Exxon Valdez Oil Spill
Restoration Project Annual Report

Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators
Restoration Project 01423
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil Spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

James L. Bodkin

Brenda E. Ballachey

U.S. Geological Survey
Alaska Science Center
1011 E. Tudor Road
Anchorage, Alaska 99503

and

Daniel Esler

Centre for Wildlife Ecology
Simon Fraser University
5421 Robertson Road, RR1
Delta, British Columbia V4K 3N2 Canada

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Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators

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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 spill. This project supports an annual survey of sea otter abundance in Prince William Sound, collection of beach-cast sea otter carcasses for estimation of survival rates, population estimates from intensive surveys in an oiled and unoiled areas, and estimates of the density and sizes of green sea urchins from those same intensive study areas. Additional evaluation of sea otter health and cytochrome P450 1A induction was approved for 2001; this component was closely linked to Project 01534, which expanded biological sampling from sea otters to include liver tissues. Studies of harlequin duck survival, oil exposure and CYP1A induction, including both field studies and captive experiments at the Alaska SeaLife Center, were approved to begin in FY2000. In this report we present the results of the third year of sea otter fieldwork, and the first winter of the harlequin duck component.

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 EVOS 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 to understand the process of population change.

In July 2000, we estimated the western Prince William Sound sea otter population at 2,658 individuals (se=294). The previous comparable estimates for western Prince William Sound were 2,475 (se=381) in 1999 and 3,119 (se=494) in 1998. We estimated population sizes of 79 (se=20) at northern Knight Island and 659 (se=189) at Montague Island in 2001. At northern Knight Island, the mean estimated summer population size has remained unchanged since 1993 (mean=77, se=2). During this same period, we have seen a significant increasing trend in population size at Montague Island from about 300 in 1993 to more than 650 in 2001. From 1999-2001, we have collected 120 sea otter carcasses from beaches in western Prince William Sound; ages at death will be determined for these animals, and will be used in estimation of survival rates. In July 2001, 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 (CYP1A) induction continued to be higher in the oiled area, similar to results from 1996-98, indicating ongoing exposure to aromatic hydrocarbons.

For the larger area of western Prince William Sound, sea otter abundance has increased since 1993, indicating progress toward recovery of the EVOS injured sea otter population. However, around northern Knight Island, where sea otter mortality was highest, no increase has been seen, suggesting that recovery may not be occurring where oil spill effects were greatest. The elevated CYP1A values and liver pathologies observed at northern Knight Island indicate toxic exposure to hydrocarbons continues and is constraining recovery of sea otters.

During harlequin duck studies in FY2001 we conducted experiments with captive birds to evaluate behavioral and metabolic responses to oil ingestion. Also, we conducted field studies in which we measured CYP1A 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 CYP1A 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 some metabolic attributes varied with oil ingestion; food consumption was elevated in oiled birds relative to controls and daily energy expenditure showed a similar pattern, although with a high degree of variation. Behavior data did not vary by time of year (early, mid, and late winter) nor by oil ingestion treatment. We modified captive bird protocol for winter 2001-02 based on our findings during the first year; revised protocols are described in this document.

During field studies, we documented significantly higher CYP1A induction in oiled areas of Prince William Sound relative to unoiled. We also found point estimates of survival that were lower in oiled areas than unoiled, although variation around these estimates were high, as this was only the first of 3 years of data collection. All aspects of the harlequin duck study generated good quantities of data and will be critical for monitoring the progress, and understanding the process, of harlequin duck population recovery.

Key words: CYP1A, demography, *Enhydra lutris*, *Exxon Valdez*, harlequin ducks, *Histrionicus histrionicus*, oil spill, population status, Prince William Sound, sea otters.

Project data: Will be addressed in the Final Report.

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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). Concerns about nearshore recovery and restoration have resulted in a suite of studies sponsored by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC), including the Nearshore Vertebrate Predator project (NVP). Principal NVP findings include an apparent lack of recovery among sea otters and harlequin ducks, both invertebrate feeders in the nearshore ecosystem. Available evidence suggests that sea otters from the spill area incurred elevated rates of mortality (Monson et al. 2000), and their exposure to hydrocarbons was higher (Ballachey et al. 2001) through at least 1998. Additionally, we identified a common pattern among several sea otter prey species consistent with reduced predation, through increased proportions of large individuals where sea otters populations were reduced (Dean et al. 2000, Dean et al. 2002). Similarly, survival rates of harlequin ducks were lower in oiled areas of PWS relative to unoiled areas (Esler et al. 2000a), densities were lower on oiled areas than expected based on habitat attributes (Esler et al. 2000b), and hydrocarbon exposure was higher in oiled areas (Trust et al. 2000). These data, for both species, suggest that continuing effects of the EVOS may be constraining full population recovery. We have continued the components of previous research that were most effective and statistically powerful at identifying if, where, and how recovery may be constrained among EVOS affected sea otters and harlequin duck populations in the nearshore. We address the need to refine and focus efforts on study components that provide the greatest resolution to ecosystem function.

Sea Otters:

We focus on sea otters (*Enhydra lutris*) through aerial surveys, collections of beach-cast carcasses and biological sampling to monitor health and biomarkers of oil exposure. In prior years, we have also examined ecological interactions between sea otters and green urchins, a preferred invertebrate prey. We selected sea otters because they were (1) injured by the oil spill and continue to show evidence for lack of a full recovery, (2) are presumably reflective of 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. For sea otters, we are monitoring both the patterns of population demographics, individual metrics of health and oil exposure, and the processes underlying change in the nearshore system.

Sea otter populations in western Prince William Sound (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. Surveys to date (1993-2000) have shown a significant increasing trend in the WPWS sea otter population, averaging about 5% per year since 1993 (power > 0.80 to detect a 1% annual change in 5 annual WPWS surveys). However, the northern Knight Island area numbers remain below pre-spill estimates, and have not shown a

significant increasing trend (Bodkin et al. in press), although our power to detect change is lower for these surveys.

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 2001). 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 a maximum of 11 sea otters (mean = 3) in this same location. Through 2001, sea otter abundance at northern Knight Island remained at about 50% of the estimated pre-spill abundance (Dean et al. 2000; USGS unpub. data). Constraints to recovery most likely are demographic, either through reduced survival among residents, or higher emigration from the oiled area (Holland-Bartels 2001). 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). 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. 2001). 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.

This project builds on previous EVOS research to develop a statistically sensitive and cost-effective program that will continue to track the WPWS sea otter population and nearshore ecosystem recovery through three avenues. First, continued surveys of sea otter abundance and mortality at appropriate intervals will allow population monitoring and testing of the predictions of previously developed EVOS Trustee Council sea otter population models (Udevitz et al. 1996, Monson et al. 2000). Further, the return of sea otter abundance to estimated pre-spill levels could define a recovery endpoint. Second, monitoring abundance and size of a key invertebrate species may allow an independent assessment of sea otter recovery through predicted responses in a prey population. Third, monitoring oil exposure and health of individual sea otters will allow us to determine a point at which lingering oil is no longer a factor in limiting recovery of the population.

Sea urchins:

The status of sea otter recovery has been assessed, in part, by conducting aerial surveys of sea otter abundance in WPWS, comparing pre- and post-spill estimates of abundance, and comparing estimates of abundance in oiled and unoiled parts of the Sound. While these data provide a foundation for assessment of recovery status, there were few pre-spill data and there were known biases in pre-spill estimates that precluded using pre- vs. post-spill comparisons in making a definitive quantitative assessment of the extent of recovery. Furthermore, recovery status could not be based solely on post-spill comparisons of oiled and unoiled areas because there are known differences in habitat between these areas, and it is uncertain whether sea otters in oiled areas could ever achieve population levels observed in unoiled parts of the Sound. As a result, in the NVP

study, and subsequently in this study, we examine prey populations as an ancillary means of assessing recovery.

Sea otters are considered keystone predators within coastal marine systems of the North Pacific that 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 that are a preferred prey. Observations of sea urchins and kelp in nearby areas with and without sea otters (Estes & Palmisano 1974, Estes et al. 1978, Duggins 1980, Breen et al. 1982, Estes & Duggins 1995), and in a given area 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 our previous 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 abundance in oiled area that persisted for nearly a decade, there was little evidence of a strong response by sea urchins to the reduction in sea otters. 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 Prince William Sound, the number of surviving sea otters may 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 Prince William Sound, 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 FY01 (Project 00423), 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 in northern Knight Island and sea otters remained about ten times more abundant at Montague Island 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. The sea urchin component was not included in the scope of work for FY01, and thus results for that component are not reported on herein.

In summary, continued monitoring of distribution and abundance, mortality, prey populations and health and oil exposure of sea otters in WPWS will be valuable in (1) providing insight into potential demographic and toxicological constraints to recovery which may improve future recovery models, (2) documenting actual recovery time for the nearshore system including sea otters, and (3) providing long-term population trend data which may be used in assessing initial damage and subsequent recovery of sea otter populations in the event of future oil spills.

Harlequin ducks:

Harlequin ducks were, and remain, particularly vulnerable to deleterious effects of the oil spill. Much of the oil from the *Exxon Valdez* 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, any perturbation (e.g., an oil spill) 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.

Also, among ducks, sea duck life histories 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 follow this pattern, having high fidelity to molting and wintering sites (Robertson 1997). High site fidelity could result in vulnerability to population effects because: (1) if residual oil spill damages exist, birds from oiled areas are vulnerable to spill effects as they return to those areas annually (i.e., these birds are affected disproportionately and are subject to cumulative effects), and (2) if dispersal and movements among areas are limited, recovery of groups of birds in oiled areas can occur only through demographic processes specific to that group (i.e., numbers are not enhanced through immigration from other areas). 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.

Vulnerability to oil spill effects is 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 from immediately post-spill through 1995 (Babcock et al. 1996, Boehm et al. 1995, Short and Babcock 1996, Wolfe et al. 1996).

Evidence from recent 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). Also, differences in CYP1A induction were detected between populations from oiled and unoiled areas (Trust et al. 2000), although this was measured on different birds than those for which survival data were collected. Further, body mass during winter showed a slight, negative relationship with CYP1A level.

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 these speculated links between oil exposure and survival probabilities, and subsequently population trends. This project is designed to explore these potential mechanisms constraining population recovery through field studies of winter survival and CYP1A induction and captive studies of metabolic, behavioral and CYP1A responses to controlled oil exposure. Further, because of their susceptibility to spill effects and high site fidelity, harlequin ducks are an ideal species for monitoring recovery of the nearshore environment.

PROJECT OBJECTIVES

Sea otters:

A. Estimate and compare sea otter abundance and population trends over time between oiled and unoiled areas within WPWS and over all of WPWS.

B. Collect age at death data from sea otters 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 (this objective was for FY00).

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 the metabolic, 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 otters at two geographical scales, WPWS and an oiled and unoiled area within WPWS. 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 2). 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 were done at the Alaska SeaLife Center in Seward.

METHODS

Sea otters:

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 allow 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 which initiated the ISU.

Replicate surveys in the intensive oiled and unoiled areas, using the same techniques described in Bodkin and Udevitz (1999) were conducted to gain precision in estimates for these two areas.

Mortality. Systematic beach surveys were conducted in April or May along shorelines in WPWS soon after snowmelt, prior to the regrowth of beach grasses, which 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 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 pre-molar) removed for age analysis. For age estimation, several longitudinal sections of the tooth were decalcified for cementum annuli readings (Bodkin et al. 1997). Matson's Laboratory (Box 308, Milltown, MT 59851) sectioned and aged all teeth.

Capture and biosampling. In summer 2001, sea otters were captured in western PWS, at Knight (oiled area) and Montague (unoiled area) islands, in the same areas as used in previous studies (NVP, 1996-98). Capture and handling methods were similar to those employed previously (Bodkin et al. in press). Sea otters were sedated, body measurements taken, a tooth collected for age determination, and a blood sample taken by jugular venipuncture. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood and frozen in liquid nitrogen for later CYP1A 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). Two biopsies were frozen immediately in liquid nitrogen and a third biopsy fixed in neutral buffered formalin. Following reversal, sea otters were released in the same vicinity as captured. Whole blood was shipped to Quest Laboratories in Portland, OR for hematology assays; serum samples were frozen and later sent as a batch to Quest for clinical chemistry assays.

In the NVP study, the RT-PCR assay (quantitative reverse transcriptase PCR assay; Vanden Heuvel et al. 1993, 1994; Snyder et al. 2001) 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 per 100 ng of total RNA. This 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).

Harlequin ducks:

Captive Studies – General. From 8 – 12 September 2000, harlequin ducks for studies at the Alaska SeaLife Center were captured during wing molt from unoiled Montague Island using a standard drive trap. Birds were banded with unique USFWS bands, body mass was recorded, and blood was sampled following the protocol below. We determined sex based on plumage characteristics and age class by bursal probing (Mather and Esler 1999). Only females in the after-second-year age class were used in captive experiments. Following capture, 25 birds were flown to the Alaska SeaLife Center in Seward and housed in outdoor pens to expose them to natural climatic and photoperiod conditions. Captured individuals were held for an adjustment period prior to any experimental manipulation or dosing. Throughout the experiment birds were fed ad libitum, but only during daylight hours. After offering the birds an array of food options, Atlantic silversides, which are a high energy density forage fish, proved to be the preferred diet. Supplemental vitamins also were given. Weights of food, by dosing treatment, were measured every day prior to delivery and upon retrieval from the pen to estimate food consumption.

During the first winter, birds were captured and dosed with oil twice weekly, from 16 October 2000 through 22 February 2001. Dosing was designed to simulate long-term, intermittent exposure, which is likely similar to exposure experienced by wild birds. Three levels of oil exposure occurred: a control group (n=7), a group receiving 1 ml oil/kg body mass on each dosing day (n=7), and a group receiving 10 ml/kg (n=7). Birds not assigned to treatments were released. Unused birds were not randomly determined; we released those that were thriving least in captivity, under the premise that using birds that were not acclimating well to captivity would introduce unnecessary variation into our experiments. Doses were based on mass of the ducks on the previous capture event. Ducks were assigned to treatments randomly and treatments were associated with each of three pens, again assigned randomly. The order of handling by dosing treatment was alternated systematically at each handling event, following a randomly determined order for the first event. Treatment birds were dosed via oral gavage, using a syringe and a gavage tube. The appropriate dose was determined by weighing the oil, after accounting for the specific gravity of the batch of weathered crude. The syringe and gavage tube were placed on a scale, the scale tared, and then the appropriate mass of oil drawn into the syringe. Control birds had a gavage tube placed in their esophagus to simulate dosing. The oil used in the study was Prudhoe Bay crude oil, weathered by continuous mixing with seawater for 10 days. The weathered oil was separated from the water prior to administration.

Birds were rounded up prior to daylight on handling days. Mass of each bird was measured at each capture event. Following dosing, birds were held for 1 hour in individual holding pens with screen bottoms to accommodate short-term passage of oil.

Captive Studies – CYP1A Induction. Induction of CYP1A was indicated by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity following methods detailed in Trust et al. (2000). This method required surgical biopsy of < 1 g liver sample. Surgeries were conducted on 23 and 24 February 2001 by veterinarian Dan Mulcahy, following standard procedures. Following biopsy, liver samples were placed individually in cryogenic vials and frozen in liquid nitrogen. Samples were shipped to Woods Hole for subsequent analysis.

Captive Studies – Metabolism. Metabolic consequences of oil exposure were quantified using two approaches: doubly-labeled water to estimate daily energy expenditure (DEE) and oxygen consumption to estimate basal metabolic rate (BMR). DEE estimation using doubly-labeled water requires injection of water with both the oxygen and water isotopically-labeled. As the hydrogen is lost only through water and oxygen through both water loss and carbon dioxide production, the difference in turnover rates between marked hydrogen and oxygen can be used to estimate metabolism. DEE was estimated once, during February. Natural abundances of ^{2}H and ^{18}O, the isotopes used in this experiment, were determined by analysis of 15µl of blood collected from each bird. Also, we conducted a pre-experiment to determine water flux prior to DEE estimation, to optimize doubly-labeled water experimentation, using 6 birds (2 from each treatment, selected randomly). To estimate water flux we injected 1 g of deuterium ($^{2}H_2O$, 99.8 AP enrichment), waited 1.5 hours for equilibration, and took 6 15 µl blood samples in 25 µl unheparinized capillary tubes from each bird. Capillary tubes were flame-sealed and refrigerated. After 24 hours, another set of samples was taken from each bird. All samples were stored at 5°C and shipped to the Centre for Isotope Research in Groningen, The Netherlands. For DEE determination, 0.65 g (amount based on water flux pre-experiment) of labeled water with 60 atom percent ^{18}O and 30 atom percent ^{2}H was injected intramuscularly in each bird. After an equilibration time of 1.5 hours a blood sample was taken and birds were released into their pens. After 48 hours birds were recaptured and blood samples taken immediately. All samples were stored at 5°C and shipped to the Centre for Isotope Research.

BMR was measured on handling days throughout the length of the experiment in an open-flow respirometry system to estimate oxygen consumption. BMR is the metabolic rate of animals at rest, in their thermal neutral zone, and in a post-absorbtive state (i.e., not actively digesting). After dosing commenced, 3 birds (1 from each treatment) had their BMR measured on each capture event, following a random order within treatments without replacement. Birds were left in the chamber for at least 60 minutes. Estimates of metabolism will be made from data collected after oxygen consumption stabilized. Following measurement, birds were released to their pen (no later than mid-day). Respirometry measurements were not taken on days when blood samples were drawn.

For respirometry measurements, birds were placed in a plexiglass metabolic chamber. The chamber was covered with a cloth in a quiet environment. Air was drawn through, in order, drierite, ascarite and, again, drierite columns to remove carbon dioxide and water before air entered the metabolic chamber. The air from the chamber was drawn through another set of scrubbers before entering the O2 analyzer. Air was pulled through the chamber by a veristatic pump at a flow rate of 1800 ml/min. A subset of air (100 ml/min) was drawn through the oxygen analyzer to determine changes in % O2 content in effluent air. The analyzer was calibrated to atmospheric oxygen (20.95%) before each bird. Chamber temperature was recorded for each trial.

Captive Studies – Behavior. Captive harlequin duck behavior was quantified using focal-animal sampling. Continuous observations of focal individuals were recorded during 30 minute sessions, the reported duration of sea duck activity bouts (Goudie and Ankney 1986). The Observer software was used to record the start and end times of each behavior. Behaviors were categorized according to Adams at al. (2000) and included feeding (including handling), locomotion (including walking, swimming, flying and diving), maintenance (including preening, scratching, stretching, wing flapping, and splash bathing), rest (including sleeping, loafing, and resting) and social (agonistic). Captive birds were identified by uniquely coded tarsus bands. Focal individuals and observation start times were randomly selected and each individual was sampled equally, i.e., there was no resampling until all birds had been sampled within a round. On each sampling day, one bird from each dosage group was observed during each diurnal period (morning, afternoon, evening). Observation start times began 30 minutes prior to sunrise and continued until 30 minutes after sunset and were adjusted to compensate for changes in photoperiod (Fischer 1998). Behavior observations were not conducted on days when birds are dosed or metabolic measurements were taken. Temperature, wind direction, wind speed, and day length were recorded at the beginning of each observation session. Behavior observations were conducted from the arrival of captive birds until the end of dosing.

Field Studies. The key data for field studies of harlequin ducks are CYP1A and survival data, which will allow for explicit tests of the hypothesis that mortality and oil exposure are related and, also, allow monitoring of these data, which were demonstrably differed between areas in NVP studies. We collected survival and exposure data from 50 birds by capturing them during the first 3 weeks of November 2000, conducting surgeries to both implant transmitters and biopsy livers, and monitoring subsequent winter survival. We also collected feathers and plumage swabs for analyses of external hydrocarbons. Five additional birds were outfitted with subcutaneous transmitters. Data collection, sample analysis, and data analysis follow protocols used during NVP studies (Esler et al. 2000a, Trust et al. 2000); thus, results from this work will be directly comparable.

We used floating mist nets (Kaiser et al. 1995) to catch flying birds in oiled (Knight Island, Green Island, Crafton Island) and unoiled (Montague Island) study areas. Captured birds were banded with uniquely coded USFWS bands, aged by bursal probing (Mather and Esler 1999), and sexed by plumage characteristics. We biopsied and radioed

females of all age classes. Age parameters will be included in all analyses to account for any survival differences due to age.

We conducted aerial radio telemetry flights from the capture and marking period through the end of March. Mortality status of birds indicated as dead was confirmed by detection of signals from upland habitats, which are not used by harlequin ducks during nonbreeding periods.

RESULTS

Sea otters:

Aerial Surveys. The previous WPWS aerial survey of sea otters occurred in July 2000 and we estimated a sea otter population size of 2658 (se=294) (Figure 3). No survey of WPWS was conducted in 2001. In July 2001, we estimated population sizes of 79 (se=20) at our oiled intensive Northern Knight Island study site and 659 (se=189) at our unoiled intensive Montague Island study site (Figure 4).

Mortality. In April 2001, we located the remains of 34 beach cast sea otters in the oil spill area of WPWS. We also collected an additional 86 beach cast animals in the same areas in April 1999 and 2000. Age estimates have been obtained for collections from 1999 & 2000, and teeth from the 2001 carcass collections have been submitted for age estimates.

Capture and Biosampling. In July 2001, we captured and sampled 16 sea otters (15 adults and 1 pup) at Knight Island (oiled area), and 18 sea otters (15 adults and 3 pups) at Montague Island (unoiled area). All animals appeared healthy at capture. Blood samples from adult animals were submitted for hematology and clinical chemistry. Blood from all 34 animals was processed to isolate PBMC, which were assayed for CYP1A. Endoscopic procedures were done on the adult otters to biopsy livers for CYP1A assays and histopathology (see report for Restoration Project 01534). During the endoscopies, livers were viewed on a TV monitor, and any changes or lesions present could be observed. The gross appearance of livers of sea otters in the unoiled area was largely normal. However, two sea otters from the oiled area (of 15 examined) showed marked liver changes, including swollen margins, irregular surfaces, and abnormally dark, mottled color. Histopathological examination of these samples showed further abnormalities, which were present at a higher incidence and greater severity in the oiled area. Histopathology was covered in the report for Restoration Project 01534, and selected results are presented in Appendix 1 of this report.

Levels of CYP1A in PBMC were elevated in the oiled area (Figure 5). The oiled area mean was 7.9 (SD = 39.5; units are molecules of CYP1A mRNA per 100 ng RNA), compared to a mean of 2.4 (SD = 8.5) for the unoiled area; this difference was significant (t-test, $t = -3.19$, $P < 0.005$).

The most notable difference observed in hematology and serum chemistry results was for gamma glutamyl transferase (GGT), a serum enzyme indicative of liver function. 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. This difference was not significant ($P < 0.11$). However, there were three sea otters from the oiled area with elevated GGT levels (> 20 IU/L; Figure 6), a pattern that is consistent with previous findings for GGT of sea otters from oiled areas.

Harlequin ducks:

Captive Studies – General. Seventy-seven harlequin ducks were captured during molt drives in September 2000, of which 25 females older than second-year were sent, via floatplane, to the Alaska SeaLife Center. During the first year of the study, several health or husbandry issues arose, including bumblefoot, vitamin E toxicosis, feather damage, and mink predation. These were addressed as they occurred, and sample size attrition did not unduly affect the research; at the end of the experiment 17 birds were in the sample, 5 each in the control and low dose groups and 7 in the high dose group. Of the 25 birds originally brought into captivity, 1 escaped, 1 was killed by a mink, 1 died shortly after arrival from malnutrition, 2 died from vitamin E toxicosis, 1 died from an impacted intestine, and 2 were released at Montague Island prior to the end of the experiment. Remaining birds were released in April 2001. During September 2001, 78 harlequin ducks were captured. Again, 25 after-second-year females were sent to the Alaska SeaLife Center. During the second winter 3 birds died prior to the beginning of experiments, one of septicemia, one of pasturella infection, and one of unknown causes. Two birds died during the experiment of viral infections, and several others displayed sublethal symptoms. Because of the virus, the bird will not be released back into PWS due to risk of infecting wild populations. It is uncertain whether the virus exists in wild populations. Two of the most-seriously infected birds were euthanized following oiling experiments in February 2002. The remaining birds will be used in experiments to investigate effects of diet on circulating levels of vitamins E and K (in light of the coagulopathy observed in the captive flock), to gain more insight into the virus, and to document variation in stress hormones. Following these experiments the birds will be euthanized.

Captive Studies – Cytochrome P450 1A Induction. CYP1A induction was measured by EROD activity at the culmination of the oil ingestion experimentation for winter 2000-01. Average (± SE) EROD activity (pmol/min/mg) for control birds (n = 5) was 86.7 (± 34.1). Values for the 1 ml/kg (n = 5) and 10 ml/kg (n = 7) treatments were highly variable, with averages of 1013.3 (± 280.3) and 364.2 (± 132.5), respectively, and widely overlapping confidence intervals. This may have been the result of passage of oil within an hour of dosing, particularly for the high (10 ml/kg) treatment, resulting in the variation among individuals and lack of difference between oil treatments. When combined, the average across the 1 ml/kg and 10 ml/kg treatments was 634.6 (± 163.7), which was dramatically different from control birds (Fig. 7).

Captive Studies – Metabolism. Basal metabolic rates were quantified over n = 27, 28, and 27 sessions for the 0, 1, and 10 ml/kg oil ingestion treatments, respectively, during

winter 2000-01. Metabolic rates (kJ/hr/kg) were similar among treatments (Fig. 8) with averages (± SE) of 26.8 (± 0.7), 27.8 (± 0.6), and 25.4 (± 0.6) for the 0, 1, and 10 ml/kg oil ingestion treatments, respectively.

Food consumption was notably higher for birds from both oil exposed treatments than unexposed control birds (Fig. 9). Masses were similar among treatments, so either (1) total energy expenditure was higher for oil exposed birds or (2) assimilation efficiency by the gut is lower for oil exposed birds or (3) a combination of both. In any case, these increased costs may be untenable under the constraints imposed during mid-winter.

Daily energy expenditure (DEE) data suggested a trend similar to food consumption (Fig. 10), with average (± SE) DEE (kJ/day) higher in the 1 ml/kg (631 ± 53) and 10ml/kg (650 ± 37) treatments than the control (594 ± 10). However, sample sizes were small (n = 5, 7, and 5, respectively) and data were variable, leading to widely overlapping confidence intervals. Because this is important information to quantify with precision, we added more DEE work for winter 2001-02 (see below).

Captive Studies – Behavior. Variation in behavior was considered among treatments and stage of winter (i.e., early, middle, and late). Behavior was similar across seasons and treatments (Table 11). Feeding behavior is the most important category, given the assumption that wild harlequin ducks are constrained in the amount of time that they can feed and, hence, even small changes in feeding behavior in response to oil exposure may be untenable in the wild. We found little evidence of variation in feeding behavior across treatments (Fig. 11). However, time spent feeding in our experiments was very low (1.5 – 1.9%; Table 1). We modified protocol for feeding during winter 2001-02, with the expectation of increasing time spent feeding and, thus, increasing our ability to detect subtle variations in feeding time among treatments.

Field Studies: During winter 2000-01 capture activities, 169 harlequin ducks were handled. Of these, 50 females (25 in each area) underwent surgery for radio transmitter implantation and liver biopsy for P450 activity. Seventeen of the liver biopsy samples thawed when a liquid nitrogen container failed; fortunately, none of the samples from oiled areas were compromised, as these are the data that are most important for tracking oil exposure over time and for quantifying the relationship between oil exposure and survival. External hydrocarbon samples were collected from all 50 birds that underwent surgery; for 35 of these we took paired samples of feathers to compare swab and feather assays of oil exposure. Also, we outfitted 5 females (1 in unoiled areas and 4 in oiled) with subcutaneous radios to test the methodology and bolster sample sizes for survival estimation. A similar protocol for field data were followed successfully during winter 2001-02. Telemetry data collection for this aspect of the project recently ended (31 March 2002) and EROD analyses for P450 induction have not been completed. Thus, FY2002 results can not be presented here.

In winter 2000-01, average (± SE) EROD activity for harlequin ducks captured in unoiled and oiled areas was 173.6 (± 54.7) and 794.0 (± 200.5), respectively. These result in nonoverlapping confidence intervals (Fig. 12) and correspond closely to EROD activity

measured in oiled and unoiled birds in captivity (Fig. 7). Analyses of survival data from oiled and unoiled areas yielded point estimates of 0.91 in unoiled areas and 0.83 in oiled areas; these had broad and overlapping confidence intervals (Fig. 13), reflecting low sample sizes that can be obtained for single years. Survival data were collected during winter 2001-02 and are scheduled to be collected during winter 2002-03.

DISCUSSION

Sea otters:

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. In 1994 and 1999, our estimates of the entire PWS sea otter population were similar, at 9,092 in 1994 (Bodkin and Udevitz 1999) and 8,355 in 1999 (J.L. Bodkin unpublished data), with broadly overlapping confidence intervals. Although changes in abundance may be evident at smaller geographic scales within PWS, our data suggest a relatively stable population of sea otters within the entire PWS region.

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 principally oiled areas, we have observed a significant trend of increasing sea otter abundance between 1993 and 2000 (Fig. 3). The lowest estimate was obtained in 1993 (2,054) and the highest in 1998 (3,119). The average annual rate of growth during this period is 0.04, about 1/2 the long-term growth rate observed in PWS (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 may reflect residual density dependent effects on food (Fukuyama et al. 2000) or space availability, or possibly residual spill effects such as continued low-level oil exposure to sea otters and/or their prey.

At Montague Island, we have seen the mean estimated summer population size significantly increase from about 300 in 1993 to more than 650 in 2001 (average annual increase = 11%, adj. $R^2 = 0.72$, $P < 0.002$). During this same period at northern Knight Island, sea otter abundance has remained unchanged (mean = 77, se = 2), and is about half the estimated pre-spill abundance of about 150 (Dean et al. 2000). 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 at Knight have exhibited elevated levels of the CYP1A biomarker, compared to Montague Island (Ballachey et al. 2001; this report). 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).

Elevated sea otter mortality in the years following the *Exxon Valdez* oil spill appears to be contributing to the lack of recovery in WPWS. 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 (Figures 3 & 4) 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. 115 pre- and 384 post-spill ages, based on carcasses collected through 1998). We anticipate having a cumulative sample size of about 150 ages at death for the years 1999-2002, and incorporating these data into the Monson et al. (2000) model in summer 2002, to estimate recent sea otter survival rates in WPWS.

Elevated levels of the CYP1A biomarker were found in 6 predator species during the NVP study (Ballachey et al. 2001, 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 difference was not statistically significant. One objective in resampling the oiled area population in the present study was to see if there had been further decline, and indeed it appears that exposure is decreasing, based on the mean and range of CYP1A values obtained in 2001. However, the sample size in 2001 (n = 15 per area) was relatively small. Even with a lower mean value, the area difference is still very highly significant in 2001, indicating that exposure to lingering hydrocarbons continues. This finding is consistent with results on harlequin ducks and, when considered together with the GGT and liver pathology results, which demonstrate severe adverse effects on health of sea otters in the oiled area, strongly implicates oil exposure as a factor limiting recovery of the sea otters in the most heavily oiled areas of western PWS. An additional capture effort is planned for summer 2002, for further examination and collection of liver tissues and blood samples.

Based on the data collected over the past decade, in conjunction with liver observations from 2001, it is apparent that GGT is a marker of toxic damage to liver tissues in sea otters. Although the difference in GGT between areas in 2001 was not statistically significant, the data nevertheless conform to a pattern observed in earlier studies (1992, 1996-98; Ballachey et al. 2001; in review), 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 again in 2001, the proportion

of otters with high GGT values is about 0.15, and no extremely high GGT values (> 100 IU/L) were detected. This decline in GGT values is consistent with declines for CYP1A, and further suggests that exposure is oil diminishing, although certainly not gone, by 2001.

Harlequin Ducks:

Data collected during winter 2000-01 are leading towards successful completion of the harlequin duck portion of the project, and provide preliminary insights into harlequin duck recovery status and potential constraints to population recovery.

Field Studies: Field studies indicated that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil through at least 2000; results from November 2001 are pending lab analysis. These findings, along with parallel findings for sea otters, reinforce one of the surprising findings from NVP studies, namely that exposure to oil following a spill and subsequent effects occur over a duration far exceeding the conventional paradigm of a year or two. The survival data collected during the first winter of harlequin studies under this project are consistent with that conclusion, with point estimates of survival lower in oiled areas than unoiled areas, similar to findings from NVP studies. However, 3 winters of data collection are necessary to have power to draw strong conclusions about variation in survival by area. Also, we will analyze survival data in relation to CYP1A induction of individuals, to more closely consider the link between oil exposure and demography.

Captive Studies: As predicted, CYP1A differed between oiled and unoiled treatments in captivity, although we didn't observe differences between 1 ml/kg and 10 ml/kg treatments, perhaps because of lack of metabolism and assimilation of oil, particularly in the high dose treatment. However, our oiling regime resulted in CYP1A induction that was very similar to that observed in birds captured and radiomarked during field studies (Figs. 7 and 12), leading us to believe that our captive work results in similar physiological responses to those wild birds are experiencing and thus our captive work will have strong inference for understanding mechanisms constraining harlequin duck population recovery in PWS.

Basal metabolic rate data from the first winter of experimental work at the Alaska Sealife Center suggested that there were no differences between oil ingestion treatments. This would be interpreted as evidence that there are not strong energetic costs of metabolizing and detoxifying oil. However, in the first winter, respirometry was conducted 3-4 days following oil exposure, which may have been beyond the period within which metabolic responses would occur. Modifications to protocol for winter 2001-02 (see below) resulted in respirometry data collection closer in time (1-2 days) following oil ingestion; we will examine these data to consider effects of time since oiling, as well as treatment dose, on basal metabolic rates.

Food consumption and DEE suggest that there may be significant metabolic consequences for birds exposed to oil. Given the speculation that harlequin ducks are unable to accommodate even small increases in metabolic costs in the wild, especially

during mid-winter, these findings have important implications for understanding population recovery. This aspect of the work received extra attention during winter 2001-02 (see below), which we expect to shed additional light on this mechanism linking oil exposure and demography.

Time spent feeding was low and similar among treatments. However, given increases in food consumption and suggestions of a similar pattern in DEE in oiled treatments, one would expect to see increased feeding by oiled birds. Perhaps birds varied the intensity of food consumption within foraging bouts, which would have been undetectable using observations. Alternatively, the subtle increase in food consumption (approximately 10%) by oiled birds may be difficult to detect with the statistical power resulting from the observation approach and relatively low proportions of time spent foraging. We modified feeding protocol for winter 2001-02 (see below) to attempt to increase time spent foraging.

Changes in Captive Studies for Winter 2001-02: Although the captive studies during the second winter occurred during FY2002, and hence outside the scope of this report, we describe modifications to experimental protocol. Generally, the study design closely followed that of the first year, with a few modifications to allow clearer insight into the study questions based on the findings from the first year. During the first winter, we found that birds passed a significant proportion of their oil dose within an hour of dosing, particularly for the high dose treatment. We also found that low and high dose treatments were similar in most of our measured endpoints, including cytochrome P4501A induction. Thus, for the second winter's experiments, we attempted to increase assimilation of oil to more closely reflect the dose delivered by: (1) dosing in the evening after birds had fed all day, under the assumption that a full gut would slow passage rates and increase assimilation; and (2) increasing the frequency and lowering the amount of dosing. We dosed birds every other day (instead of twice weekly) using an amount of oil that would result in consistent doses between year 1 and year 2 over a 2 week period.

Also, feeding behavior during the first winter constituted a very small proportion of the duck's daily time budget (Fig. 11). We were concerned that (1) this would make it more difficult to detect subtle differences in time spent feeding among treatments, and (2) this was a poor simulation of the time spent feeding in the wild, which might influence our inference. Therefore, we changed food delivery by presenting the food in the pools rather than in trays, so that the ducks had to dive to get food, which we speculated would increase time spent foraging by increasing search time and, also, increasing energetic costs that in turn would require more food intake. We also fed primarily Antarctic krill, rather than the Atlantic silversides used during the first winter. Krill have a lower energy density than silversides and are smaller, which we assumed would also contribute to increasing the proportion of time spent foraging. Due to the changes in feeding protocol, behavior data collection was modified. Feeding behavior was divided into searching, diving and handling. Also, pacing and passive swimming (resting in the water) were added as behavioral categories to allow more accurate recording of behaviors unique to captivity.

Because of differences in our dosing regime, timing of respirometry relative to dosing changed between year 1 (3 to 4 days after dosing) and year 2 (1 to 2 days after dosing). This will allow us to consider time since dosing as a covariate explaining variation in BMR.

From the first winter's studies, we detected hints of increased DEE in oiled treatments relative to controls, but high variability within all treatments (Fig. 10). During the second winter we conducted 2 rounds of doubly-labeled water work to increase sample sizes and, hence, improve our statistical power to detect effects of oil ingestion on energetics.

During the second winter, we also added new experiments to evaluate effects of external oiling on our study endpoints. Preliminary results of efforts to quantify residual oil in Prince William Sound have encountered oil at a higher frequency than expected (NOAA, unpubl. data). The persistence of oil in intertidal areas raises concerns that continued external exposure to oil is occurring in wild harlequin ducks and may be related to reduced overwinter survival. Previous work with waterfowl has found that external oil increases heat production (Jenssen 1994). The effects of external oiling vary with species and harlequin ducks are particularly at risk given their reliance on diving to obtain food, their small body mass, and low thermal conductance. No previous work has addressed the basal metabolic, daily energy expenditure, and behavioral effects of external oiling, particularly in comparison to the effects of oil ingestion on these same parameters.

We exposed birds externally to three levels of weathered Prudhoe Bay crude oil: 1 ml, 2.5 ml and 5 ml. Birds were randomly selected for inclusion in each treatment, with the constraint that equal numbers in each external treatment were drawn from each of the 3 internal oil dosing groups. In other words, 2 birds from each of the 3 ingestion treatments were in each of the 3 external oil treatments, for a total of 18 birds used in the external oil experiment. Exposure levels were based on the potential range of exposure that may occur in the wild from low-level release of residual oil. Similar levels of exposure have been used in previous studies and will permit comparison of results. Each bird was exposed one time. Exposure occurred while the bird was at rest in a container containing 10 l of seawater. Oil was injected into the container through a tube below the surface of the water using a syringe to create a film of oil on the water's surface. The bird was held in the container for 20 minutes before oil was introduced and remained in the container until most of the oil was absorbed by its plumage (no more than forty minutes). The container was covered and kept in a quiet environment during exposure. Oil remaining in the container after the bird was removed was wiped with an oil absorbent cloth, and will be extracted and quantified in the lab. After exposure the bird was placed in the enclosure on the ODL. Collection of behavioral and physiological data occurred over the next week, following the protocol described for the ingestion experiments.

A new aspect of external oil experiments was respirometry in water. This was important because thermoregulatory effects of plumage oiling may be dramatically higher in water than air. Respirometry on water was done with fresh seawater (6 C) placed into a modified cooler. Air temperatures for all respirometry work, both air and water trials, was between 0 and 5 C. All birds were cleaned seven days after oil exposure. Cleaning

was done by repeated washing in a 2% Dawn detergent solution as described in *Protocol for the Care of Oil-affected Birds* (Univ. California Davis).

CONCLUSIONS

Results of aerial surveys of sea otter abundance have identified a significant increase of about 600 animals since 1993 in the oiled portions of WPWS. However, at Knight Island where oiling and sea otter mortality were highest, we have detected no similar increases, suggesting recovery has been delayed for more than a decade. The overall PWS sea otter population appears stable at about 8-9,000 since 1994. Recent findings of substantial quantities of subsurface oil in intertidal areas of western PWS (J. Rice, NOAA-ABL, pers. comm.), and adverse effects of residual oil on the growth and survival of clams (Fukuyama et al. 2001), strongly support the role of oil in delaying recovery of the nearshore marine community, at least at northern Knight Island. Our findings of relatively low mortality rates, even for sea otters born after 1989 (Monson et al. 2000), are consistent with our observations of elevated biomarkers and liver alterations in sea otters from the oiled area. In concert, these results strongly implicate continuing exposure to lingering oil as a primary factor limiting recovery of the sea otters. Continuing studies scheduled for 2002, and potential studies proposed for 2003, will further clarify the linkages between presence of lingering oil on shorelines, bioavailability of that oil, exposure and effects on health of individual sea otters, and recovery of the otter population.

Harlequin duck captive and field studies are proceeding successfully, and large quantities of data have been collected in all aspects of the project. These data will result in better understanding of constraints to harlequin duck population recovery in PWS. Preliminary data strongly indicate that exposure to *Exxon Valdez* oil continued in oiled areas of PWS through 2000, based on cytochrome P4501A induction. The survival analysis requires 3 years of data collection to adequately address survival differences between oiled and unoiled areas; however, the pattern of reduced survival in oiled areas observed during NVP studies (Esler et al. 2000a) closely matches the point estimates of survival from winter 2000-2001. If this pattern continues over the subsequent two winters, this would constitute strong evidence that demographic effects of the spill continue and population recovery will be further delayed. Data from captive studies from the second winter need to be compiled with first year results and rigorously analyzed before firm conclusions are drawn. However, there are indications that differences exist between oil dose treatments (especially metabolism) that may be mechanisms linking oil exposure and survival in wild harlequin ducks.

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Table 1. Diurnal time budget of captive Harlequin Ducks exposed to oil, winter 2000-2001

			% of time[d]							
Treatment[a]	Winter stage[b]	n^c	Feeding	Drinking	Locomotion	Resting	Maintenance	Social	Water	Land
0	Early	65	1.6 (0.2)[e]	0.3 (0.1)	13.9 (2.7)	64.2 (3.7)	19.8 (2.6)	0.1 (0.0)	18.7 (3.2)	81.3 (3.2)
1	Early	63	2.0 (0.3)	0.2 (0.1)	14.4 (2.9)	70.3 (4.0)	13.0 (2.3)	0.1 (0.0)	14.7 (3.4)	85.3 (3.4)
10	Early	65	1.8 (0.3)	0.2 (0.1)	17.2 (3.0)	62.0 (4.0)	18.7 (2.8)	0.1 (0.0)	19.2 (3.2)	80.8 (3.2)
0	Mid	58	1.2 (0.1)	0.1 (0.1)	10.8 (2.1)	66.3 (4.1)	21.6 (3.2)	0.0 (0.0)	14.1 (3.1)	85.9 (3.1)
1	Mid	61	2.0 (0.3)	0.3 (0.1)	21.7 (3.9)	63.7 (4.3)	12.3 (2.0)	0.0 (0.0)	22.2 (4.2)	77.8 (4.2)
10	Mid	63	1.4 (0.2)	0.2 (0.1)	9.3 (2.4)	71.5 (3.9)	17.6 (3.0)	0.0 (0.0)	9.1 (2.7)	90.9 (2.7)
0	Late	37	1.4 (0.2)	0.3 (0.1)	9.8 (3.3)	73.3 (4.2)	15.2 (2.7)	0.0 (0.0)	9.5 (3.6)	90.5 (3.6)
1	Late	38	1.5 (0.3)	0.3 (0.1)	23.4 (5.4)	64.4 (5.9)	10.4 (3.0)	0.0 (0.0)	24.4 (5.4)	75.6 (5.4)
10	Late	40	1.3 (0.3)	0.4 (0.2)	5.8 (1.8)	73.0 (4.7)	19.5 (4.4)	0.0 (0.0)	3.8 (1.8)	96.2 (1.8)
0	Total	160	1.4 (0.1)	0.2 (0.1)	11.9 (1.5)	67.1 (2.3)	19.4 (1.7)	0.1 (0.0)	14.9 (1.9)	85.1 (1.9)
1	Total	161	1.9 (0.2)	0.2 (0.1)	19.2 (2.3)	66.4 (2.7)	12.2 (1.4)	0.0 (0.0)	19.8 (2.4)	80.2 (2.4)
10	Total	169	1.5 (0.1)	0.3 (0.1)	11.4 (1.6)	68.3 (2.4)	18.4 (1.9)	0.0 (0.0)	11.7 (1.7)	88.3 (1.7)

[a] doses listed as mL/kg body mass

[b] Early winter (17 Oct-08 Dec), mid winter (09 Dec-31 Jan), late winter (01 Feb-21 Feb)

[c] sampling unit was each 30 minute observation period

[d] behaviors reported as percent of sampling period

[e] SE in parentheses

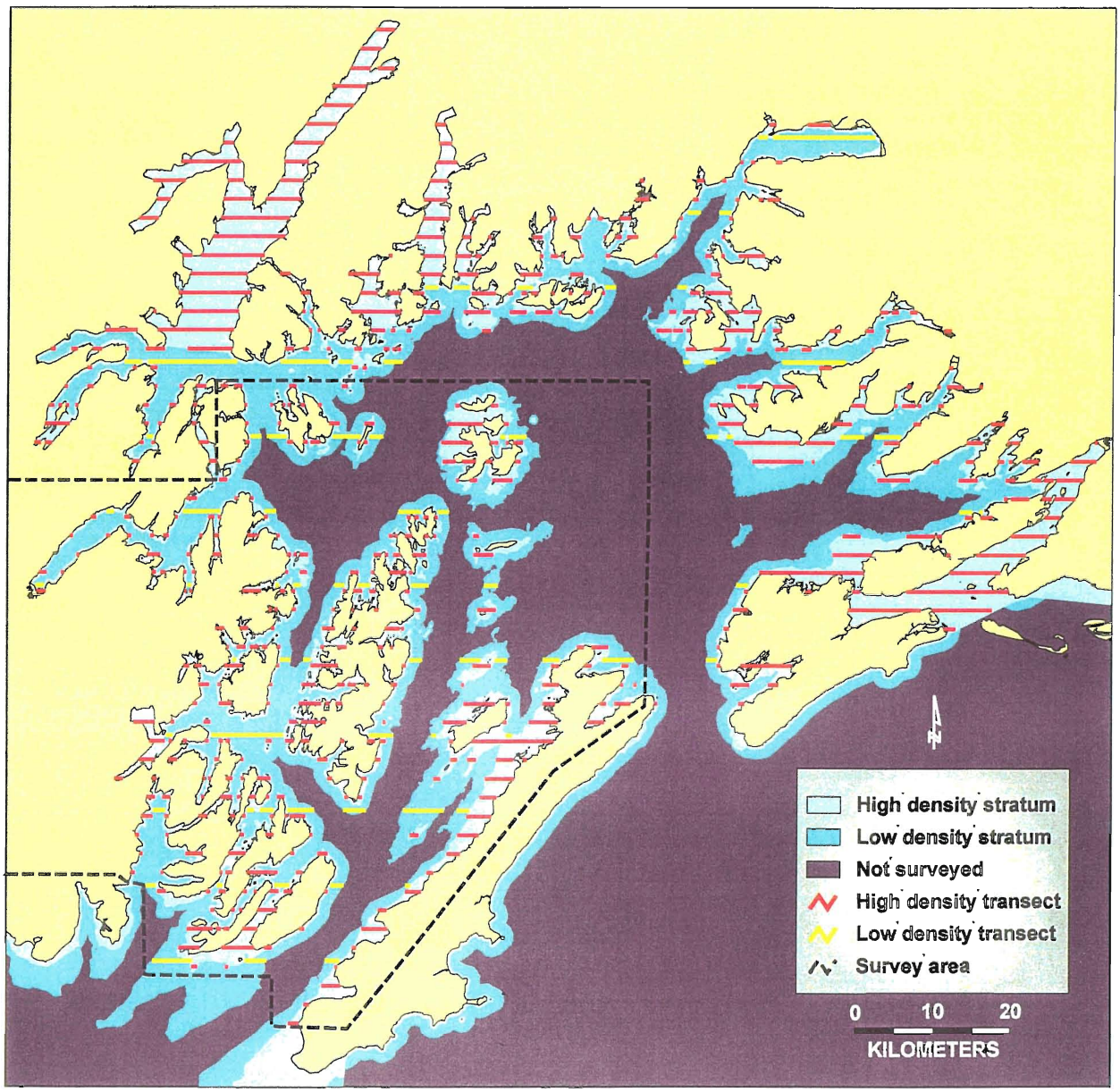

Fig. 1. Western Prince William Sound sea otter survey area, 2000.

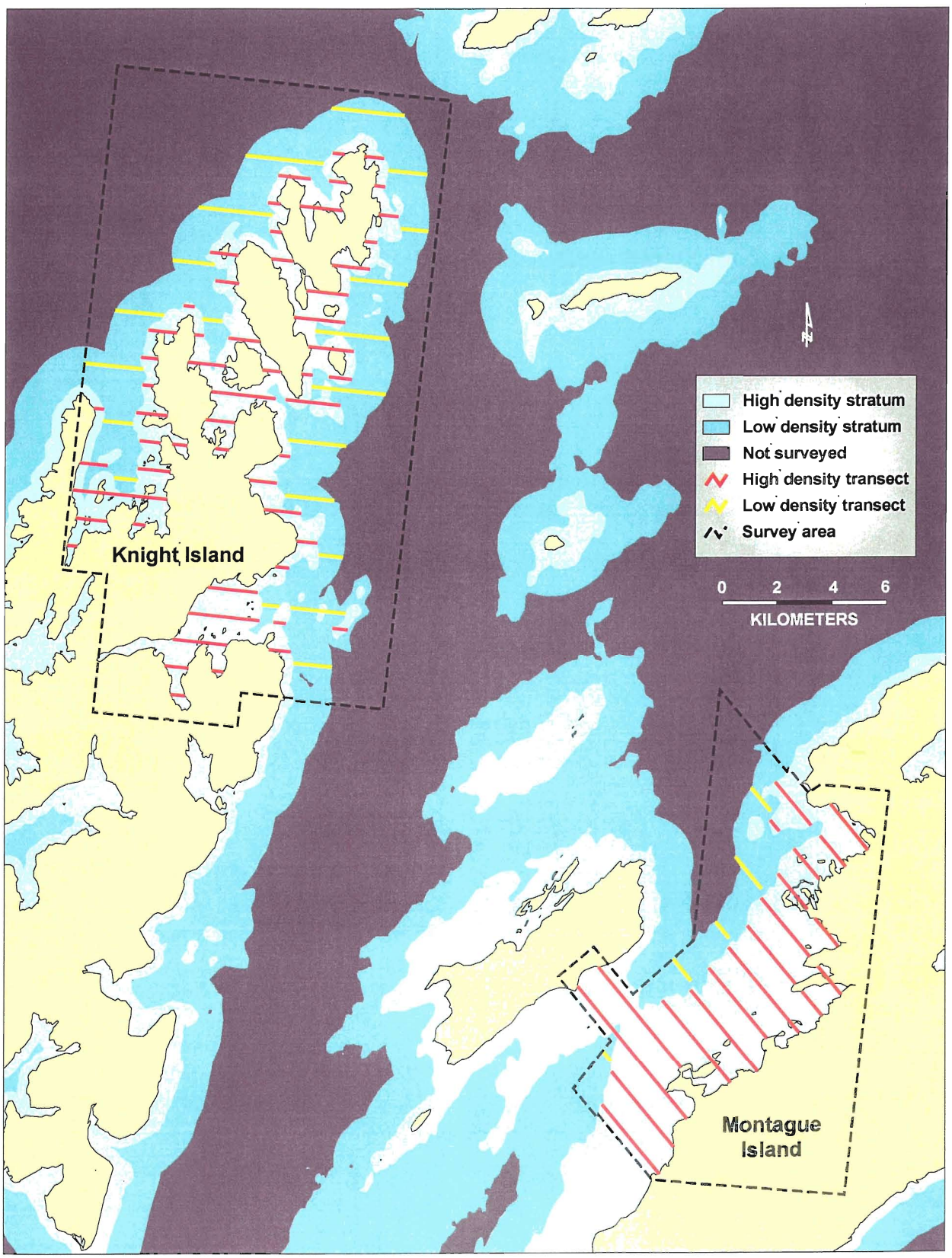

Fig. 2. Knight (oiled) and Montague (unoiled) study areas.

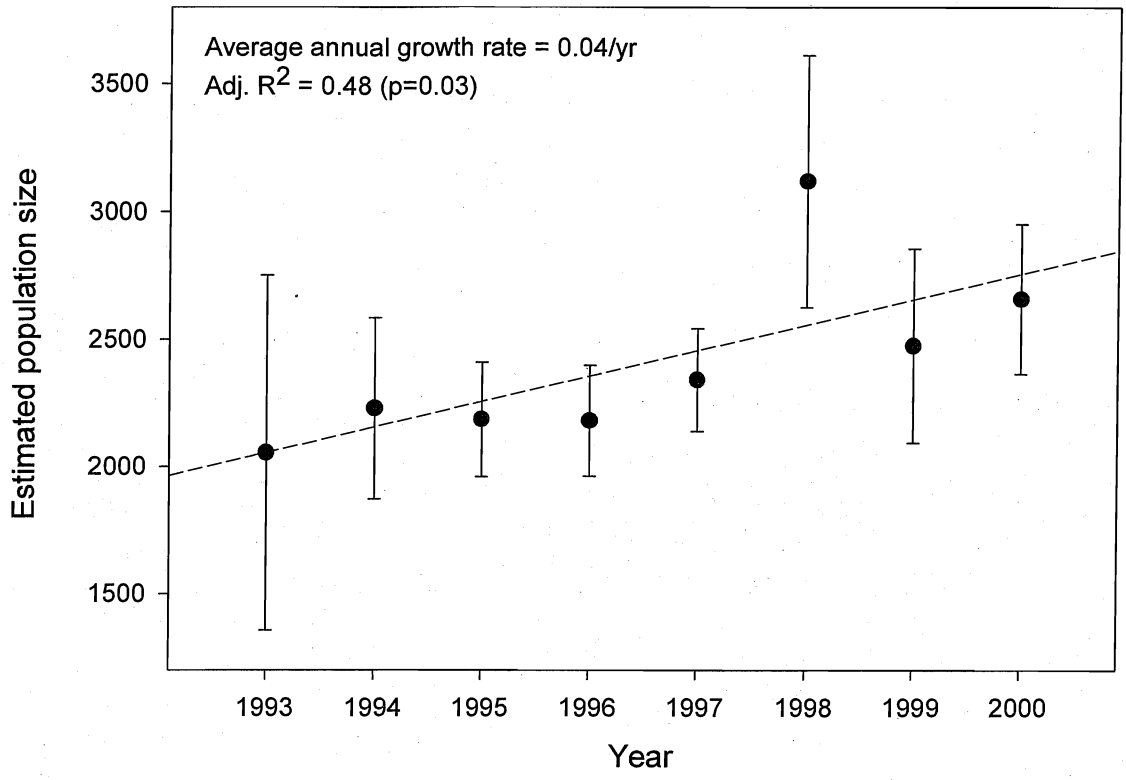

Figure 3. Estimates of sea otter abundance in western Prince William Sound, 1993-2000.

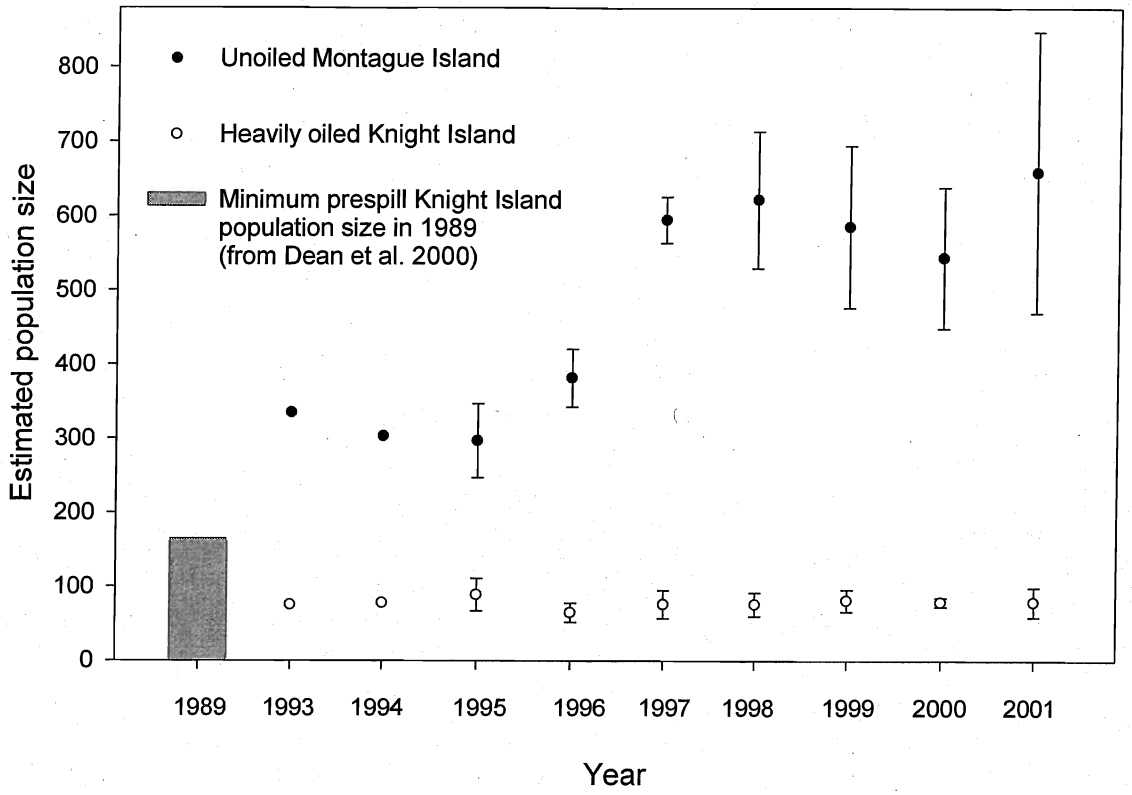

Figure 4. Estimates of sea otter abundance at Knight and Montague study areas, 1993-2001.

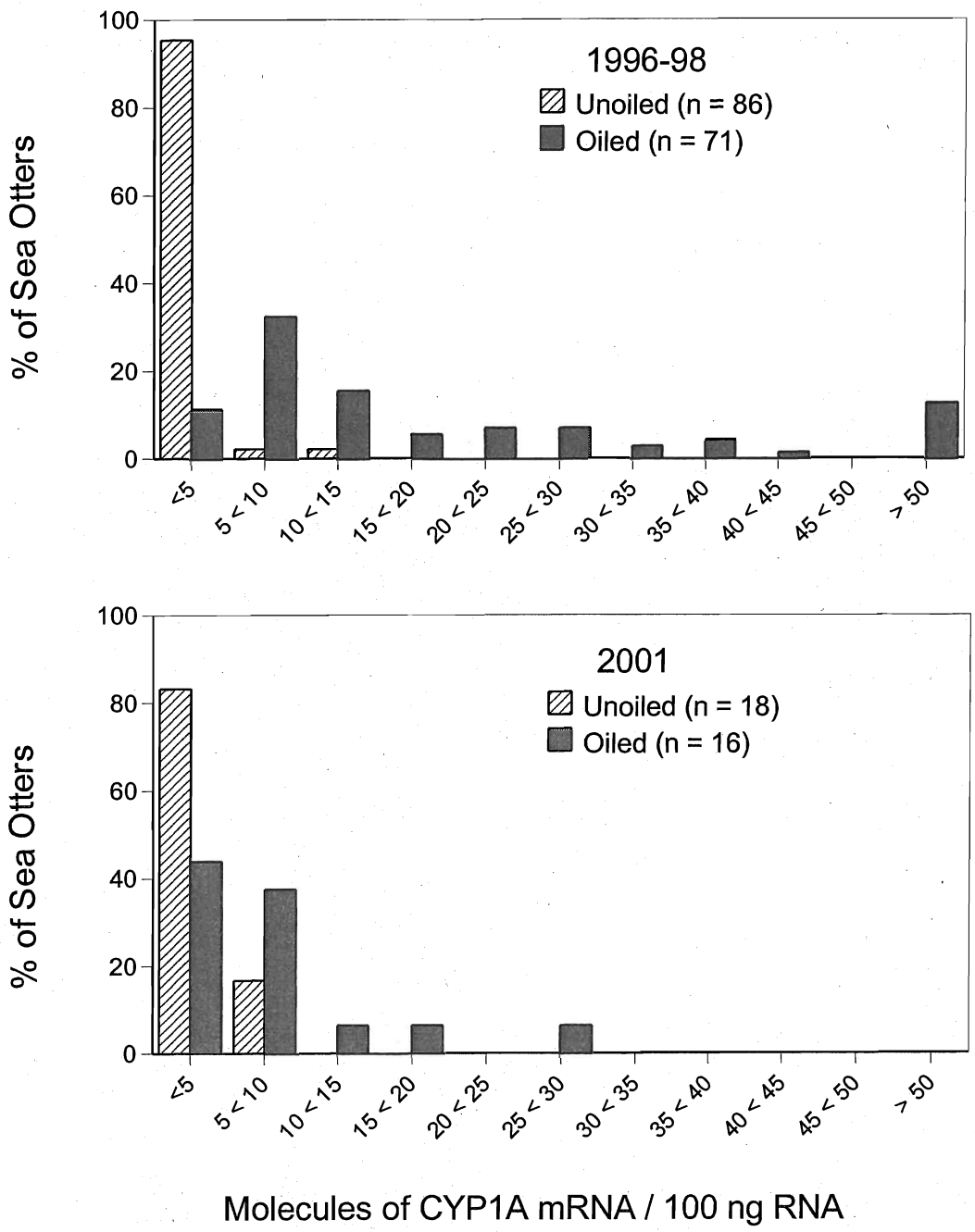

Figure 5. CYP1A values for sea otters from oiled (northern Knight) and unoiled (Montague) study areas, in 1996-98 (NVP data) and in 2001. Units are molecules of CYP1A mRNA x 10^6 per 100 ng total RNA.

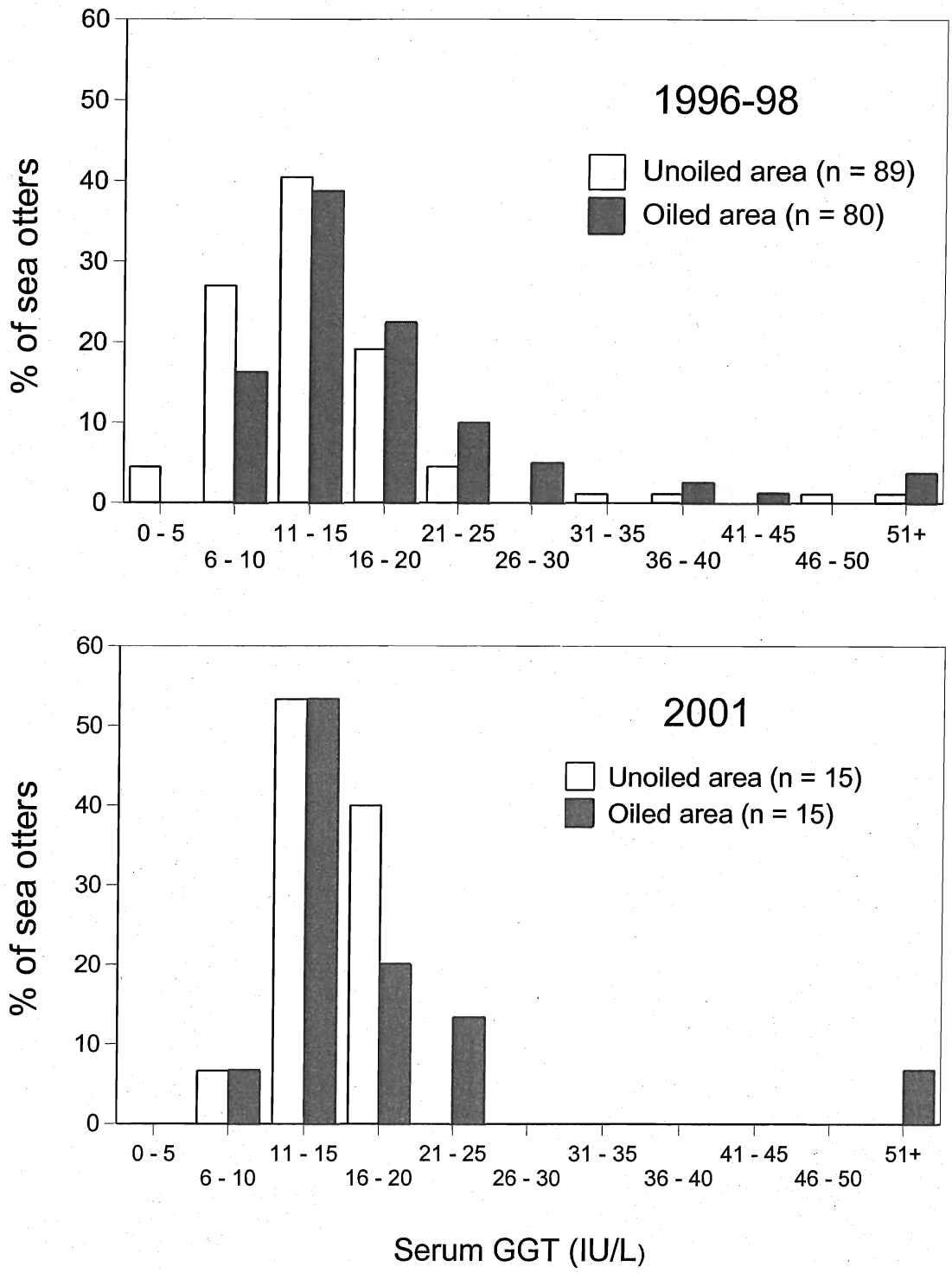

Figure 6. Serum GGT (gamma glutamyl transferase) values for sea otters from oiled (northern Knight) and unoiled (Montague) study areas, in 1996-98 (NVP data) and in 2001.

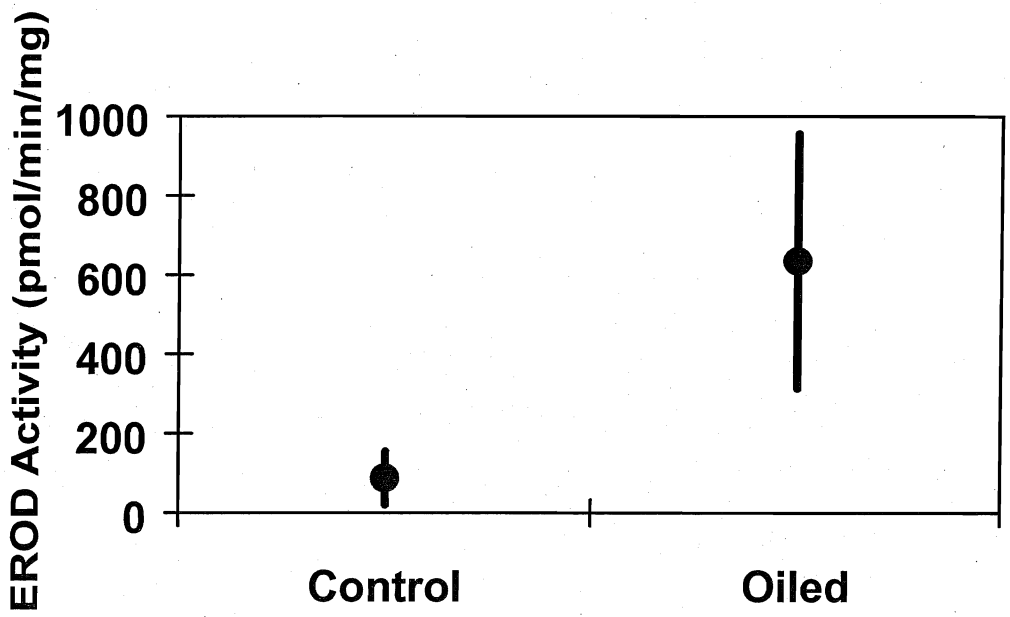

Figure 7. Cytochrome P4501A induction (± 95% confidence intervals) of harlequin ducks held in captivity at the Alaska SeaLife Center, winter 2000-01. The oiled category combines birds ingesting 1 ml/kg and 10 ml/kg of crude oil twice weekly.

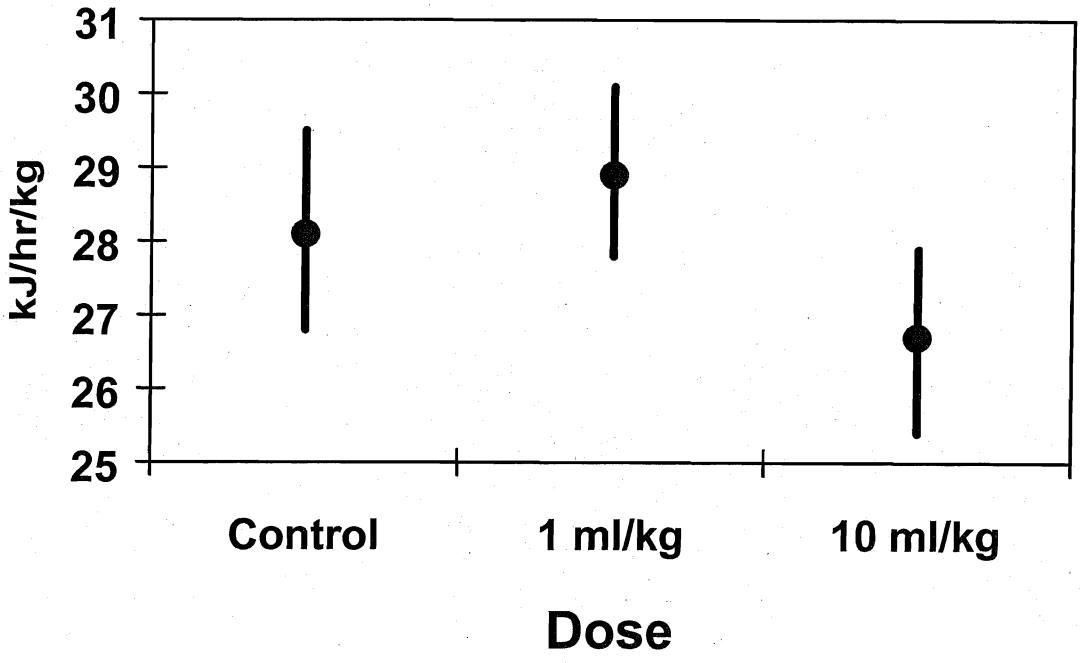

Figure 8. Basal metabolic rates (± 95% confidence intervals) of harlequin ducks held in captivity at the Alaska SeaLife Center, winter 2000-01, by twice weekly dosing regimes of crude oil.

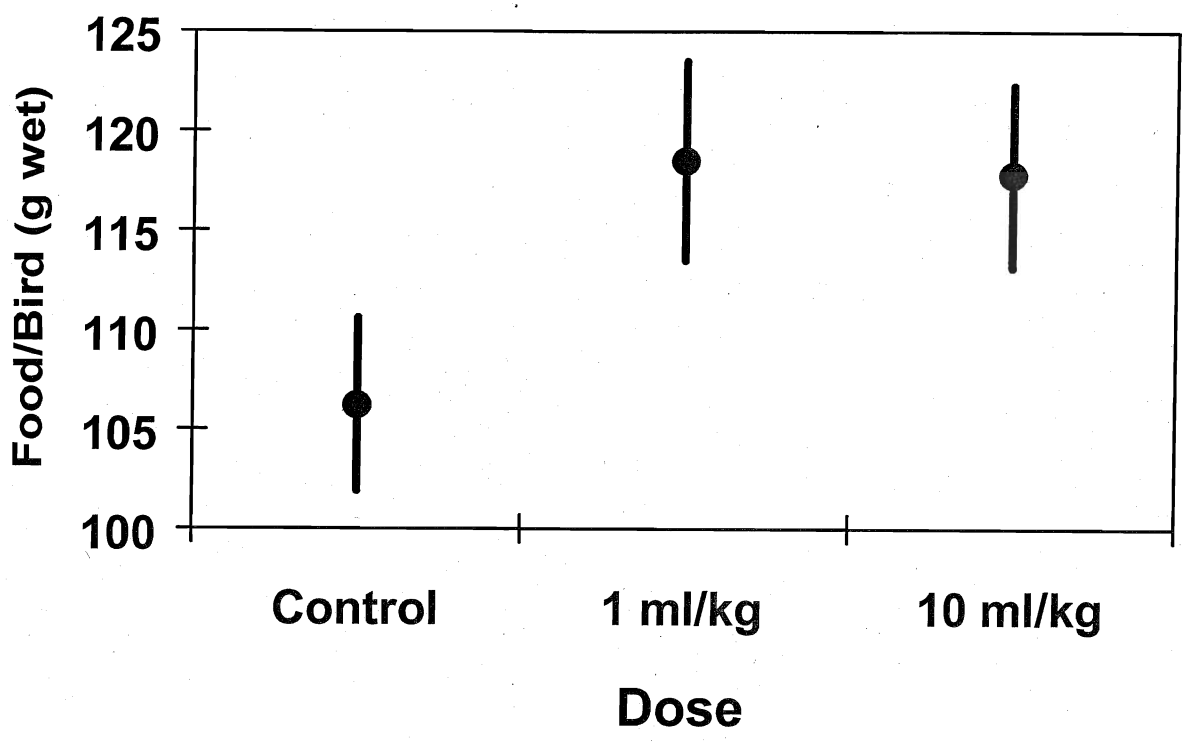

Figure 9. Food consumption (± 95% confidence intervals) of harlequin ducks held in captivity at the Alaska SeaLife Center, winter 2000-01, by twice weekly dosing regimes of crude oil.

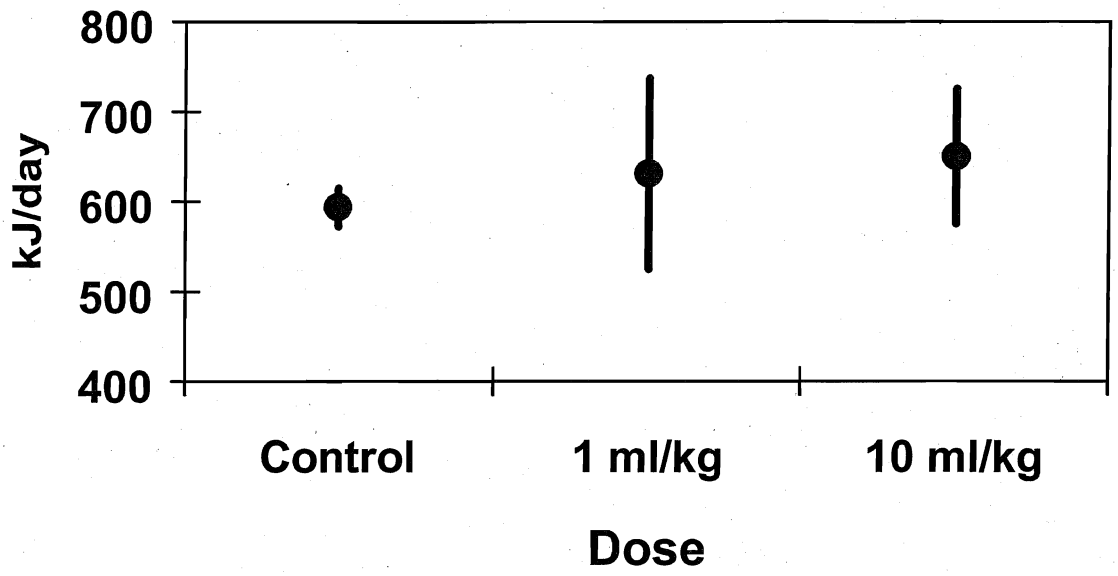

Figure 10. Daily energy expenditure (± 95% confidence intervals) of harlequin ducks held in captivity at the Alaska SeaLife Center, winter 2000-01, by twice weekly dosing regimes of crude oil.

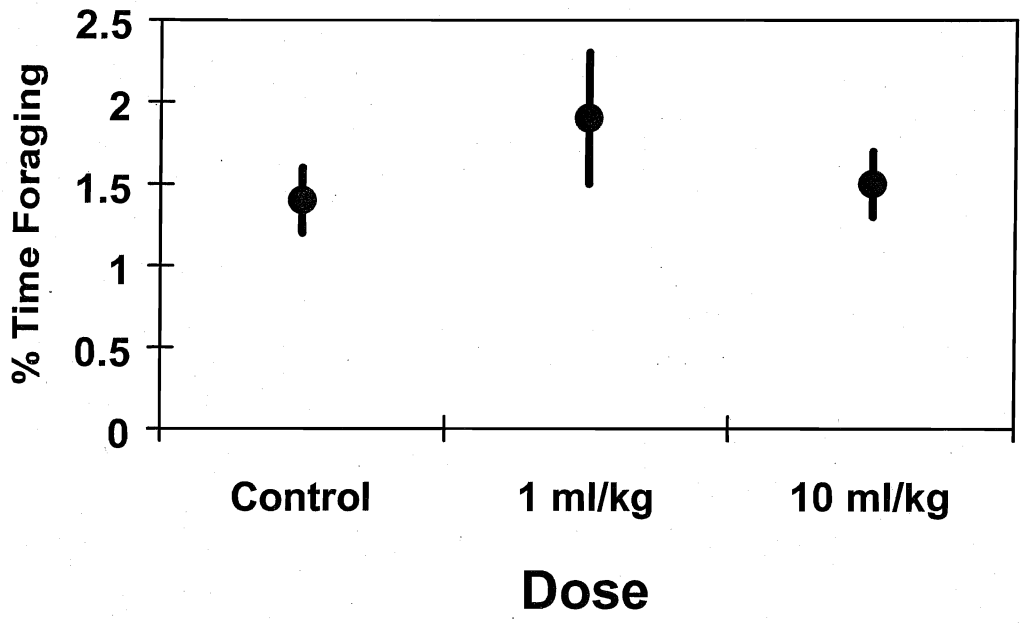

Figure 11. Feeding behavior (± 95% confidence intervals) of harlequin ducks held in captivity at the Alaska SeaLife Center, winter 2000-01, by twice weekly dosing regimes of crude oil.

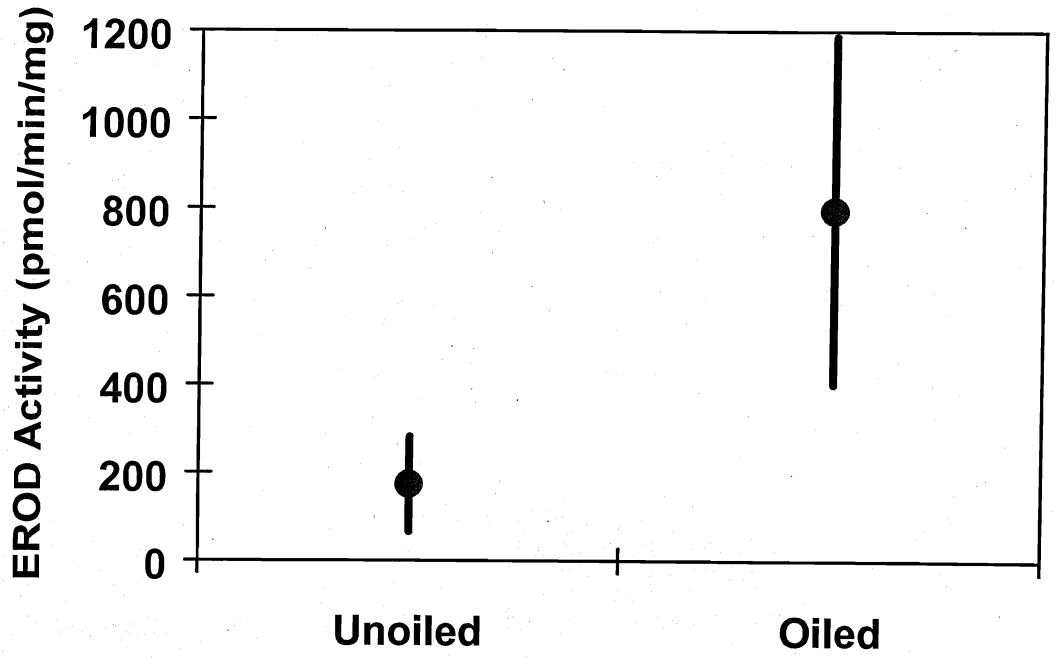

Figure 12. Cytochrome P4501A induction (± 95% confidence intervals) of harlequin ducks captured in unoiled and oiled areas of Prince William Sound, November 2000.

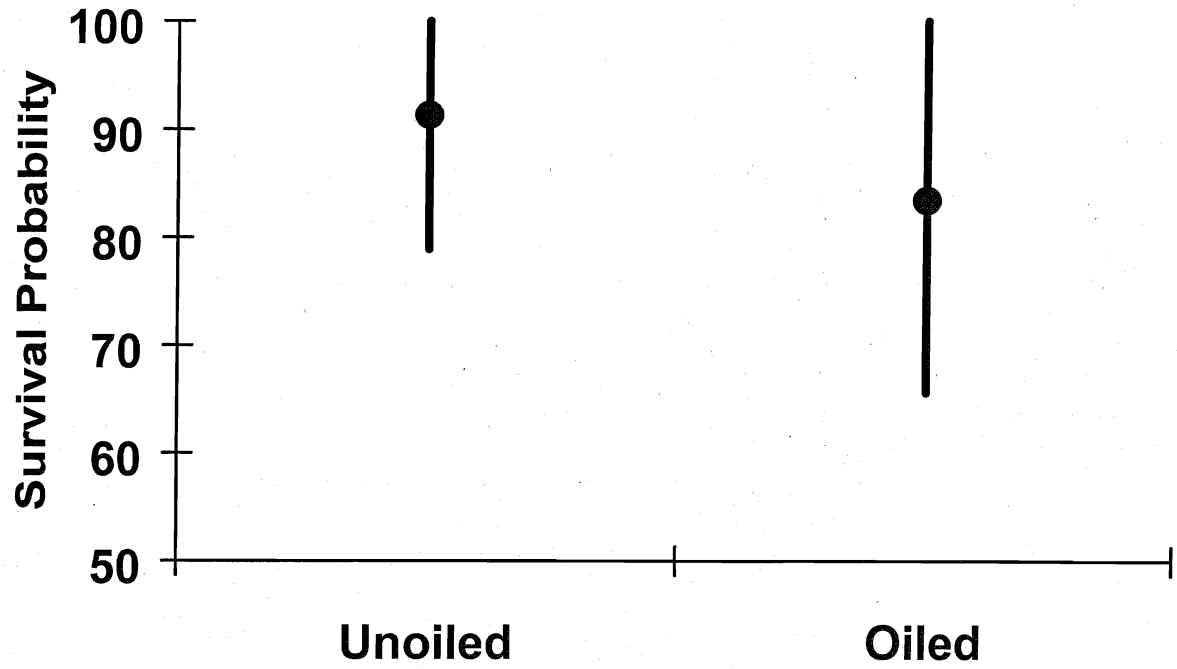

Figure 13. Survival probabilities (± 95% confidence intervals) of female harlequin ducks from unoiled and oiled areas of Prince William Sound, November 2000 – March 2001.

APPENDIX

Annual Report for Restoration Project 01423

HISTOPATHOLOGY OF SEA OTTER LIVER TISSUES COLLECTED IN JULY 2001

Brenda E. Ballachey

U.S. Geological Survey
Alaska Science Center
1011 E. Tudor Road
Anchorage, Alaska 99503

and

Paul W. Snyder

Department of Veterinary Pathology
Purdue University
West Lafayette, IN

Summary

We have done a preliminary examination of liver tissues collected in July 2001, from sea otters in oiled and unoiled areas of western Prince William Sound. Microscopically, the livers had a spectrum of lesions that included degenerative, regenerative and inflammatory changes. Those lesions included: 1) telangiectasis; 2) lympohistiocytic inflammation; 3) vacuolar and fatty degeneration; 4) necrosis; 5) foci of regeneration; and 6) apoptosis. The inflammatory, degenerative, necrotic, and regenerative lesions were present in both groups (oiled and non-oiled area animals) to varying degrees of severity. There was no discernable difference between between oiled and unoiled area animals with regard to inflammatory lesions. In general, the degenerative, regenerative and necrosis were more severe in the oiled area animals. The telangiectasis (SO-01-21 and SO-98-29) and apoptosis (SO-01-28) lesions were limited to specific animals captured in the oiled area. Examples of lesions are shown in the four attached figures. To finalize the histopathology examinations, two pathologists will independently grade the slides for these six lesions as none, minimal, mild, moderate, and severe. Further imformation on liver samples collected from sea otters in 2001 is provided in the Final Report for Project 01534.

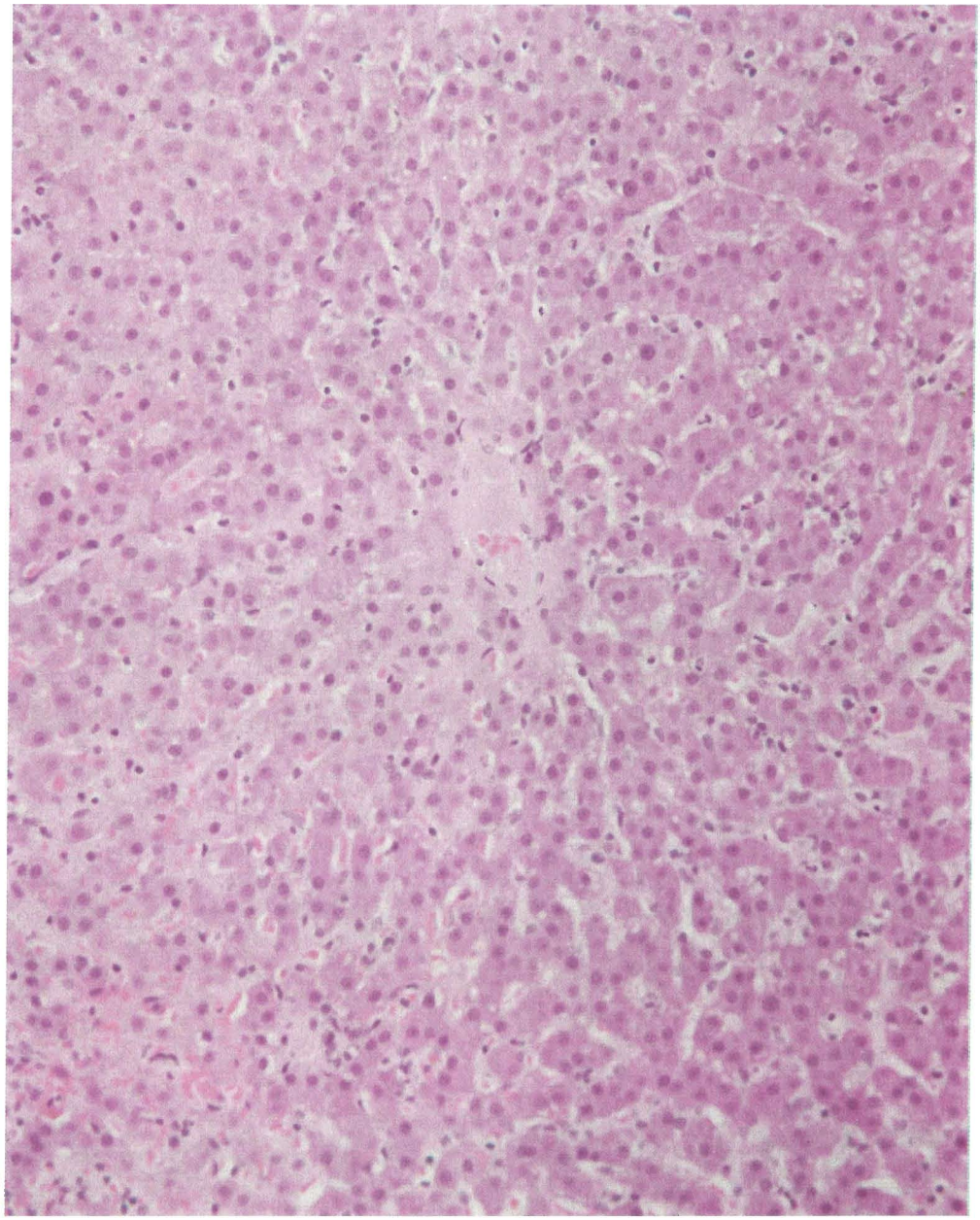

Figure APP-1. Sea otter SO-01-07.1, from unoiled area. Normal liver.

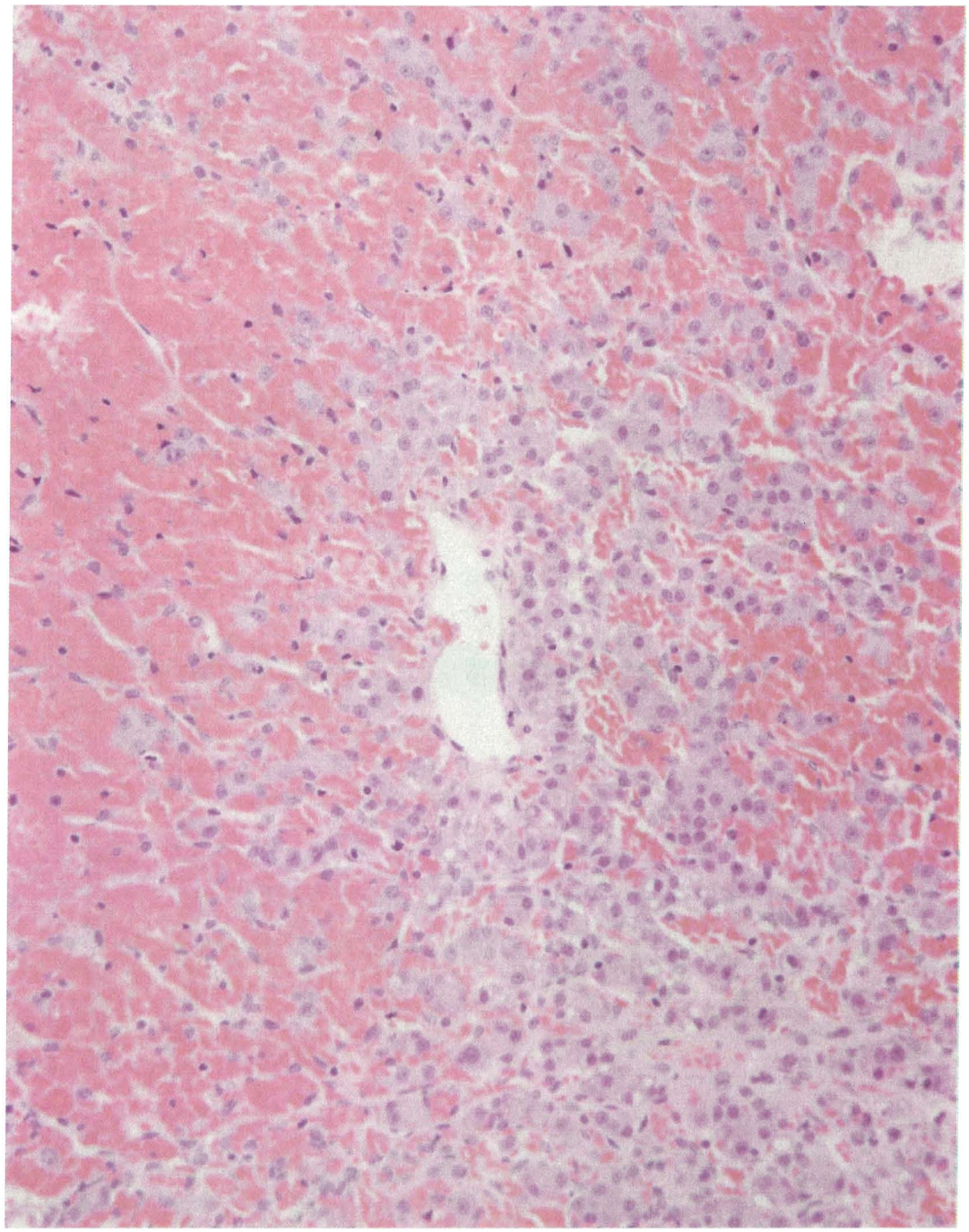

Figure APP-2. Sea otter SO-01-21.3, from oiled area. Severe telangiectasis.

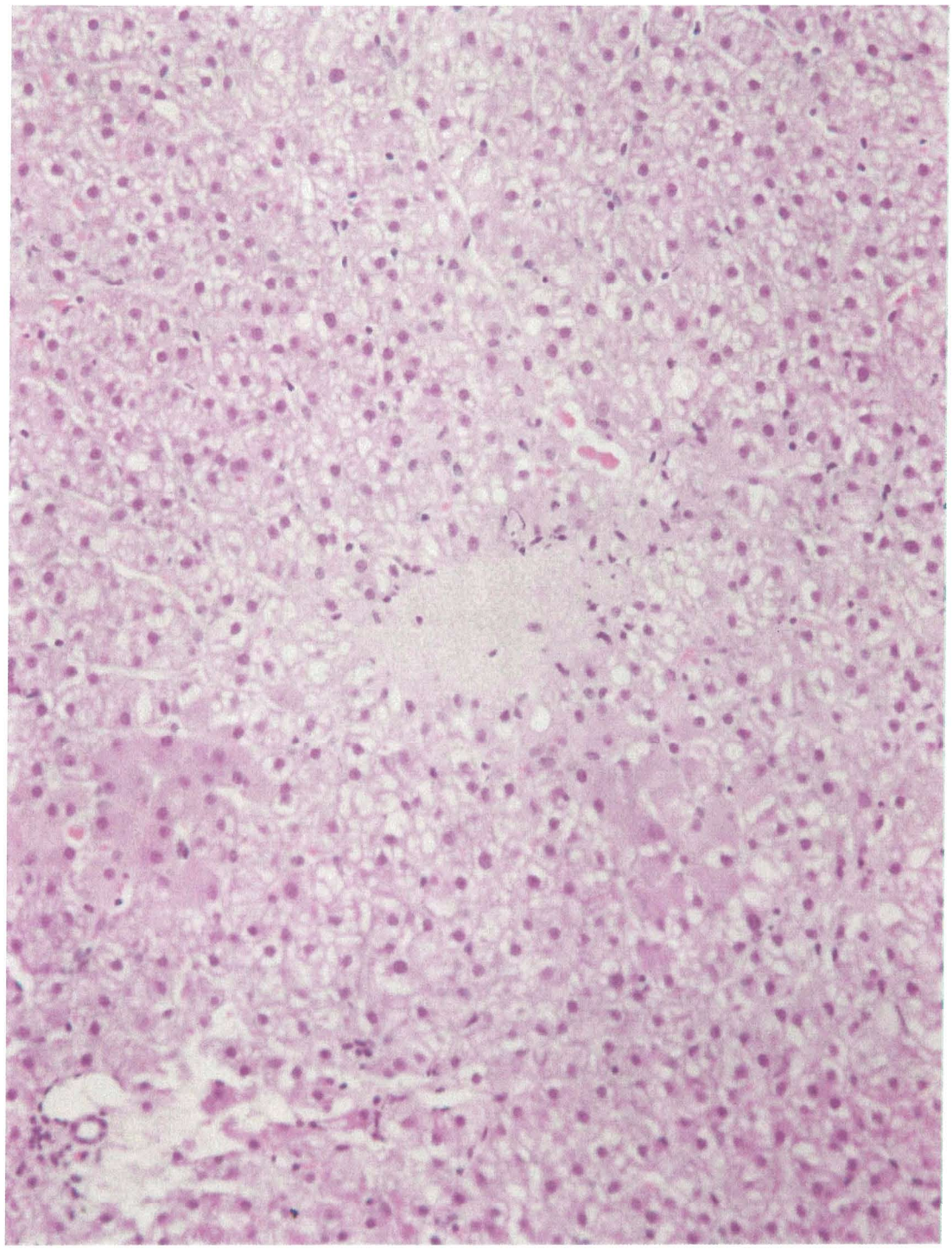

Figure APP-3. Sea otter SO-01-27.1, from oiled area. Eosinophilic foci suggestive of regeneration (repair).

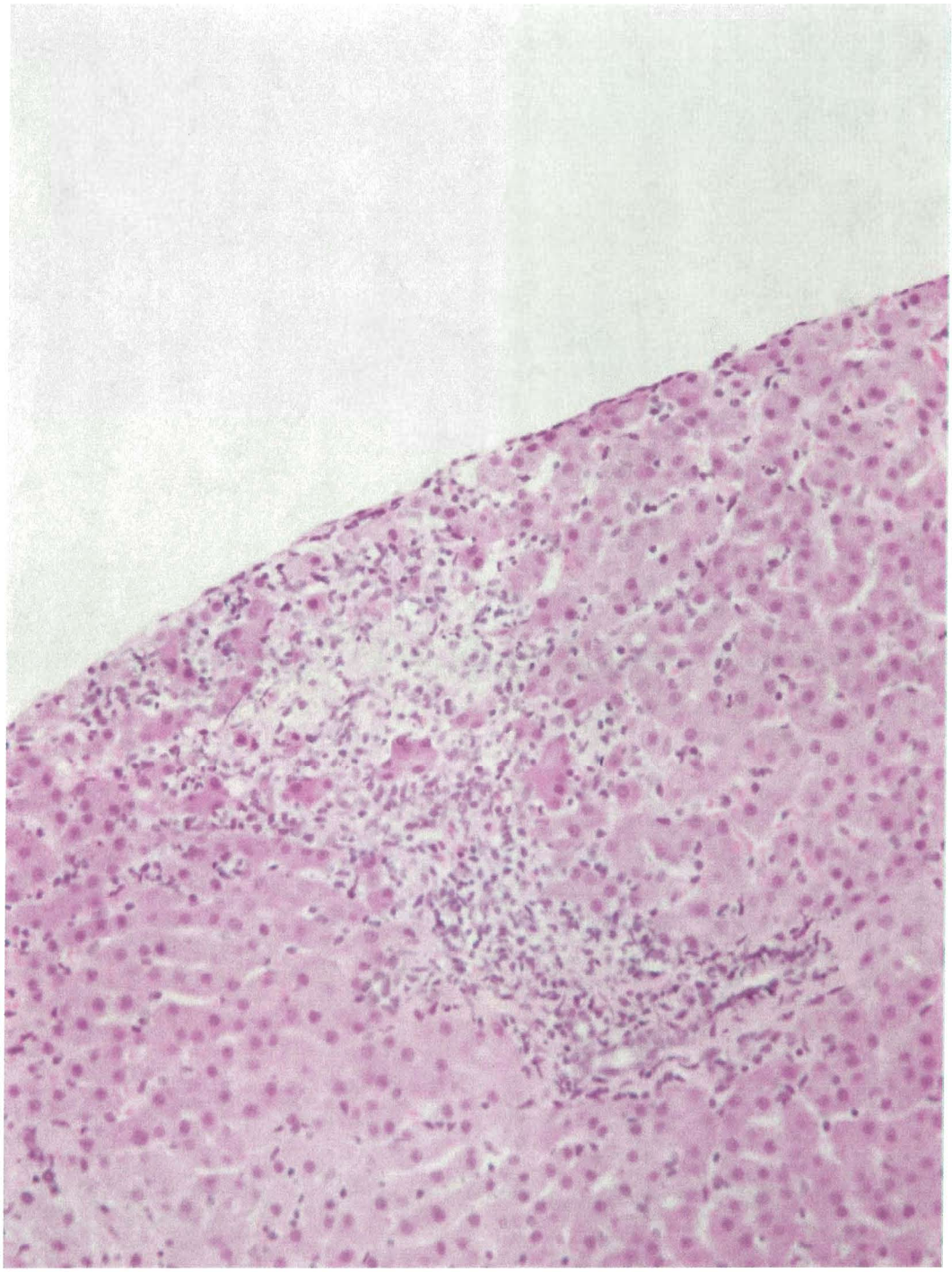

Figure APP-4. Sea otter SO-01-28.1, from oiled area. Inflammation and necrosis.