. We speculate that harsher climatic conditions in more northerly areas could be affecting winter survival rates (Ricklefs 1973). Using data from Esler et al. (2000a), where cumulative winter survival was estimated to be 0.837 for radio-marked adult females on Montague Island (1995-1997), we partitioned our estimate of female survival into breeding and wintering components. Our estimate of breeding season survival estimate would range from 0.81 to 0.88, depending upon whether apparent or true annual survival was used in the calculations. This estimate is nearly identical to those obtained for harlequin ducks breeding

in Oregon (Bruner 1997), the only published study on breeding season survival rates, suggesting that over-winter survival may have a stronger influence on harlequin duck populations at the northern portion of their range.

By tracking a large number of radio-marked individuals over an extensive ( $> 7000 \text{ km}^2$ ) study area, we were able to quantify the scale of within-winter movements, as well as the degree of connectivity among population segments at multiple spatial scales. For harlequin ducks, winter populations are clearly subdivided demographically. We determined home ranges for female harlequin ducks during winter, and found them to be among the most restricted of any migratory bird species (Dugger et al. 1994, Warnock and Takekawa 1995, Gordon 2000, Sanzenbacher and Haig 2002). Long distance ( $>30\text{-}40 \text{ km}$ ) dispersal events were rare, and contrary to studies in coastal British Columbia (Regehr 2003), age related differences were not evident. Exchange rates among population segments were high at smaller spatial scales, however, most movements were to adjacent *sites*, and dispersal rates declined significantly as the geographic scale of reference was increased to the *zone* and *area* scales. At the *area* scale, which included island or mainland regions of Prince William Sound (e.g. Montague or Green Island), site fidelity was 95%. Based on calculations derived from radio failure rates, we estimated that fewer than 2% of all females dispersed from Prince William Sound during any given winter.

Although the results described above indicate that biologically relevant population structure exists at relatively small scales, molecular studies have indicated that there is little variation in allele frequencies at a regional scale (Scribner et al. 1998, Lanctot et al. 1999) or along the Pacific coast (Scribner *in preparation*). Lanctot et al. (1999) discussed four potential reasons why genetic variation among wintering aggregations of harlequin ducks is so low. These included: (1) the possibility of a recent expansion of refugial populations; (2) barriers to gene flow which are too recent to allow for genetic differentiation; (3) habitat alterations that may have lead to episodic dispersal in the past; and (4) low levels of continuing dispersal occurring among population segments. Based on results of this study, we can conclude that dispersal rates within Prince William Sound are sufficient to homogenize populations genetically. However, low levels of continuing dispersal are unlikely to account for the lack genetic variation at larger spatial scales.

The first three putative mechanisms discussed by Lanctot et al. (1999) are all related to evolutionary history and historical demography (Awise 2000). While we accept that each may be useful for explaining the paucity of genetic structure apparent in Pacific coast populations, we also contend that unless caution is applied when interpreting such results misleading conclusions might be drawn about the interconnectedness of present day populations. Original concepts used to define management units in wildlife conservation were typically related to the identification of local self-sustaining population segments, recognized by their “radius of mobility” or “rate of spread into unoccupied range” (Leopold 1933, Dizon et al. 1992). The data required to define local self-sustaining population segments has been difficult to obtain, and genetic markers have enjoyed widespread use as proxies that imply reproductive isolation (Moritz 1994). However, overemphasis should not be placed on genetic markers when attempting to delineate management units. Our study demonstrates that the amount of reproductive isolation required for genetic structure to develop is much higher than the amount required for demographic independence of population segments. Simply put, while a few migrants per generation will

homogenize populations genetically (Slatkin 1985), similarly low rates of exchange would not link them demographically.

Implications--One of our primary objectives in this part of the study was to address the degree to which high rates of site fidelity might produce a lag in the time required for populations to recover from the *Exxon Valdez* oil spill. Our data indicate that harlequin duck site fidelity is remarkably high between and within years. In turn, this suggests that full recovery from the spill, defined as return to prespill conditions, may be constrained by lack of immigration into spill-affected areas, and that recovery must occur primarily through recruitment of young individuals into the area. Given the life history of harlequin ducks, this process may take decades, even in the absence of continuing direct effects from the spill.

## CONCLUSIONS

The //423 studies described here, along with the preceding NVP work, offer an unprecedented documentation of the response of wildlife populations to a major oil spill. These studies describe the patterns and duration of population change, as well as the underlying physiological, behavioral, and demographic processes leading to observed patterns. Observations for sea otters and harlequin ducks are remarkably similar, seemingly due to common habitats and food types.

One of the more unexpected results from this body of research was the duration of exposure to residual *Exxon Valdez* oil. A conventional assumption about effects of major spills was that direct effects of oil exposure on wildlife were limited to a relatively short (1 to 2 year) period. However, residual *Exxon Valdez* oil persisted in nearshore sediments through at least 2001 (Short et al. 2003). Our studies show that this residual oil remained bioavailable and that sea otters and harlequin ducks were exposed to it for more than a decade after the spill. Sea otters and harlequin ducks are particularly susceptible to long-term exposure, given their occurrence in nearshore habitats and diet of benthic invertebrates that reside in and on sedimentary habitats where oil persists. Our data clearly indicate that some wildlife populations do not meet conventional assumptions regarding long-term exposure following oil spills. The duration of exposure has important implications for risk assessment of oil spills and their ecosystem effects.

By the end of the //423 studies, we were seeing indications that exposure was decreasing. Harlequin duck CYP1A induction converged between oiled and unoiled areas during 2001 and 2002. And, although sea otter CYP1A remained elevated in oiled areas through 2002, the magnitude of the difference between areas was diminishing. These data suggest that constraints to population recovery that are related to effects of oil exposure should concurrently diminish.

Another important finding from the NVP and //423 studies was the population-level demographic differences between oiled and unoiled areas associated with differences in oil exposure. Sea otter abundance was markedly reduced at northern Knight Island due to initial effects of the spill. However, in the absence of direct effects, we predicted that sea otter numbers would recover over time at Knight Island, given available food resources there (Dean et al. 2002). This has not occurred, presumably as a result of increased mortality among prime-age individuals (Monson et al. 2000). Similarly, harlequin duck mortality was significantly higher on oiled areas than unoiled areas through 1998, corresponding to decreasing population trends and reduced densities on oiled areas (Esler et al. 2002). We suggest that the observed demographic differences were a direct result of continued exposure to residual oil. The harlequin duck data from the latter years of the study corroborate this conclusion: in 2001 and 2002, when oil exposure had diminished, survival estimates between areas also were similar, suggesting that oil exposure was a primary cause of divergent demographics between areas in previous years. We also found little support for the conventional assumption that the predominant effects of spills are primarily due to immediate, acute mortalities resulting from massive oil exposure and subsequent toxicosis or hypothermia. Overall, our sea otter and harlequin duck results suggest that longer-term, chronic exposure may have more subtle but potentially stronger influences on population demography than acute effects. Again, this finding is important from a risk assessment perspective, and has important implications for predicting population-level effects of low-level, chronic oiling that may result not only from residual contamination from major spills, but potentially also from a wide variety of pollutant sources.

Our data indicate a number of mechanisms by which oil exposure could affect sea otter and harlequin duck survival and, hence, populations. Individuals exposed to oil displayed a range of behavioral and physiological responses. For example, in sea otters from oiled areas we consistently measured elevated GGT, suggesting an effect of exposure on liver function. For harlequin ducks, winter body mass was negatively correlated with CYP1A during NVP studies (Esler et al. 2002). Studies of captive harlequin ducks indicated that birds ingesting oil did not show dramatic behavior changes, although we acknowledge that we could not fully simulate the conditions that they would experience in PWS. However, captive birds with oiled plumage showed distinct changes in behavior, at a level that we suggest would be unsustainable in the wild, indicating that external exposure to even small amounts of oil would almost certainly influence survival.

Given indications of declining oil exposure in sea otters and harlequin ducks, we believe that direct effects of oil exposure on demography have moderated, and may now be of diminishing consequence. However, return to pre-spill numbers or other recovery endpoints requires not only that direct effects end, but that sufficient time passes for normal demographic processes to allow necessary increases in abundance. Another of the prior assumptions of spill-induced reductions in population abundance was that immigration of individuals from unaffected areas would facilitate recovery. Our collective findings from sea otters and harlequin ducks strongly suggest that species-specific life history and behavior can limit the role of immigration as a factor in recovery. In light of the high fidelity to seasonal (harlequin duck) or annual (sea otter) home ranges we conclude that recovery on oiled areas will occur primarily through recruitment of young individuals and not via immigration. Given the life history of these species, this demographic response will likely require a protracted period. For both species, it would be prudent to continue monitoring population abundance and oil exposure to track the process of population recovery from effects of the spill.

Ecosystem recovery remains an elusive goal, for a number of reasons. First, defining the state of the ecosystem at the time of the spill as an endpoint of recovery is problematic, as the system was so poorly described at that time. Second, ecosystems are inherently dynamic entities, and the assumption that the system after the acute effects of the spill subsided, would soon approximate the system as it occurred prior to March 1989 is tenuous at best. This problem is exacerbated by chronic spill effects, or other factors that delay recovery. As the time from the initial perturbation increases, the probability of ecosystem level change increases, thus escalating the difficulty of defining, much less attaining that pre-perturbation condition. And lastly, while populations may serve as reasonable surrogates in defining ecosystem recovery, not all species were affected equally, and some post-spill population trajectories likely reflect processes quite independent of the spill (e.g. some long-term pinniped and seabird population trends). Our findings suggest that physiological responses of birds and mammals to chronic hydrocarbon exposure may be useful yardsticks for evaluating the extent to which residual oil may be constraining ecosystem recovery. Further, the collective findings of the suite of EVOS nearshore studies clearly indicate that selection of species from which to evaluate ecosystem recovery requires careful consideration, as each species affords a unique view into the nearshore ecosystem.

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## LITERATURE CITED

- Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior* 69:227-263.
- Anderson, M. G., J. M. Rhymer, and F. C. Rohwer. 1992. Philopatry, dispersal, and the genetic structure of waterfowl populations. Pages 365-395 *in* B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu, editors. *Ecology and management of breeding waterfowl*. University of Minnesota Press, Minneapolis, Minnesota, USA.
- Avise, J. C. 2000. *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge, Massachusetts, USA.
- Babcock, M. M., G. V. Irvine, P. M. Harris, J. A. Cusick, and S. D. Rice. 1996. Persistence of oiling in mussel beds three and four years after the Exxon Valdez oil spill. *American Fisheries Society Symposium* 18:286-297.
- Ballachey, B. E., J. L. Bodkin, S. Howlin, K. A. Kloecker, D. H. Monson, A. H. Rebar, and P. W. Snyder. 2002a. Hematology and serum chemistry of sea otters in oiled and unoiled areas of Prince William Sound, Alaska, 1996-98. Appendix BIO-01 in 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. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska.
- , -----, P. W. Snyder and T. A. Kondratyuk. 2003a. Lingerin Oil: Bioavailability and Effects to Prey and Predators. Exxon Valdez Oil Spill State/Federal Restoration Draft Final Report (Project 030585, Part II), U.S. Geological Survey, Anchorage, Alaska.

- , P. W. Snyder and T. A. Kondratyuk. 2003b. Comparison of cytochrome P450 1A induction in blood and liver cells of sea otters. Exxon Valdez Oil Spill State/Federal Restoration Final Report (Project 01534), U.S. Geological Survey, Anchorage, Alaska.
- , J. J. Stegeman, P. W. Snyder, G. M. Blundell, J. L. Bodkin, T. A. Dean, L. Duffy, D. Esler, G. Golet, S. Jewett, L. Holland-Bartels, A. H. Rebar, P. A. Seiser, and K. A. Trust. 2002b. Oil exposure and health of nearshore vertebrate predators in Prince William Sound following the Exxon Valdez oil spill. Chapter 2 in Holland-Bartels, L.E., editor. 2002. Mechanisms of impact and potential recovery of nearshore vertebrate predators following the 1989 Exxon Valdez oil spill, volume 1. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska.
- Ben-David, M., L. K. Duffy, and R. T. Bowyer. 1999. Responses of river otters to oil contamination: a controlled study of biological stress markers. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 98/99348), Institute of Arctic Biology, University of Alaska Fairbanks, Alaska, USA.
- , T. M. Williams, and O. A. Ormseth. 2000. Effects of oiling on exercise physiology and diving behavior of river otters: a captive study. *Canadian Journal of Zoology* 78:1380-1390.
- Bennetts, R. E., J. D. Nichols, J. D. Lebreton, R. Pradel, J. E. Hines, and W. M. Kitchens. 2001. Methods for estimating dispersal probabilities and related parameters using marked animals. Pages 3-17 *in* J.E. Clobert, E. Danchin, A. A. Dhondt, and J. Nichols, editors. *Dispersal: causes, consequences and mechanisms of dispersal at the individual, population and community level*. Oxford University Press, Oxford, U. K.

- Bjørnstad, O. N., H. P. Andreassen, and R. A. Ims. 1998. Effects of habitat patchiness and connectivity on the spatial ecology of the root vole (*Microtus oeconomus*). *Journal of Animal Ecology* 67:127-141.
- Bodkin, J. L., J. A. Ames, R. J. Jameson, A. M. Johnson and G. M. Matson. 1997. Accuracy and precision in estimating age of sea otters using cementum layers in the first premolar. *Journal of Wildlife Management* 61:967-973.
- , B. E. Ballachey, M. A. Cronin and K. T. Scribner. 1999. Population demographics and genetic diversity in remnant and re-established populations of sea otters. *Conservation Biology* 13(6):1278-1385.
- , -----, T. A. Dean, A. K. Fukuyama, S. C. Jewett, L. McDonald, D. H. Monson, C. E. O'Clair, and G. R. VanBlaricom. 2002. Sea otter population status and the process of recovery from the 1989 Exxon Valdez oil spill. *Marine Ecology Progress Series* 241:237-253.
- , A. M. Burdin and D. A. Ryzanov. 2000. Age and sex specific mortality and population structure in sea otters. *Marine Mammal Science* 16(1):201-219.
- , G.G. Esslinger and D.H. Monson. 2004. Foraging depths of sea otters and implications to coastal marine communities. *Marine Mammal Science* 20(2)
- , and M. S. Udevitz. 1994. An intersection model for estimating sea otter mortality along the Kenai Peninsula. Pages 81-95 in T. R. Loughlin, editor. *Marine mammals and the Exxon Valdez*. Academic Press. San Diego, California, USA.
- , and -----. 1999. An aerial survey method to estimate sea otter abundance. Pages 13-26 in G. W. Garner, S. C. Amstrup, J. L. Laake, B. J. F. Manly, L. L. McDonald, and D. G.

- Robertson, editors. Marine mammal survey and assessment methods. AA Balkema, Rotterdam, Netherlands.
- Boehm, P. D., P. J. Mankiewicz, R. Hartung, J. M. Neff, D. S. Page, E. S. Gilfillan, J. E. O'Reilly, and K. R. Parker. 1996. Characterization of mussel beds with residual oil and the risk to foraging wildlife 4 years after the Exxon Valdez oil spill. *Environmental Toxicology and Chemistry* 15:1289-1303.
- , D. S. Page, E. S. Gilfillan, W. A. Stubblefield, and E. J. Harner. 1995. Shoreline ecology program for Prince William Sound, Alaska following the Exxon Valdez oil spill: Part 2-chemistry and toxicology. Pages 347-397 in P. G. Wells, J. N. Butler, and J. S. Hughes, editors. *Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters*. ASTM STP 1219. American Society for Testing Materials, Philadelphia, Pennsylvania, USA.
- Breen, P.A., T. A. Carson, J. B. Foster and E. A. Stewart. 1982. Changes in subtidal community structure associated with British Columbia sea otter transplants. *Marine Ecology Progress Series* 7:13-20.
- Brodeur, S., J. P. L Savard, M. Robert, P. Laporte, P.Lamothe, R. D. Titman, S. Marchand, S. Gilliland, and G. Fitzgerald. 2002. Harlequin duck *Histrionicus histrionicus* population structure in eastern Nearctic. *Journal of Avian Biology* 33: 127-137.
- Brodsky, L. M., and P. J. Weatherhead. 1985. Variability and behavioural response of wintering black ducks to increased energy demands. *Canadian Journal of Zoology* 63:1657-1662.
- Brownie, C., J. E. Hines, J. D. Nichols, K. H. Pollock, and J. B. Hestbeck. 1993. Capture-recapture studies for multiple strata including non-Markovian transitions. *Biometrics* 49:1173-1187.

- Bruner, H. J. 1997. Habitat use and productivity in the central Cascade Range of Oregon. Thesis, Oregon State University, Corvallis, Oregon, USA.
- Bunck, C. M., C. L. Chen, and K. H. Pollock. 1995. Robustness of survival estimates from radio-telemetry studies with uncertain relocation of individuals. *Journal of Wildlife Management* 59:790-794.
- Burger, J. 1997. Effects of oiling on feeding behavior of Sanderlings and Semipalmated Plovers in New Jersey. *Condor* 99:290-298.
- , and N. Tsioura. 1998. Experimental oiling of Sanderlings (*Calidris alba*): behavior and weight changes. *Environmental Toxicology and Chemistry* 17:1154-1158.
- Burnham, K. P., and D. R. Anderson. 2002. Model selection and multimodel inference: a practical information theoretic approach. Second edition. Springer-Verlag, New York, New York, USA.
- Calow, P. 1991. Physiological costs of combating chemical toxicants: ecological implications. *Comparative Biochemistry and Physiology* 100C:3-6.
- Carls, M. G., M. M. Babcock, P. M. Harris, G. V. Irvine, J. A. Cusick, S. D. Rice. 2001. Persistence of oiling in mussel beds after the Exxon Valdez oil spill. *Marine Environmental Research* 51:167-190.
- Connor, E. F., S. H. Faeth, and D. Simberloff. 1983. Leafminers on oak: the role of immigration and in situ reproductive recruitment. *Ecology* 64: 191-204.
- Cooke, F., G. J. Robertson, C. M. Smith, R. I. Goudie, and W. S. Boyd. 2000. Survival, emigration, and winter population structure of Harlequin Ducks. *Condor* 102:137-144.
- Coon, N. C., and M. P. Dieter. 1981. Responses of adult mallard ducks to ingested South Louisiana crude oil. *Environmental Research* 24:309-304

- Cormack, R. M. 1964. Estimates of survival from the sighting of marked animals. *Biometrika* 51:429-438.
- Coulson, J. C. 1984. The population dynamics of the Eider Duck *Somateria mollissima* and evidence of extensive non-breeding by adult ducks. *Ibis* 126: 525-543.
- Custer, T. W., C. M. Custer, R. K. Hines, D. W. Sparks, M. J. Melancon, D. J. Hoffman, J. W. Bickham, and J. K. Wickliffe. 2000. Mixed-function oxygenases, oxidative stress, and chromosomal damage measured in Lesser Scaup wintering on the Indiana Harbor Canal. *Archives Environmental Contamination Toxicology* 38:522-529.
- Cuthill, I. C., and A. I. Houston. 1997. Managing time and energy. Pages 97-120 in J. R. Krebs, editor. *Behavioural ecology: an evolutionary approach*. Blackwell Sciences, Oxford, U.K.
- Dean, T. A., J. L. Bodkin, S. C. Jewett, D. H. Monson and D. Jung. 2000. Changes in sea urchins following a reduction in sea otter density as a result of the Exxon Valdez oil spill. *Marine Ecology Progress Series* 199:281-291.
- , -----, A.K. Fukuyama, S.C. Jewett, D.H. Monson, C.E. O'Clair, and G.R. VanBlaricom. 2002. Food limitation and the recovery of sea otters in Prince William Sound. *Marine Ecology Progress Series*. 241:255-270
- , S. C. Jewett, D. R. Laur and R. O. Smith. 1996. Injury to epibenthic invertebrates resulting from the Exxon Valdez oil spill. Pages 424-439 in S. D. Rice, R. B. Spies, D. A. Wolfe, and B. A. Wright, editors. *Proceedings of the Exxon Valdez oil spill symposium*. American Fisheries Society Symposium 18.
- Dizon, A. E., C. Lockyer, W. F. Perrin, D. P. Demaster, and J. Sisson. 1992. Rethinking the stock concept: a phylogeographic approach. *Conservation Biology* 6: 24-36.

- Duffy, L. K., M. K. Hecker, G. M. Blundell, and R. T. Bowyer. 1999. An analysis of the fur of river otters in Prince William Sound, Alaska: oil related hydrocarbons eight years after the Exxon Valdez oil spill. *Polar Biology* 21:56-58.
- Dugger, B. D., K. J. Reinecke, and L. H. Fredrickson. 1994. Late winter survival of female mallards in Arkansas. *Journal of Wildlife Management* 58: 94-99.
- Duggins, D. O. 1980. Kelp beds and sea otters: an experimental approach. *Ecology* 61:447-453.
- Dzus, E. H., and R. G. Clark. 1996. Effects of harness-style and abdominally implanted transmitters on survival and return rates of mallards. *Journal of Field Ornithology* 67:549-557
- Easton, W. C., and, H. C. Murray. 1981. Effects of crude oil on avian intestinal function. *Canadian Journal of Physiology and Pharmacology* 59:1063-1068.
- Ekker, M., B. M. Jenssen, and K. Winge. 1989. Behavioral and thermoregulatory responses of external contamination of eiders, *Somateria molissima*, under semi-natural conditions: a pilot study. Pages 301-306 in K. E. Zachariassen, editor. *Biological effects of chemical treatment of oil-spills at sea*. Trondheim, Norway.
- Esler, D. 2000. Applying metapopulation theory to conservation of migratory birds. *Conservation Biology*. 14: 366-372.
- , T. D. Bowman, T. A. Dean, C. E. O'Clair, S. C. Jewett, and L. L. McDonald. 2000a. Correlates of harlequin duck densities during winter in Prince William Sound, Alaska. *Condor* 102:920-926.

- , -----, K. A. Trust, B. E. Ballachey, T. A. Dean, S. C. Jewett, and C. E. O'Clair. 2002. Harlequin duck population recovery following the Exxon Valdez oil spill: progress, process and constraints. *Marine Ecology Progress Series* 241: 271-286.
- , D. M. Mulcahy, and R. L. Jarvis. 2000b. Testing assumptions for unbiased estimation of survival of radiomarked harlequin ducks. *Journal of Wildlife Management* 64:591-598.
- , J. A. Schmutz, R. L. Jarvis, and D. M. Mulcahy. 2000c. Winter survival of adult female harlequin ducks in relation to history of contamination by the Exxon Valdez oil spill. *Journal of Wildlife Management* 64:839-847.
- Estes, J. A., and D. O. Duggins. 1995. Sea otters and kelp forests in Alaska: generality and variation in a community ecological paradigm. *Ecological Monographs* 65:75-100.
- , and J. F. Palmisano. 1974. Sea otters: their role in structuring nearshore communities. *Science* 185:1058-1060.
- , N. S. Smith and J. F. Palmisano. 1978. Sea otter predation and community organization in the western Aleutian Islands, Alaska. *Ecology* 59:822-833.
- , M. T. Tinker, T. M. Williams and D. F. Doak. 1998. Increasing food chain length and the collapse of kelp forest ecosystems in western Alaska. *Science* 282:3473-3476.
- Exxon Valdez Oil Spill Trustee Council. 1999. Exxon Valdez Oil Spill Trustee Council Status Report. Anchorage, Alaska.
- Fischer, J. B. 1998. Feeding behavior, body condition, and oil contamination of wintering harlequin ducks at Shemya Island, Alaska. Thesis, University of Massachusetts, Amherst, Massachusetts, USA.
- , and C. R. Griffin. 2000. Feeding behavior and food habits of wintering harlequin ducks at Shemya Island, Alaska. *Wilson Bulletin* 112:318-325.

- Flemming, J. W., L. Sileo, and J. C. Franson. 1982. Toxicity of Prudhoe Bay crude oil to sandhill cranes. *Journal of Wildlife Management* 46:474-478.
- Flint, P. L., M. R. Petersen, C. P. Dau, J. E. Hines, and J. D. Nichols. 2000. Annual survival and site fidelity of Steller's eiders molting along the Alaska Peninsula. *Journal of Wildlife Management* 64: 261-268.
- Fukuyama, A. K., G. Shigenaka, and R. Z. Hoff. 2000. Effects of residual oil on intertidal *Protothaca staminea*: mortality, growth, and bioaccumulation of hydrocarbons in transplanted clams. *Marine Pollution Bulletin* 40:1042-1050.
- Galt, J. A., W. J. Lenr, and D. L. Payton. 1991. Fate and transport of the Exxon Valdez oil spill. *Environmental Science and Technology* 25:202-209.
- Garrott, R. A. L. L Eberhardt, and D. M. Burn. 1 993. Mortality of Sea Otters in Prince William Sound following the Exxon Valdez Oil Spill. *Marine Mammal Science* 9(4):343-359.
- Garshelis, D. L. 1997. Sea otter mortality estimated from carcasses collected after the Exxon Valdez oil spill. *Conservation Biology* 11(4):905-916.
- Gordon, C. E. 2000. Movement patterns of wintering grassland sparrows in Arizona. *AUK* 117:748-759.
- Gordon, C. J. 2003. Role of environmental stress in the physiological response to chemical toxicants. *Environmental Research* 92:1-7.
- Goudie, R. I., and C. D. Ankney. 1986. Body size, activity budgets, and diets of sea ducks wintering in Newfoundland. *Ecology* 67:1475-1482.
- , S. Brault, B. Conant, A. V. Kondratyev, M. R. Petersen, and K. Vermeer. 1994. The status of sea ducks in the North Pacific rim: toward their conservation and management.

Transactions of the North American Wildlife and Natural Resources Conference 59:27-49.

Grand, J. B., P. L. Flint, M. R. Petersen, and L. Moran. 1998. Effect of lead poisoning on spectacled eider survival rates. *Journal of Wildlife Management* 62:1103-1109.

Grue, C. E., S. C. Gardner, and P. L. Gibert. 2002. On the significance of pollutant-induced alterations in the behaviour of fish and wildlife. Pages 1-90 *in* G. Dell'Omo editor. *Behavioural ecotoxicology*. John Wiley and Sons, West Sussex, U.K.

Haramis, G. M., D. G. Jorde, and C. M. Bunck. 1993. Survival of hatching-year female canvasbacks wintering in Chesapeake Bay. *Journal of Wildlife Management* 57:763-771.

Hartung, R. 1967. Energy metabolism in oil-covered ducks. *Journal of Wildlife Management* 31:798-804.

Hayes, M. O., and J. Michel. 1999. Factors determining the long-term persistence of Exxon Valdez oil in gravel beaches. *Marine Pollution Bulletin* 38:92-101.

Heath, J. P., W. A. Montevecchi, and G. J. Robertson. 2004. Temporal allocation of foraging effort over environmental conditions by harlequin ducks wintering in Newfoundland. Pages 00-00 *in* G. J. Robertson, and P. W. Thomas, editors. *Harlequin ducks in the Northwest Atlantic*. Canadian Wildlife Service Occasional Paper No. 000.

Heinz, G. H. 1989. How lethal are sublethal effects? *Environmental Toxicology and Chemistry* 8:463-464.

Hestbeck, J. B., J. D. Nichols, and R. A. Malecki. 1991. Estimates of movement and site fidelity using mark-resight data of wintering Canada geese. *Ecology* 72:523-533.

Highsmith, R. C., T. L. Rucker, M. S. Stekoll, S. M. Saupe, M. R. Lindeberg, R. N. Jenne, and W. P. Erickson. 1996. Impact of the Exxon Valdez oil spill on intertidal biota. Pages

- 212-237 in S. D. Rice, R. B. Spies, D. A. Wolfe, and B. A. Wright, editors. Proceedings of the Exxon Valdez oil spill symposium. American Fisheries Society Symposium 18.
- Holland-Bartels, L. H. 2002. Mechanisms of impact and potential recovery of nearshore vertebrate predators. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration Project 95025-99025), US Geological Survey-Biological Resources Division, Anchorage, Alaska.
- Holmes, W. N., J. Cronshaw, and J. Gorsline. 1978. Some effects of ingested petroleum on seawater-adapted ducks (*Anas platyrhynchos*). *Environmental Research* 17:177-190.
- , Gorsline, and J. Cronshaw. 1979. Effects of mild cold stress on the survival of seawater-adapted mallard ducks (*Anas platyrhynchos*) maintained on food contaminated with petroleum. *Environmental Research* 20:425-444.
- Hooge, P.N. and B. Eichenlaub. 2000. Animal movement extension to Arcview. ver. 2.0. Alaska Science Center - Biological Science Office, U.S. Geological Survey, Anchorage, AK, USA.
- Irvine G. V., D. H. Mann, J. W. Short. 1999. Multi-year persistence of oil mousse on high energy beaches distant from the *Exxon Valdez* spill origin. *Mar Poll Bull* 38:572-584.
- Jenssen, B. M. 1989. Effects of ingested crude and dispersed crude oil on thermoregulation in ducks (*Anas platyrhynchos*). *Environmental Research* 48:49-56.
- , 1994. Effects of oil pollution, chemically treated oil, and cleaning on the thermal balance of birds. *Environmental Pollution* 86:207-215.
- , and M. Ekker 1990. Effects of plumage-oiling on thermoregulation in common eiders residing in air and water. In S. Myrreget, editor. Transactions of the 19th IUGB congress. Norwegian Institute for Nature Research, Trondheim, Norway.

- , and -----. 1991a. Dose dependent effects of plumage-oiling on thermoregulation of common eiders *Somateria mollissima* residing in water. *Polar Research* 10:579-584.
- , and -----. 1991b. Effects of plumage contamination with crude oil dispersant mixtures on thermoregulation in common eiders and mallards. *Archives of Environmental Contamination and Toxicology* 20:398-403.
- Jewett S. C., T. A. Dean, R. O. Smith and A. Blanchard. 1999. The Exxon Valdez oil spill: Impacts and recovery in the soft-bottom benthic community in and adjacent to eelgrass beds. *Marine Ecology Progress Series* 185:59-83.
- , -----, B. R. Woodin, M. K. Hoberg, and J. J. Stegeman. 2002. Exposure to hydrocarbons ten years after the Exxon Valdez oil spill: evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. *Marine Environmental Research* 54(1):21-48.
- Johnson, D. H., J. D. Nichols, and M. D. Schwartz. 1992. Population dynamics of breeding waterfowl. Pages 446-485 in B. D. J. Batt, A. D. Afton, M. G. Anderson, C. D. Ankney, D. H. Johnson, J. A. Kadlec, and G. L. Krapu, editors. *Ecology and management of breeding waterfowl*. University of Minnesota Press, Minneapolis, Minnesota, USA.
- Jolly, G. M. 1965. Explicit estimates from capture-recapture data with both death and immigration stochastic model. *Biometrika* 52:225-247.
- Kaiser, G. W., A. E. Derocher, S. Crawford, M. J. Gill, and I. A. Manley. 1995. A capture technique for marbled murrelets in coastal inlets. *Journal of Field Ornithology* 66:321-333.

- Kennedy, S. W., A. Lorenzen, C. A. James, and B. T. Collins. 1993. Ethoxyresorufin-O-deethylase and porphyrin analysis in chicken embryo hepatocyte cultures with a fluorescence multi-well plate reader. *Analytical Biochemistry* 211:102-112.
- Kernohan, B. J., R. A. Gitzen, and J. J. Millspaugh. 2001. Analysis of animal space use and movements. Pages 126-168. *in* J.J. Millspaugh and J.M. Marzulloff, editors. *Radio Tracking and Animal Populations*. Academic Press, San Diego, California, USA.
- Konar, B. 2000. Limited effects of a keystone species: long term trends of sea otters and kelp forests at the Semichi Islands, Alaska. *Marine Ecology Progress Series* 199:271-280.
- Korschgen, C. E., K. P. Kenow, A. Gendron-Fitzpatrick, W. L. Green, and F. J. Dein. 1996. Implanting intra-abdominal radio transmitters with external whip antennas in ducks. *Journal of Wildlife Management* 60:132-137.
- , S. J. Maxson, and V. B. Kuechle. 1984. Evaluation of implanted radio transmitters in ducks. *Journal of Wildlife Management* 48:982-987.
- Krementz, D. G., P. W. Brown, F. P. Kehoe and C. S. Houston. 1997. Population dynamics of white-winged scoters. *Journal of Wildlife Management* 61:222-227.
- , J. E. Hines and D. F. Caithamer. 1996. Survival and recovery rates of American eiders in Eastern North America. *Journal of Wildlife Management* 60:855-862.
- Kvitek, R. G., P. J. Iampietro, and C. E. Bowlby. 1998. Sea otters and benthic prey communities: A direct test of the sea otter as a keystone predator in Washington State. *Marine Mammal Science* 14:895-902.
- , and J. S. Oliver. 1988. Sea otter foraging habits and effects on prey populations and communities in soft bottom environments. Pages 22-47 *in* G. R. VanBlaricom and J. A. Estes, editors. *The community ecology of sea otters*. Springer-Verlag, Berlin, Germany.

- Lancot, R., B. Goatcher, K. Scribner, S. Talbot, B. Pierson, D. Esler, and D. Zwiefelhofer. 1999. Harlequin duck recovery from the Exxon Valdez oil spill: a population genetics perspective. *Auk* 116:781-791.
- Laur, D. R., A. W. Ebeling and D. A. Coon. 1988. Effects of sea otter foraging on subtidal reef communities off Central California. Pages 151-168 *in* G. R. VanBlaricom and J. A. Estes, editors. *The community ecology of sea otters*. Springer-Verlag, Berlin, Germany.
- Leberon, J. D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: a unified approach with case studies. *Ecological Monographs* 62: 67-118.
- Leighton, F. A. 1993. The toxicity of petroleum to birds. *Environmental Review* 1:92-103.
- Leopold, A. 1933. *Game management*. University of Wisconsin Press, Madison, Wisconsin, USA.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 2000. *SAS System for mixed models*. Fourth edition. SAS Institute, Cary, North Carolina, USA.
- Lorenzen, A., S. W. Kennedy, L. J. Bastien, and M. E. Hahn. 1997. Halogenated aromatic hydrocarbon-mediated porphyrin accumulation and induction of Cytochrome P4501A in chicken embryo hepatocytes. *Biochemical Pharmacology* 53:373-384.
- Lowry, L. F., and J. S. Pearse. 1973. Abalones and sea urchins in an area inhabited by sea otters. *Marine Biology* 23:213-219.
- Marty G. D, J. W. Short, D. M. Dambach, N. H. Willits, R. A. Heintz, S. D. Rice, J. J. Stegeman, and D. E. Hinton. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously

- exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology* 75:989-1007.
- Mather, D. D., and D. Esler. 1999. Evaluation of bursal depth as an indicator of age class of harlequin ducks. *Journal of Field Ornithology* 70:200-205.
- McCullagh, P., and J. A. Nelder. 1989. *Generalized linear models*. Second edition. Chapman and Hall, New York, New York, USA.
- McEwan, E. H., and A. F. C. Koelink. 1973. The heat production of oiled mallards and scaup. *Canadian Journal of Zoology* 51:27-31.
- , and P. M. Whitehead. 1980. Uptake and clearance of petroleum hydrocarbons by the glaucous-winged gull (*Larus glaucescens*) and the mallard duck (*Anas platyrhynchos*). *Canadian Journal of Zoology* 58:723-726.
- Monson, D. H., D. F. Doak, B. E. Ballachey, A. Johnson, and J. L. Bodkin. 2000. Long-term impacts of the Exxon Valdez oil spill on sea otters, assessed through age-dependent mortality patterns. *Proceedings National Academy of Sciences, USA*. 97(12):6562-6567.
- Moritz, C. 1994. Defining 'evolutionarily significant unit' for conservation. *Trends in Ecology and Evolution* 9:373-375.
- Mulcahy, D. M., and D. Esler. 1999. Surgical and immediate postrelease mortality of harlequin ducks (*Histrionicus histrionicus*) implanted with abdominal radio transmitters with percutaneous antennae. *Journal of Zoo and Wildlife Medicine* 30: 397-401.
- Munk, J. E., and R. A. MacIntosh. 1993. Continuing studies of green sea urchin growth and recruitment near Kodiak, Alaska. *Journal of Shellfish Research* 12:145.
- Nichols, J. D., and A. Kaiser. 1999. Quantitative studies of bird movement: a methodological review. *Bird Study* 46(suppl):289-298.

- , and K. H. Pollock. 1990. Separate estimation of recruitment from immigration versus in situ reproduction using Pollock's robust design. *Ecology* 71:21-26.
- Noldus Information and Technology. 1991. The Observer Software. Version 2.01. Wageningen, The Netherlands.
- Olsen, G. H., F. J. Dein, G. M. Haramis, and D. G. Jorde. 1992. Implanting radio transmitters in wintering canvasbacks. *Journal of Wildlife Management* 56:325-328.
- Ormseth, O. A., and M. Ben-David. 2000. Ingestion of crude oil: effects on digesta retention times and nutrient uptake in captive river otters. *Journal of Comparative Physiology B* 170:419-428.
- Paquette, G. A., J. H. Devries, R. B. Emery, D. W. Howerter, B. L. Joynt, and T. P. Sankowski. 1997. Effects of transmitters on reproduction and survival of wild mallards. *Journal of Wildlife Management* 61:953-961.
- Peakall, D. B. 1996. Disrupted patterns of behavior in natural populations as an index of ecotoxicity. *Environmental Health Perspectives* 105 (Supplement 2):331-335.
- Peterson, C. H. 2000. The Exxon Valdez oil spill in Alaska: acute, indirect, and chronic effects on the ecosystem. *Advances in Marine Biology* 39:1-103.
- Pietz, P. J., G. L. Krapu, R. J. Greenwood, and J. T. Lokemoen. 1993. Effects of harness transmitters on behavior and reproduction of wild mallards. *Journal of Wildlife Management* 57:696-703.
- Pollock, K. H., S. R. Winterstein, C. M. Bunck, and P. D. Curtis. 1989a. Survival analysis in telemetry studies: the staggered entry design. *Journal of Wildlife Management* 53:7-15.
- , -----, and M. J. Conroy. 1989b. Estimation and analysis of survival distributions for radio-tagged animals. *Biometrics* 45:99-109.

- Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco and R. T. Paine. 1996. Challenges in the quest of keystones. *BioScience* 46:609-620.
- Ramsey, F. L., and D. W. Schafer. 2002. *The statistical sleuth, a course in methods of data analysis*. Second edition. Duxbury, Pacific Grove, California, USA.
- Ranta E., V. Kaitala, and P. Lundberg. 1997. The spatial dimension in population fluctuations. *Science* 278: 1621-1623.
- Rattner, B. A. 1981. Tolerance of adult mallards to subacute ingestion of crude petroleum oil. *Toxicology Letters* 8:337-342.
- Rebar, A. H., B. E. Ballachey, D. L. Bruden, and K. A. Kloecker. 1996. Hematology and clinical chemistry of sea otters captured in Prince William Sound, Alaska, following the Exxon Valdez oil spill. Exxon Valdez Oil Spill State/Federal Damage Assessment Final Report, Marine Mammal Study 6-17. Natl. Biol. Serv., Alaska Science Center, 1011 E. Tudor Road, Anchorage, AK 99503.
- Regehr, H. M. 2003. Movement patterns and population structure of Harlequin Ducks wintering in the Strait of Georgia, British Columbia. Dissertation, Simon Fraser University, Burnaby, British Columbia, Canada.
- Ricklefs, R. E. 1973. Fecundity, mortality, and avian demography. Pages 366-435 in D. S. Farner, editor. *Breeding Biology of Birds*. National Academy of Sciences, Washington, DC, USA.
- Rifkin, A. B., A. Firpo, and D. R. Alonso. 1984. Coordinate induction of Cytochrome P-448 mediated mixed function oxidases and histopathologic changes produced acutely in chick

embryo liver by polychlorinated biphenyl congeners. *Toxicology and Applied Pharmacology* 72:343-354.

Robertson, G. J. 1997. Pair formation, mating system, and winter philopatry in harlequin ducks.

Dissertation, Simon Fraser University, Burnaby, British Columbia, Canada.

-----, and F. Cooke 1999. Winter philopatry in migratory waterfowl. *Auk* 116: 20-34.

-----, and R. I. Goudie. 1999. Harlequin Duck (*Histrionicus histrionicus*). In A. Poole and F.

Gill, editors. *The Birds of North America*, No. 466. *The Birds of North America*,

Philadelphia, Pennsylvania, USA.

Rosenberg, D H., and M. J. Petrula. 1998. Status of harlequin ducks in Prince William Sound,

Alaska after the Exxon Valdez oil spill, 1995-1997. Exxon Valdez Oil Spill Restoration

Project Final Report, Restoration Project 97427, Alaska Department of Fish and Game,

Division of Wildlife Conservation, Anchorage, Alaska, USA.

Rotella, J. J., D. W. Howerter, T. P. Sankowski, and J. H. Devries. 1993. Nesting effort by wild

mallards with 3 types of radio transmitters. *Journal of Wildlife Management* 57:690-695.

Sanzenbacher, P. M., and S. M. Haig. 2002. Residency and movement patterns of wintering

Dunlin in the Willamette Valley of Oregon. *Condor* 104: 271-280.

SAS Institute Inc. 1999. SAS/STAT Software, Version 8.0. SAS Institute, Cary, North

Carolina, USA.

Scribner, K. T., R. Lanctot, S. L. Talbot, B. J. Pierson, and K. Dickson. 1998. Genetic variation

among populations of Harlequin Ducks (*Histrionicus histrionicus*): are eastern

populations distinct? A final report to Region 5, US Fish and Wildlife Service.

- Seaman, D. E., J. J. Millspaugh, B. J. Kernohan, G. C. Brundige, K. J. Raedeke, and R. A. Gitzen. 1999. Effects of sample size on kernel home range estimates. *Journal of Wildlife Management* 63:739-747.
- Seber, G. A. F. 1965. A note on the multiple recapture census. *Biometrika* 52:249-259.
- Short, J. W., and M. M. Babcock. 1996. Prespill and postspill concentrations of hydrocarbons in mussels and sediments in Prince William Sound. *American Fisheries Society Symposium* 18:149-166.
- , M. R. Lindeberg, P. M. Harris, J. M. Maselko, J. J. Pela, and S. D. Rice. 2003. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. *Environmental Science and Technology*.
- Silverman, B. W. 1986. *Density estimation for statistics and data analysis*. First edition. Chapman and Hall, London U. K.
- Slatkin M. 1985. Rare alleles as indicators of gene flow. *Evolution* 39: 53-65.
- Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J. and Klenk, D. C. 1985. Measurement of protein using bicinchoninic acid. *Analytical Biochemistry* 150:76-85.
- Snyder, P. W., T. Kondratyuk, B. E. Ballachey, and J. Vanden Heuvael. 2002. CYP1A gene expression in sea otters (*Enhydra lutris*): a quantitative reverse transcriptase-polymerase chain reaction to measure CYP1A mRNA in peripheral blood mononuclear cells. Appendix BIO-02.1 *in* 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. Exxon Valdez Oil Spill Restoration Project Final Report (Restoration

Project 99025), U.S. Geological Survey, Alaska Biological Science Center, Anchorage, Alaska.

- Stegeman, J. J., R. L. Binder, and A. Orren. 1979. Hepatic and extrahepatic microsomal electron transport components and mixed-function oxygenases in the marine fish (*Stenotomus versicolor*). *Biochemical Pharmacology* 28:3431-3439.
- Stubblefield, W. A., G. A. Hancock, W. H. Ford, and R. K. Ringer. 1995. Acute and subchronic toxicity of naturally weathered Exxon Valdez crude oil in mallards and ferrets. *Environmental Toxicology and Chemistry* 14:1941-1950.
- Trust, K. A., D. Esler, B. R. Woodin, and J. J. Stegeman. 2000. Cytochrome P4501A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. *Marine Pollution Bulletin* 40:397-403.
- , A. Fairbrother, and M. J. Hooper. 1994. Effects of 7,12-dimethylbenz[a]anthracene on immune function and mixed-function oxygenase activity in the European starling. *Environmental Toxicology and Chemistry*. 13:821-830.
- Tsai, K., K. H. Pollock, and C. Brownie. 1999. Effects of violation of assumptions for survival analysis methods in radiotelemetry studies. *Journal of Wildlife Management* 63:1369-1375.
- Udevitz, M. S., B. E. Ballachey, and D. L. Bruden. 1996. A population model for sea otters in western Prince William Sound. Exxon Valdez oil spill restoration project final report (restoration project 93043-3), Natl. Biol. Serv., Anchorage, AK. 34pp.
- Vanden Heuval, J. P, G. C. Clark, M. C. Kohn, A. M. Tritscher, and W. F. Greenlee. 1994. Dioxin-responsive genes: examination of dose-response relationships using quantitative reverse transcriptase-polymerase chain reaction. *Cancer Research* 54:62-68.

- Vanden Heuval, J. P., G. C. Clark, C. L. Thompson, Z. McCoy, C. R. Miller, G. W. Lucier, and D. A. Bell. 1993. CYP1A mRNA levels as a human exposure biomarker: use of quantitative polymerase chain reaction to measure CYP1A expression in human peripheral blood lymphocytes. *Carcinogenesis* 14:2003-2006.
- Ward, D. H., and P. L. Flint. 1995. Effects of harness-attached transmitters on premigration and reproduction of brant. *Journal of Wildlife Management* 59:39-46.
- Warnock, S. E., and J. Y. Takekawa. 1995. Habitat preferences of wintering shorebirds in a temporally changing environment: Western sandpipers in the San Francisco Bay Estuary. *Auk* 112: 920-930.
- Watson, J. C. 1993. The effects of sea otter (*Enhydra lutris*) foraging on shallow rocky communities off Northwestern Vancouver Island, British Columbia. Thesis, University of California, Davis, California, USA.
- Weis, J. S., G. Smith, T. Zhou, C. Santiago-Bass, and P. Weis. 2001. Effects of contaminants on behavior: biochemical mechanisms and ecological consequences. *BioScience* 51:209-217.
- White, G. C. and K. P. Burnham. 1999. Program MARK -- survival estimation from populations of marked animals. *Bird Study* 46(Suppl):120-138.
- Williams, T. M., D. J. O'Connor, and S. W. Nielsen. 1995. The effects of oil on sea otters: histopathology, toxicology, and clinical history. Chapter one *in* T.M. Williams and R.W. Davis, editors. *Emergency Care and Rehabilitation of Oiled Sea Otters: A Guide for Oil Spills Involving Fur-bearing Marine Mammals*. University of Alaska Press, Fairbanks, Alaska, USA.

- Wolfe, D. A., M. M. Krahn, E. Casillas, S. Sol, T. A. Thompson, J. Lunz, and K. J. Scott. 1996. Toxicity of intertidal and subtidal sediments contaminated by the Exxon Valdez oil spill. American Fisheries Society Symposium 18:121-139.
- Wolfinger, R. 1993. Covariance structure in general mixed models. Communications in Statistics: Simulation and Computation 22:1079-1106.
- Woodin, B. R., R. M. Smolowitz. and J. J. Stegeman. 1997. Induction of Cytochrome P4501A in the intertidal fish (*Anoplarchus purpureus*) by Prudhoe Bay Crude Oil and environmental induction in fish from Prince William Sound. Environmental Science and Technology 31:1198-1205.