# *Exxon Valdez* Oil Spill Restoration Project Annual Report

# Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators Restoration Project 00423 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.

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Patterns and Processes of Population Change in Selected Nearshore Vertebrate Predators

# Restoration Project 00423 Annual Report

<u>Study History:</u> 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, 95025, 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 beachcast sea otter carcasses, 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 CYP1A induction was approved for 2001. 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 second year of sea otter field work, and provide a preliminary report on the harlequin duck component.

<u>Abstract</u>: 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, was 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 2,992 individuals (se=480). The previous comparable estimates for Western Prince William Sound were 2,475 (se=381) in 1999 and 2,852 (se=440) in 1998. We estimated population sizes of 79 (se=6) at Northern Knight Island and 544 (se=95) at Montague Island in 2000. 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 about 600 in 1998, subsequently decreasing slightly to about 540 in 2000 (avg. annual increase 1993-2000 =12%, adj.  $R^2$ = 0.67, P<0.01). In 1999 and 2000 we collected 86 sea otter carcasses from beaches in western Prince William Sound.

The significant increases we have detected since 1993 in and around the spill area continue to indicate progress toward recovery of the EVOS injured sea otter population.

However, the lack of a concurrent increase around northern Knight Island through 2000, where sea otter mortality was highest, indicate that recovery may not be occurring where oil spill effects were greatest.

Between 1996 and 2000, we also examined changes in sea urchin populations at Knight and Montague Islands. In 1996 and 1997 at Knight Island, more than 40% of the sea urchins examined (N=2176) were larger than 20 mm (the minimum size generally consumed by sea otters) compared to less than 20 % (N=678) at Montague Island. However, in 1998, 1999, and 2000, there was a marked increase in the number of larger sea urchins, especially at Montague Island, and there was a higher proportion of larger sea urchins (30 mm or larger) at Montague Island compared to Knight Island. These differences in the size distributions of sea urchins at Knight and Montague Island appear to be influenced more by the magnitude and timing of recruitment events rather than differences in effects of sea otter predation. Relatively few sea urchins greater than 40 mm in diameter were noted at either Knight or Montague Islands in any year, suggesting that larger sea urchins may be susceptible to sea otter predation at both locations, and that even the relatively low numbers of sea urchins.

Primary harlequin duck objectives for FY2000 were to capture harlequin ducks during wing molt to establish a captive flock for experiments at the Alaska SeaLife Center and to prepare for field and captive studies during winter 2000-01. Capture of molting females was successful, and the captive experiments proceeded as planned. Experiments involved different internal oil dosing regimes and subsequent evaluation of behavior, metabolism, P450, and blood chemistry responses. Similarly, field studies during winter 2000-01 were successfully completed, which included capture of flighted birds, sampling for external hydrocarbons, surgeries to biopsy livers for measures of P450 induction and implant radios, and subsequent telemetry monitoring. Data collection for the first winter recently finished, at the end of March 2001, and thus data have not been summarized or analyzed; these will be reported in the FY2001 annual report. However, all aspects of the study generated good quantities of data and will be critical for monitoring the progress, and understanding the process, of harlequin duck population recovery.

<u>Key words</u>: demography, *Enhydra lutris*, *Exxon Valdez*, harlequin ducks, *Histrionicus histrionicus*, oil spill, population status, Prince William Sound, sea otters, sea urchin, *Stronglycentrotus droebachiensis* 

*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 (Holland-Bartels et al.2000) through at least 1998. Additionally, we identified a common pattern among several sea otter prev species consistent with reduced predation, through increased proportions of large individuals where sea otters populations were reduced (Dean et al. 2000, Dean et al. in press). [Mention sea otter oil exposure here?] 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 on ecological interactions between sea otters and green sea 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 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 (Holland-Bartels et al. 1999), 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 et al. 1997, Holland-Bartels et al. 1998, Holland-Bartels et al. 1999). 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 2000, sea otter abundance at northern Knight Island remained at about 50% of the estimated pre-spill abundance (Dean et al. 2000). Constraints to recovery most likely are demographic, either through reduced survival among residents, or higher emigration from the oiled area (Holland-Bartels 2000). Analysis of age at death of beach cast sea otters found before and after the spill, implicate elevated mortality of sea otters that survived the spill as well as those born after 1989, as a factor contributing to delayed recovery (Monson et al. 2000).

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 two 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.

## 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 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 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 this report, we extend 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 provides 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, continued monitoring of sea otter distribution and abundance, mortality and otter prey populations in WPWS will be valuable in (1) providing insight into potential demographic 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.

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

#### METHODS

#### Sea otters:

<u>Aerial surveys</u>. 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.

<u>Mortality</u>. Systematic beach surveys were conducted in April or May along shorelines in WPWS soon after snow melt, 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, 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.

# 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. 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, 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.

## Harlequin ducks:

<u>Captive Studies – General.</u> In FY2000, harlequin ducks for captive 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. For the remainder of the fiscal year, 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 FY2001, 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.

<u>Captive Studies – Metabolism</u>. 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 <sup>2</sup>H and <sup>18</sup>O, the isotopes used in this experiment, were determined by analysis of  $15\mu$ l 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 (<sup>2</sup>H<sub>2</sub>O, 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 <sup>18</sup>O and 30 atom percent <sup>2</sup>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 research at 5°C and shipped to the research at 5°C and shipped 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.

<u>Captive Studies – Behavior</u>. 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), maintenace (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.

Captive Studies - Hematology and Serum Chemistry. Blood was drawn on 7 occasions to evaluate variation in hematology and serum chemistry in relation to oil dosing treatment. The first sample was taken in the field upon capture. Others were taken at the Alaska SeaLife Center (16 October, 23 October, 13 November, 18 December, 23 February, and 12 March). On each occasion we will drew 1.5cc of blood from jugular veins of all birds. Two whole blood smears were made; slides were labeled, in pencil, with the bird's USFWS band number and date. 0.5 ml of whole blood was placed in an heparinized (green top) microtainer, mixed vigorously, labeled with the bird's band number, and placed in the refrigerator until shipment or analysis. The blood smears and whole blood were analyzed immediately at the SeaLife Center for labile hematological parameters. For the first sampling occasion (in the field), the blood smears and heparinized microtainer were sent in with the birds for immediate analysis. 0.5 ml of blood were placed in both a heparinized (green top) and clot tube (red top) microtainer, for plasma and serum analyses, respectively. After 15 minutes, the green and red top microtainers were centrifuged for 10 minutes. Disposable pipettes (1 for each microtainer) were used to draw off plasma and serum into separate vials, which were then labeled with bird number and date and frozen until analysis. During the first sampling occasion in the field, we placed an additional ml of whole blood in a red top microtainer and, after settling and centrifugation, drew off serum for screening for Newcastle's and influenza. These were labeled and frozen for analysis. Similarly, serum samples for Newcastle's and influenza were taken at the last sampling session and analyzed prior to return of the birds to Montague Island.

<u>Captive Studies – Cytochrome P450 1A Induction</u>. Induction of cytochrome P450 1A will be indicated by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity following methods detailed in Trust et al. (2000). This method required surgical biopsy of a 0.1 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 at  $< 70^{\circ}$ C. Samples were shipped to Woods Hole in liquid nitrogen for subsequent analysis. We also collected blood lymphocytes from all birds to evaluate a method for quantifying P450 induction via quantification of mRNA; this is a collaborative effort with California State University to develop a blood-based, and hence less invasive, approach for evaluating P450 induction in birds. Results from this approach will be contrasted with results from EROD activity results.

<u>Field Studies.</u> 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.

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:

In July 2000 we estimated the WPWS sea otter population at 2992 (se=480) individuals, including pups (Figure 3). We estimated population sizes of 79 (se=6) at our oiled intensive Northern Knight Island study site and 544 (se=95) at our unoiled intensive Montague Island study site in 2000 (Figure 4).

In April 2000 we located the remains of 58 beach cast sea otters in the oil spill area of WPWS. We also report on the remains of an additional 28 animals recovered in the same area in April 1999. We could not obtain reliable age estimates from 18 of the 86 carcasses recovered. Sea otters aged 0-1 (juveniles) comprised 34% of our sample, adults age 2-8 comprised 34%, and aged adults, (>8 years) comprised 32%. The frequency distribution of ages at death for 68 sea otter carcasses is reported in Figure 5.

### 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). 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 2). Relatively few large (greater than 40 mm) sea urchins were found at either Knight or Montague Island in any year (Figure 3). 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.

#### Harlequin ducks:

Activities in FY2000 were limited to capture of birds during wing molt to establish the captive flock at the Alaska SeaLife Center. However, we also report here types and quantities of data that were collected during the winter 2000-01 study period, to allow evaluation of progress towards project goals. Full analysis and interpretation of these data will appear in the FY2001 annual report.

<u>Captive Studies</u>: Seventy-seven harlequin ducks were captured during molt drives, of which 25 females older than second-year were sent, via floatplane, to the Alaska SeaLife Center. During the course 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. Table 3 summarizes the types of data collected during this study, reflecting our efforts to maximize data generation from the captive birds.

<u>Field Studies</u>: During winter 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.

# DISCUSSION

### Sea otters:

A remnant PWS sea otter population survived the commercial fur harvest of sea otters that ended early in the 20<sup>th</sup> 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 was apparently 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 2000 (2,992). The average annual rate of growth during this period is 0.05, 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 600 in 1998 (avg. annual increase =16%, adj.  $R^2$ = 0.68, P<0.01). Since 1998, the estimated population size has declined, although not significantly, from 623 to 544. The average growth rate at Montague Island between 1993 and 2000 was 12% (adj.  $R^2$  = 0.67, P<0.01). 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 cytochrome P450

enzyme, a biomarker of oil exposure, compared to Montague Island (Holland-Bartels 1999). 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 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 2 & 3) 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). We anticipate having a cumulative sample size of about 100 ages at death for the years 1999-2001 and anticipate incorporating these data into the Monson et al. (2000) model during winter 2001-02 to estimate recent sea otter survival rates in WPWS.

### Sea urchins:

Based on the relative lack of large sea urchins in 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 Prince William Sound was related, in part, to impacts associated with the oil spill. While 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.

#### CONCLUSIONS

Results of aerial surveys of sea otter abundance have identified a significant increase of about 900 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 evidence of continued exposure to oil at Knight Island sea otters and adverse effects of residual oil on the growth and survival of clams strongly support the role of oil in delaying recovery of the nearshore marine community, at least at northern Knight Island. It appears as though episodic, and possibly localized recruitment of sea urchins, as well as continued predation may confound predictions of prey population responses to reduced predator densities.

Harlequin duck captive and field studies are proceeding successfully, and large quantities of data are being collected in all aspects of the project. These data will result in better understanding of constraints to harlequin duck population recovery in PWS.

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Table 1. Mean density of sea urchins (number m<sup>-2</sup>) from intertidal sites on northern Knight Island and Montague Islands from 1996 through 1998.

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	N	Mean	<u>SD</u>		<u>N</u>	Mean	<u>SD</u>
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	. <u>F</u>	<b>Probability</b>
Year	3.02	0.03
Area	0.72	0.54

Table 2. Changes in mean sea urchin density at sites in Knight and Montague Islands sampled in 1997, 1999, and 2000.

	K	night Island		Mo	ntague Isla	and
	N	Mean	<u>SD</u>	$\underline{\mathbf{N}}$	Mean	<u>SD</u>
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	<u>F</u>	Probability
Year	0.78	0.33
Area	< 0.01	0.99

Data Type	Data Quantity/Frequency
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Captive Studies	
Oil Dosing Bouts	-twice weekly, 16 Oct 2000 – 22 Feb 2001 (n=37)
Body Mass	-at every handling (n=44)
Metabolism – Respirometry	-83 sessions
Metabolism – Doubly-labeled Water	-for 17 birds
<b>Metabolism – Food Consumption</b>	-daily, 8 Sep 2000 – 23 Feb 2001
<b>Behavior Observations</b>	-490 sessions (245 hours)
P450 1A – EROD Activity	-for 17 birds
P450 1A – mRNA	-for 17 birds
Hematology/Serum Chemistry	-7 times, from capture to release
Field Studies	
Females Radioed	-55

Table 3. Listing of data collected during harlequin duck studies, winter 2000-01.

Telemetry Flights-from 12 Nov 2000 – 2 April 2001P450 1A – EROD Activity-for 33 birdsExternal Oil – Plumage Swab-for 50 birds

**External Hydrocarbons – Feathers** 

-for 35 birds



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 (<u>+</u> se) in western PWS 1993-2000



Fig. 4. Estimates of sea otter abundance ( $\pm$  se) for intensive study sites at Knight and Montague Is., 1993-2000 (no estimates of precision in 1993 and 1994).



Figure 5. Age at death distributions of WPWS beach cast sea otters in 1999/2000