

*Exxon Valdez* Oil Spill  
Restoration Project Final Report

Evaluating Harlequin Duck Population Recovery:  
CYP1A Monitoring and a Demographic Population Model

Project 070816

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March 2010

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**Study History:** Harlequin ducks have been studied extensively in Prince William Sound during the restoration phase following the *Exxon Valdez* oil spill, leading to one of the most thorough considerations of wildlife population injury and recovery following a major oil spill ever undertaken. These efforts have included population monitoring by the U.S. Fish and Wildlife Service and the Alaska Department of Fish and Game, as well as a series of directed research projects designed to elucidate the process of, and constraints to, population recovery. These studies demonstrated that harlequin ducks were exposed to lingering oil over a much longer time frame (i.e., through at least 2005, 16 years following the spill) than expected at the time of the spill, based on elevated levels of cytochrome P4501A induction in birds from oiled areas. In addition, several lines of evidence suggested that population injury occurred through at least 1998. Specifically, female winter survival probabilities were found to differ between oiled and unoiled areas, and densities were shown to be lower in oiled than unoiled areas after accounting for habitat-related effects. More recent data have indicated that female winter survival did not differ between oiled and unoiled sites during 2000-03, suggesting that direct effects of oil exposure on demographic properties may have abated.

In response to the data described above, the current work was proposed to add to the existing cytochrome P4501A monitoring data to track the timeline of exposure to lingering oil, and to assemble the demographic data collected during previous restoration studies into a quantitative population model to allow consideration of the timing and magnitude of oil spill injury, the mechanisms by which injury occurred and population recovery was constrained, and the current status of recovery, including predictions for timing of full recovery.

**Abstract:** We found that cytochrome P4501A induction during winters 2005, 2006-07, and 2009 was higher in harlequin ducks captured in areas of Prince William Sound oiled by the 1989 *Exxon Valdez* spill, relative to unoiled areas. Cytochrome P4501A induction did not vary in relation to age, sex, or mass of individuals, nor did it vary strongly by season in samples collected early and late in the winter. We interpret these results to indicate that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil up to 20 years after the original spill. Population modeling efforts indicated that female mortality related to chronic exposure to lingering oil was nearly double that estimated during the acute phase (the weeks and months immediately post-spill). Full recovery to pre-spill numbers was estimated to require between 13 and 32 years, with the most likely recovery scenario requiring roughly 24 years. Taken together, the data presented in this report add to a growing body of literature suggesting that effects of oil spills on wildlife

have the potential to be expressed over much longer time frames than previously assumed.

**Key Words:** biomarker, cytochrome P4501A, demography, *Exxon Valdez* oil spill, harlequin ducks, *Histrionicus histrionicus*, oil exposure, population model, Prince William Sound, recovery.

**Project Data:** Data will be kept in digital format (MS Excel) at the Centre for Wildlife Ecology, Simon Fraser University, Delta, BC, Canada and the USGS Alaska Science Center, Anchorage, Alaska.

**Citation:** Esler, D. and S. A. Iverson. 2010. Evaluating harlequin duck population recovery: CYP1A monitoring and a demographic population model. *Exxon Valdez* Oil Spill Restoration Project Final Report (Project 070816), Centre for Wildlife Ecology, Simon Fraser University, Delta, British Columbia, Canada.

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## EXECUTIVE SUMMARY

Extensive research and monitoring supported by the *Exxon Valdez* Oil Spill Trustee Council has led to a thorough understanding of the response of harlequin duck populations to the 1989 *Exxon Valdez* spill, and the process of (and constraints to) recovery. The information presented in this report adds to that body of work and, further, uses results generated during earlier studies to construct a comprehensive population model.

The work reported here can be divided into 2 main objectives: (1) measurement of cytochrome P4501A induction through winter 2009, as a measure of the degree of exposure to lingering oil; and (2) construction of a population model using previously collected demographic data to provide insight on the process and timing of harlequin duck recovery. The report, and this executive summary, treats these sequentially and as largely independent objectives.

### *Cytochrome P450 Monitoring*

Induction of cytochrome P4501A (CYP1A) in vertebrates occurs in response to exposure to a limited number of compounds, including polycyclic aromatic hydrocarbons such as those found in crude oil. Because CYP1A induction is both specific and sensitive, it has been used to evaluate exposure to inducing compounds in many cases of environmental contamination, including that of the *Exxon Valdez* oil spill. Elevated CYP1A has been demonstrated in several species in areas of Prince William Sound oiled by the *Exxon Valdez* spill relative to unoiled areas, including harlequin ducks.

In this study, CYP1A induction was determined by measuring hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity, which is a well-established method and is the same approach used in earlier *Exxon Valdez* studies and in similar studies of harlequin ducks and other sea ducks elsewhere. During winter 2006-07, we captured 50 harlequin ducks in oiled areas of Prince William Sound and 40 in unoiled areas, split evenly between early winter (November) and late winter (March and April). We also captured approximately 20 per area during March 2009. Small liver biopsies were surgically removed from each individual, frozen immediately in liquid nitrogen, and subsequently shipped to collaborators at the University of California Davis for EROD analysis.

We found that variation in CYP1A induction was strongly related to area, with average (pmol/min/mg  $\pm$  SE) EROD activity during November of 101.7 ( $\pm$  20.4) in oiled areas and 25.7 ( $\pm$  6.7) in unoiled areas. Similarly, during March/April, EROD activity was 77.7 ( $\pm$  11.1) and 16.7 ( $\pm$  2.2) in oiled and unoiled areas, respectively. We tested for effects of attributes of individuals (age, sex, and mass) on variation in EROD and found that none of these had significant explanatory value. Also, we found that season (early versus late winter) also was not an important parameter for explaining variation in EROD. For

March 2009 samples, we found that EROD activity differed between areas and averaged  $85.8 (\pm 30.2)$  in oiled areas and  $19.4 (\pm 5.1)$  in unoiled areas.

We interpret these results as strong evidence that harlequin ducks continue to be exposed to residual *Exxon Valdez* oil, as much as 20 years after the spill. Although evidence of exposure can not be interpreted to be indicative of injury to individuals or populations, continued exposure to oil does suggest that deleterious effects are possible. This work adds to the body of literature describing elevated cytochrome P4501A in several nearshore vertebrates in oiled areas, and confirms that exposure to lingering oil occurred over a much longer time frame than assumed at the time of the spill.

### *Population Modeling*

Population models are useful tools for understanding the pathways and relative effects of various factors on population size and trajectory, and these models are commonly used in wildlife and fisheries science and conservation. We used previously collected demographic data (survival, productivity, and dispersal) in a projection matrix model to consider harlequin duck population ecology in Prince William Sound in relation to the *Exxon Valdez* oil spill. This exercise provided a comprehensive and quantitative framework for assimilating data, which allowed evaluation of timing and extent of mortality related to the oil spill, determination of the sensitivity of populations to variation in demographic attributes, and estimates of the timeline of recovery under different scenarios and assumptions.

The model was focused on females only, given the male-biased sex ratio in the population and thus the assumption that females are the limiting sex in terms of population variation. The model included age-related (hatch year versus after hatch year) variation in demographic attributes. We constructed models in a stepwise fashion, progressing from simple models to more complex versions that allowed consideration of underlying assumptions and identification of important mechanisms of population change. We considered several different permutations in which maximum rates of fertility varied, the degree of correlation between survival and productivity varied, and the degree of movement between oiled and unoiled areas varied. We then contrasted 3 model permutations, representing best-case, worst-case, and most-likely combinations of inputs.

We estimated that female mortalities due to chronic exposure to lingering oil were nearly double those that died during acute phase of the spill, i.e., the weeks to months after the original spill (772 and 400, respectively). The timeline to full recovery was estimated to be 24 years under the most-likely formulation of model inputs, with a range of 13 to 32 years for the best-case and worst-case scenarios, respectively.

Our findings confirm assertions that effects of oil spills on wildlife populations are expressed over much longer time frames than previously assumed. Specifically, the degree and duration of mortality associated with chronic exposure to residual oil were much higher than anticipated at the time of the spill and greatly exceeded acute mortality,

which is more obvious and has been the primary concern following most oil spills. This result needs to be incorporated into a new paradigm of understanding of the effects of contaminants on wildlife. Our findings also provided new insights on the mechanisms of recovery. For example, we determined that movements between oiled and unoiled areas were more important to population recovery than we had expected; despite high site fidelity, the much larger number of birds outside of the spill zone provided a pool of individuals to facilitate a “rescue effect” for the depressed, oiled population. Finally, our best estimate of recovery time suggested that full recovery should be accomplished at roughly 24 years post-spill, i.e., near the present time. This, along with the best-case and worst-case scenarios, placed bounds on the duration of oil spill effects on populations that heretofore had not been available.

## REPORT STRUCTURE

The remainder of this report is structured as 2 independent manuscripts, which have been accepted for publication in *Environmental Toxicology and Chemistry* and *Ecological Applications*. These manuscripts, respectively, correspond to the 2 major objectives described in the original proposal: (1) monitoring of cytochrome P4501A, and (2) construction of a population model to evaluate recovery. More specific objectives from the proposal are listed below under headings that correspond to the 2 manuscripts:

***Cytochrome P4501A Monitoring***-- (1) To measure cytochrome P4501A induction in harlequin ducks in oiled and unoiled areas of Prince William Sound to determine the degree of exposure to residual oil from the *Exxon Valdez* oil spill. (2) To evaluate seasonal variation in CYP1A induction.

***Population Modeling***-- (3) To create a population model for harlequin ducks that uses existing demographic data to evaluate (a) the relative magnitudes of acute and chronic oil spill effects, (b) the importance of various demographic constraints on population recovery (i.e., reduced survival, low dispersal, low productivity), (c) the current status of population recovery, and (d) a projected timeline for full recovery.

# 1. CYTOCHROME P4501A BIOMARKER INDICATION OF OIL EXPOSURE IN HARLEQUIN DUCKS UP TO 20 YEARS AFTER THE *EXXON VALDEZ* OIL SPILL

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## ABSTRACT

Hydrocarbon-inducible cytochrome P4501A (CYP1A) expression was measured, as ethoxyresorufin-*O*-deethylase (EROD) activity, in livers of wintering harlequin ducks (*Histrionicus histrionicus*) captured in areas of Prince William Sound, Alaska, USA oiled by the 1989 *Exxon Valdez* spill, and in birds from nearby unoiled areas, during 2005 to 2009 (up to 20 years following the spill). The present work repeated studies conducted in 1998 that demonstrated that in harlequin ducks using areas that received *Exxon Valdez* oil, EROD activity was elevated nearly a decade after the spill. The present findings strongly supported the conclusion that average levels of hepatic EROD activity were higher in ducks from oiled areas than those from unoiled areas during 2005 to 2009. This result was consistent across four sampling periods; further, results generated from two independent laboratories using paired liver samples from one of the sampling periods were similar. EROD activity did not vary in relation to age, sex, or body mass of individuals, nor did it vary strongly by season in birds collected early and late in the winter of 2006 to 2007, indicating that these factors did not confound inferences about observed differences between oiled and unoiled areas. We interpret these results to indicate that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil up to 20 years after the original spill. This adds to a growing body of literature suggesting that oil spills have the potential to affect wildlife for much longer time frames than previously assumed.

## INTRODUCTION

Effects of the 1989 *Exxon Valdez* oil spill on wildlife populations and communities in Prince William Sound, Alaska have been intensively studied, and debated, over the two decades since the spill. One of the more remarkable and unanticipated findings from this body of work was the length of time (at least a decade) over which animals were exposed to residual oil and showed depression of various population demographic attributes (Bodkin et al. 2002, Esler et al. 2002, Peterson 2001, Peterson et al. 2003). Peterson et al. (2003) considered these results to represent a paradigm shift in the way in which oil contamination is thought to affect the environment; in particular, chronic, delayed, and indirect effects of oil spills appear to have much longer and larger consequences on wildlife populations and communities than previously assumed.

Research has continued in areas of Prince William Sound affected by the *Exxon Valdez* spill, to document the process and timeline of population and ecosystem recovery. Spatial and temporal extents of wildlife exposure to lingering *Exxon Valdez* oil have been inferred from indicators of induction of certain members of the cytochrome P450 1 gene subfamily (CYP1A). Vertebrate CYP1A genes are induced by larger polycyclic aromatic hydrocarbons (PAHs), including those found in crude oil, and halogenated aromatic hydrocarbons, including planar polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and difurans (Payne et al. 1987, Goksøyr 1995, Whitlock 1999). Because CYP1A is strongly induced by a limited number of compounds, it can be a particularly useful biomarker, i.e., a measurable physiological response by an organism, for evaluating exposure to those chemicals (Whyte et al. 2000). Although CYP1A induction does not necessarily indicate deleterious effects on individuals or populations (Lee and Anderson 2005), elevated CYP1A levels indicate exposure to inducing compounds and, hence, at least the potential for associated toxic consequences, including subtle effects that may be difficult to detect in nature (Carls et al. 2005). Therefore, indicators of CYP1A have been part of many considerations of environmental effects of contamination, including those associated with the *Exxon Valdez* oil spill.

Indicators of induction of CYP1A mRNA, protein or activity have been used routinely to evaluate exposure to PAHs, PCBs, and dioxins in fish (Stegeman et al. 1986, Gooch et al. 1989, Goksøyr 1995, Spies et al. 1996, Marty et al. 1997, Woodin et al. 1997, Collier et al. 1996, Wiedmer et al. 1996, Jewett et al. 2002, Carls et al. 2005). Although such studies are less common for birds and mammals, indicators of CYP1A levels have been used successfully as biomarkers of exposure of these taxa to inducing compounds, including PAHs (Lee et al. 1985, Peakall et al. 1989, Rattner et al. 1994, Trust et al. 1994; Ben-David et al. 2001; Miles et al. 2007).

In the case of the *Exxon Valdez* oil spill, indicators of CYP1A induction have been used to examine exposure to lingering oil for a number of vertebrates (e.g., Trust et al. 2000, Jewett et al. 2002). These studies demonstrated that, within Prince William Sound,

CYP1A expression levels in many species were higher in areas oiled by the *Exxon Valdez* spill relative to unoiled areas nearly a decade after the spill. The authors of these studies concluded that oil remaining in the environment, particularly in intertidal areas, was encountered and ingested by some nearshore vertebrates. This conclusion is consistent with confirmation of the occurrence of residual *Exxon Valdez* oil in intertidal sediments of Prince William Sound during the same period in which elevated CYP1A was indicated (Short et al. 2004), as well as calculations that intertidal-foraging vertebrates would be likely to encounter lingering oil repeatedly through the course of a year (Short et al. 2006).

Harlequin ducks (*Histrionicus histrionicus*) were one of the species showing indication of elevated CYP1A induction in oiled areas of Prince William Sound relative to unoiled areas (Trust et al. 2000). Harlequin ducks are marine birds that spend most of their annual cycle in intertidal and shallow subtidal zones of temperate and subarctic areas of the Pacific coast of North America (Robertson and Goudie 1999). They are common in Prince William Sound during the nonbreeding season (average of 14,500 individuals between 1990 and 2005; McKnight et al. 2006), and are at higher risk of exposure to residual *Exxon Valdez* oil than many other seabirds, given their exclusive occurrence in nearshore habitats where a disproportionate amount of oil was deposited (Galt et al. 1991, Wolfe et al. 1994) and where lingering oil has remained (Hayes and Michel 1999, Short et al. 2004).

In addition to higher likelihood of exposure, a number of natural history and life history characteristics make harlequin duck individuals and populations particularly sensitive to oil pollution (Esler et al. 2002). These include a diet consisting of invertebrates that live on or in nearshore sediments, a life history strategy predicated on high survival rates, and a small body size, relative to other sea ducks, that may limit their flexibility when faced with increased energetic demands. Consistent with these sensitivities to effects of oil contamination, demographic problems were observed in oiled areas of Prince William Sound during the same period in which elevated CYP1A was indicated, including reductions in population trends (Rosenberg and Petrula 1998), densities (Esler et al. 2000a), and female survival (Esler et al. 2000b) relative to unoiled areas. It was concluded that continued exposure to lingering oil was likely a constraint on population recovery (Esler et al. 2002).

Because of the history of elevated indicators of CYP1A induction (Trust et al. 2000), continued occurrence of lingering oil in intertidal habitats where harlequin ducks occur (Short et al. 2004), and vulnerability of harlequin ducks to effects of oil exposure (Esler et al. 2002), the present study was conducted to follow up on the original research describing elevated biomarkers of CYP1A in this species. In that study, Trust et al. (2000) found that average ( $\pm$  standard error [SE]) CYP1A expression levels, measured by hepatic 7-ethoxyresorufin-*o*-deethylase (EROD) activity, were significantly higher in wintering harlequin ducks captured in areas oiled by the *Exxon Valdez* spill than those captured in nearby unoiled areas ( $204.6 \pm 20.3$  and  $70.7 \pm 21.5$  pmol/min/mg protein, respectively). Samples for the Trust et al. (2000) study were collected in March and April of 1998, nine years after the oil spill. Our primary objective for the present study was to



repeat this work during 2005 to 2009, 16 to 20 years after the *Exxon Valdez* oil spill, to evaluate whether differences in EROD activity persisted.

In addition to assessment of temporal variation, potential effects of individual attributes (age, sex, and body mass) and season on variation in CYP1A induction also were considered. Age, sex, and season have been shown to affect CYP1A induction in some fish (Sleiderink et al. 1995, Goksøyr and Larsen 1991, , Lindstrom-Seppa and Stegeman 1995, Whyte et al. 2000, Kammann et al. 2005), and thus these factors should be accounted for when evaluating sources of variation in CYP1A induction (Lee and Anderson 2005).

## METHODS

### *Capture and Sample Collection*

To facilitate comparisons, the present study closely followed the design and procedures of Trust et al. (2000). We captured wintering harlequin ducks using a modified floating mist net (Kaiser et al. 1995) during four capture periods: March 2005, November 2006, March and April 2007, and March 2009. Birds were captured in a number of areas oiled during the *Exxon Valdez* spill, including Bay of Isles (60° 22' N, 147° 40' W), Crafton Island (60° 29' N, 147° 57' W), Green Island (60°18'N, 147° 24' W), and Foul Pass (60° 29' N, 147° 38' W). Also, birds were captured on nearby northwestern Montague Island (60° 15' N, 147° 12' W), which was not oiled and thus was considered a reference site. Harlequin ducks in Prince William Sound exhibit high site fidelity during winter, with 94% remaining all winter on the same island or coastline region where they were originally captured and only 2% moving between oiled and unoiled areas (Iverson and Esler 2006). We assume that this level of movement had little influence on our ability to draw inferences about differences in EROD activity between areas. Captured birds were placed in portable pet carriers and transported by skiff to a chartered research vessel for processing. Each individual was marked with a uniquely-numbered, U.S. Fish and Wildlife metal tarsus band; the band number was used to identify the data and samples for that individual. Sex of each bird was determined by plumage and cloacal characteristics, and age class was determined by the depth of the bursa of Fabricius for females and bursal depth and plumage characteristics for males (Mather and Esler 1999, Smith et al. 1998). Age class was summarized as either hatch-year (HY), i.e., hatched the previous breeding season, or after-hatch-year (AHY). Numbers of individuals used in analyses of CYP1A induction are indicated in Table 1, by year, season, age class, sex, and area (oiled versus unoiled).

Small (< 0.5 g) liver biopsies were surgically removed by a veterinarian from each harlequin duck while they were under general anesthesia using vaporized and inhaled Isoflurane. Once removed, liver samples were immediately placed into a labeled cryovial and frozen in liquid nitrogen. All samples were maintained in liquid nitrogen or a -80° C freezer until they were shipped to the lab in liquid nitrogen.

During March 2005 sampling, two liver biopsies were taken from each individual and sent to different laboratories for EROD analysis. Dual samples were taken to allow inter-laboratory comparisons for validation of analytical integrity and inference of exposure between oiled and unoiled areas. One of the paired samples was sent to Woods Hole Oceanographic Institution (WHOI), where all pre-2005 samples were analyzed, and the other to the University of California Davis (UCD), where analyses were conducted in subsequent years. Both labs conducted all analyses without *a priori* knowledge of the areas from which the samples were collected and neither lab had access to the data from the other.

### *Laboratory Analyses*

CYP1A induction was determined by measuring hepatic 7-ethoxyresorufin-*o*-deethylase activity, which is a catalytic function principally of hydrocarbon-inducible CYP1A enzymes. Birds possess two CYP1A genes (CYP1A4 and CYP1A5) and the expression of both appears to be inducible by AH receptor agonists in some species. Both CYP1As also catalyze EROD in some species (Kubota, A. personal communication). In studies of captive harlequin ducks, EROD activity was confirmed to be significantly higher in birds chronically ingesting weathered Prudhoe Bay crude oil, compared to controls (Esler 2008). Similarly, oil-dosed Steller's eiders (*Polysticta stelleri*), another sea duck, had roughly 4-fold increased EROD activity compared to controls (Miles et al. 2007). EROD activity analysis procedures followed standard methods used in previous studies, described in detail by Trust et al. (2000) for analyses at WHOI and Miles et al. (2007) for those at UCD. The measure of EROD activity is expressed in picomoles per minute per milligram of protein, i.e., pmol/min/mg protein.

### *Statistical Analyses*

Four analyses were conducted to evaluate variation in EROD activity. First, results from the WHOI and UCD laboratories were compared for paired samples collected during March 2005 to determine if consistent results were obtained. Next, EROD activity was evaluated in relation to capture location (oiled or unoiled area) and individual attributes (age, sex, and body mass) for the March 2005 samples using data from both WHOI and UCD laboratories. Third, EROD activity was evaluated in relation to location of capture, individual attributes, and season for samples obtained during November 2006 and March/April 2007 and analyzed at the UCD laboratory. Finally, variation in EROD activity was analyzed in relation to capture location and individual attributes for birds captures during March 2009. Separate analyses were run for data from each winter because we wished to compare results from both labs in 2005, so analyzing 2005 separately facilitated direct comparison of inferences from each data set. Also, considerable variation can occur between laboratory runs (Esler 2008); this does not affect contrasts between treatments within runs, but could complicate interpretation across runs. Finally, different sets of explanatory variables were used among winters, as seasonal effects could only be evaluated using data from winter 2006-07. For consistency, results from the UCD lab were used as the primary data, although results and inferences were contrasted across both labs for the 2005 data.

## Laboratory comparison

EROD activity of liver samples collected during March 2005 was compared between laboratories using the paired samples collected from 40 individuals. Laboratory results were contrasted using a simple linear regression, with the expectation that there would be low unexplained variation around this relationship if the two labs were giving similar results. We recognize that different analytical runs, even within a lab, may generate different absolute values of EROD activity (Esler 2008), but one would still expect the different labs to have a strong correlation, even if the slope differs from 1.

## EROD activity March 2005

For the 2005 data, our primary interest was to determine whether area (oiled versus unoiled) explained variation in EROD activity, after accounting for any effects of age class, sex, and body mass. Least squares general linear models (GLM) were used to estimate variation explained by each of a candidate set of models that included different combinations of variables of interest, and an information-theoretic approach was used for model selection and inference (Burnham and Anderson 2002) in which support for various model configurations is contrasted using Akaike's Information Criterion (AIC). Age, sex, and body mass variables (which we termed *individual attributes*) were included or excluded as a group, i.e., models either included all of these variables or none of them. We used singular and additive combinations of area and individual attribute effects, resulting in a candidate model set including: (1) EROD = area; (2) EROD = individual attributes; and (3) EROD = area + individual attributes. We also included a null model, which consisted of estimates of a mean and variance across all of the data; strong support for the null model would indicate that variables considered in other candidate models did not explain important variation in the response.

The model with the lowest AIC value corrected for small sample size ( $AIC_c$ ) was considered to have the strongest support from the data among the models considered. Another metric,  $AIC_c$  weight ( $w$ ), was calculated for each model; these sum to 1.0 across the entire model set and provide a measure of relative support for candidate models. The variables included in the models with highest support are considered to explain important variation in the response. Parameter likelihoods, which are the sums of  $w$  for all models including a given parameter, indicate the relative support for that variable, taking into account model uncertainty. Parameter likelihoods close to 1 indicate strong support. Finally, weighted parameter estimates and associated unconditional standard errors were calculated, which are estimates of the size, direction, and associated variation of effects of variables after accounting for model uncertainty.

## EROD activity winter 2006/2007

A similar analysis was conducted for data collected during November 2006 and March and April 2007. All of these samples were run concurrently at the UCD lab, and thus there was no inter-lab or inter-run variation to consider. Using GLMs and information-

theoretic methods of model selection and inference, as described above, we evaluated variation in EROD in relation to singular and additive combinations of individual attributes, area, and, in this case, season (November versus March and April). Therefore, the candidate set included the following 8 models: (1) EROD = individual attributes; (2) EROD = area; (3) EROD = season; (4) EROD = individual attributes + area; (5) EROD = individual attributes + season; (6) EROD = area + season; (7) EROD = individual attributes + area + season; and (8) EROD = null.

### EROD activity March 2009

Our analysis of data from samples collected during March 2009 was the same as that conducted for March 2005. Note that data were generated only from the UCD lab for March 2009 samples.

## RESULTS

### *Laboratory comparison*

Based on paired liver biopsies from harlequin ducks captured in March 2005, we found a strong correlation ( $r^2 = 0.70$ ; Figure 1) between results reported from WHOI and those reported independently from UCD. Averages ( $\pm$  SE) differed somewhat by laboratory (birds captured on areas oiled by the *Exxon Valdez* spill =  $194.9 \pm 30.1$  at WHOI and  $161.3 \pm 31.2$  at UCD, and those captured from unoiled areas =  $96.6 \pm 14.4$  at WHOI and  $55.3 \pm 13.7$  at UCD), although area differences were readily apparent in both data sets (Figure 2). Similarly, the slope of the relationship between the two labs (using results from WHOI as the response) was less than one ( $0.71 \pm 0.08$ ), indicating that the different labs reported data with somewhat different absolute values, as reported elsewhere (Esler 2008). However, the close correlation, as well as the similarity in GLM results using both data sets (below), indicated that data from both labs supported the same inference about differences in EROD activity levels in relation to individual attributes and areas.

### *EROD activity March 2005*

Variation in EROD activity of harlequin ducks captured in March 2005 was strongly associated with whether they were from oiled or unoiled areas. Based on UCD analyses, the model with area as the only explanatory variable received nearly ten times the support of any other model, with a  $w$  of 0.87 (Table 2). The group of individual attribute variables did not explain meaningful variation in EROD, as both models including individual attributes had small  $w$  and received less support than the null model (i.e., had larger  $AIC_c$  values; Table 2). Analyses from WHOI corroborated these conclusions; the order of candidate models and the relative support for each closely matched those based on UCD analyses (Table 2).

Parameter likelihood values also supported the importance of area for explaining variation in March 2005 EROD activity. Using the UCD analysis results, the area

parameter was strongly supported, with a parameter likelihood of 0.91 (Table 3). Also, the weighted parameter estimate indicated that areas differed by an average of 96.0 pmol/min/mg protein, with EROD activity markedly higher in oiled areas (Figure 2). Parameter likelihood values for individual attributes were small, and the weighted parameter estimates were smaller than the corresponding unconditional standard errors (Table 3), further indicating that they did not have strong explanatory value. As with the model ranking described above, the patterns in parameter likelihoods, weighted parameter estimates, and unconditional SE based on WHOI results mirrored those from UCD (Table 3; Figure 2), strengthening confidence in the inference drawn from these analyses.

#### *EROD activity winter 2006/2007*

For samples from winter 2006-2007, analyzed only in the UCD lab, we found that the model with area as the only explanatory variable was best supported, with a  $w$  of 0.51 (Table 4). However, the model with area and season as explanatory variables also received considerable support, with an  $AIC_c$  value that was only 0.4 from the best-supported model and a  $w$  of 0.41. None of the other candidate models received substantial support, including all other models with season as an explanatory variable. Consistent with these findings, the parameter likelihood value for area was 1.0, indicating that only models including area received any meaningful support from the data (Table 3). The weighted parameter estimate for area indicated that EROD was significantly higher in oiled areas compared to unoiled (Table 3; Figure 3). Parameter likelihoods for individual attributes were low and the size of unconditional SE exceeded the weighted parameter estimates in all cases (Table 3), which confirmed that these variables did not explain important variation in the response. The parameter likelihood for season indicated moderate support (Table 3), based on the inclusion of season in the second-best model; however, the weighted parameter estimate for season was relatively small (-7.7 pmol/min/mg protein) and 95% confidence intervals around this estimate ( $\pm 1.96*SE$ ) broadly overlapped zero, further suggesting the lack of importance of season for explaining variance in EROD. Figure 3 graphically illustrates the modest seasonal difference in EROD, as well as the consistent and large difference by area.

#### *EROD activity March 2009*

Consistent with earlier sampling periods, the best supported model for March 2009 data indicated differences in average EROD activity between areas, with a  $w$  of 0.73 (Table 5). As in previous winters, average EROD activity was higher in oiled areas relative to unoiled areas (Fig. 3). The null model received a modest amount of support ( $w = 0.21$ ), but less than a third of the support received by the model including an area effect. The importance of the area term is reflected in the parameter likelihood of 0.76 (Table 3). Models including individual attributes received very little support, indicating that age, sex, and mass were not strong correlates with EROD, which was confirmed by the low parameter likelihood for these explanatory variables.

## DISCUSSION

We found that hepatic CYP1A levels in harlequin ducks captured during 2005 - 2009, based on EROD activity, were unequivocally higher in areas oiled during the *Exxon Valdez* spill than in nearby unoiled areas. This conclusion was strongly supported over multiple sample periods, as well as by two independent labs for one of the sampling periods. Our results are consistent with the findings of Trust et al. (2000) from eleven years prior that harlequin ducks were exposed to CYP1A inducers more frequently or in higher concentrations at oiled areas relative to unoiled areas. We interpret the current results as evidence that harlequin ducks continued to be exposed to residual oil from the *Exxon Valdez* spill through at least 2009, 20 years after the spill. This interval of time is much longer than conventional assumptions about duration of bioavailability of spilled oil (Peterson et al. 2003). Evidence of continued exposure indicates that deleterious effects on individuals or populations also are possible to have persisted over this time frame, although we recognize that exposure can not necessarily be inferred to indicate damage (Lee and Anderson 2005).

Similar spatial patterns of CYP1A induction have been described for other vertebrates in Prince William Sound, including Barrow's goldeneyes (*Bucephala islandica*; Trust et al. 2000), adult pigeon guillemots (*Cephus columba*; Golet et al. 2002), river otters (*Lontra canadensis*; Bowyer et al. 2003), and two demersal fishes (Jewett et al. 2002), masked greenlings (*Hexagrammos octogrammus*) and crescent gunnels (*Pholis laeta*), within a decade of the *Exxon Valdez* spill. This body of evidence overwhelmingly supports the conclusion that harlequin ducks, along with other nearshore vertebrates, were being exposed to CYP1A-inducing compounds in areas of Prince William Sound, Alaska that received oil during the *Exxon Valdez* spill.

Some authors have questioned the source of CYP1A inducing compounds in Prince William Sound (Harwell and Gentile 2006), recognizing that there may be multiple CYP1A-inducing compounds from multiple sources within a given area (Lee and Anderson 2005). Several authors (Page et al. 1996, 1997, Boehm et al. 2001, Harwell and Gentile 2006) have argued that non-*Exxon Valdez* sources of PAHs are more abundant and more likely to induce CYP1A responses than residual Exxon Valdez oil. However, the spatial correspondence between elevated CYP1A induction and history of contamination during the *Exxon Valdez* oil spill strongly suggests causation. Also, other studies have indicated that PAHs in the areas where elevated CYP1A was observed in vertebrates are predominately from the *Exxon Valdez* spill (Short et al. 2004), supporting the inference that *Exxon Valdez* oil was the inducing agent. Recent studies have indicated that sites with residual *Exxon Valdez* oil had bioavailable PAHs that elicited CYP1A induction when experimentally injected into fish (Springman et al. 2008). Other potential CYP1A inducers, specifically PCBs, were very low and below concentrations that would induce CYP1A induction, consistent with broad-scale atmospheric deposition (Short et al. 2008). In addition, Trust et al. (2000) considered the potential role of PCBs in observed CYP1A induction in sea ducks in Prince William Sound and found that plasma concentrations were very low and generally were not related to EROD activity. In addition, Short et al. (2006) calculated that, given the distribution of residual *Exxon*

*Valdez* oil through 2003, benthic foraging vertebrates were likely to encounter lingering oil, further suggesting that residual *Exxon Valdez* oil was the inducing compound.

Vertebrates that inhabit the intertidal and shallow subtidal environments, particularly those that consume benthic organisms, were most likely to have elevated CYP1A (Bodkin et al. 2002, Esler et al. 2002). This is presumably due, in part, to that fact that intertidal areas of Prince William Sound received a large portion of the spilled *Exxon Valdez* oil (Galt et al. 1991, Wolfe et al. 1994) and sequestered lingering oil a decade or more post-spill (Hayes and Michel 1999, Short et al. 2004). Also, because certain molluscan invertebrates have a limited capacity to metabolize PAHs (e.g., Chaty et al. 2004) and are known to bioaccumulate PAHs (Short and Harris 1996, Fukuyama et al. 2000, Rust et al. 2004), predators such as harlequin ducks may be more likely to ingest PAHs with their prey. Also, invertivores disturb sediment during foraging, which is a potential mechanism for release of hydrocarbons and ingestion.

Consistent with predictions of increased exposure to residual oil and vulnerability to subsequent effects, as well as empirical evidence of exposure (Trust et al. 2000, Bodkin et al. 2002), invertivorous, nearshore-dwelling vertebrates have been shown to have population demographic attributes outside of the normal range during the period since the *Exxon Valdez* oil spill. For example, sea otter numbers in heavily oiled regions of Prince William Sound were well below estimates of pre-spill numbers (Bodkin et al. 2002). Also, sea otter survival in oiled areas was depressed through at least 1998 (Monson et al. 2000). Similar evidence of post-spill demographic problems was described for harlequin ducks (Esler et al. 2002). Densities of wintering harlequin ducks in 1996 and 1997 were lower than expected in oiled areas of Prince William Sound, after accounting for effects of differing habitat (Esler et al. 2000a). Also, survival of wintering female harlequin ducks was lower in oiled areas than unoiled (Esler et al. 2000b) during 1995 to 1998. More recent estimates have indicated that harlequin duck survival during winters 2000 to 2003 did not differ between oiled and unoiled areas (Esler and Iverson 2010), suggesting that despite the evidence of continued exposure reported herein, oil-induced effects on demographic rates may be diminishing.

In addition to potential relationships between oil exposure and demographic rates (Esler et al. 2002), more subtle effects at the suborganismal and molecular level are plausible. Rainbow trout (*Oncorhynchus mykiss*) showed increased mortality in response to viral challenge when they had been exposed to a CYP1A inducer (Springman et al. 2005). In mammals, CYP1A1 is known to activate PAH to toxic and mutagenic derivatives (Nebert et al. 2004). In birds, Trust et al. (1994) identified effects of PAHs on immune function and mixed-function oxygenase activity (e.g., EROD) in European starlings (*Sturnus vulgaris*). In controlled dose experiments, crude oil and PAHs have been linked to impaired reproduction, depressed weight gain, increased organ weight, increased endocrine activity, or mixed-function oxygenase activity in several avian taxa (Hoffman 1979, Naf et al. 1992, Peakall et al. 1980, Peakall et al. 1981). Induction of CYP1A gene expression does not in itself represent an adverse effect, and with some substrates or inducers could be principally an adaptive response. However, it can be a marker of exposure to PAHs demonstrated to have adverse effects on birds. Associations between

aryl hydrocarbon receptor agonist activation and subsequent effects, including possible involvement of the multiple CYP1 genes that are expressed in birds, have not been fully explored in relation to the effects of the Exxon Valdez oil spill and research is warranted to appropriately assess those effects on harlequin ducks and other species at risk of exposure.

In summary, the EROD levels reported here provide strong evidence of CYP1A induction in harlequin ducks from oiled areas, which we conclude is due to continued exposure to residual *Exxon Valdez* oil, and that harlequin ducks remain at risk of potential deleterious consequences of that exposure. This work extends the timeline of exposure to 18 years post-spill, and adds to the body of evidence describing the previously unanticipated duration of exposure and potential effects of the *Exxon Valdez* oil spill. We note that oil from other contamination events also has been reported to persist over long periods of time (Corredor et al. 1990, Burns et al. 1994, Vandermeulen and Singh 1994, Reddy et al. 2002, Peacock et al. 2005). We agree with Peterson et al. (2003) that the conventional paradigm that the duration of presence of residual oil and associated effects is limited to a few years should be abandoned and replaced with the recognition that these may occur over decades. We recommend that monitoring of indicators of CYP1A induction in harlequin ducks in Prince William continue until EROD in oiled areas has returned to background levels, to fully describe the timeline over which exposure occurs.

## ACKNOWLEDGEMENTS

This research was supported primarily by the *Exxon Valdez* Oil Spill Trustee Council. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council. We thank those who helped with field work, under frequently difficult winter conditions, including: A. Birmingham, J. Bond, T. Bowman, M. Kirk, N. Slosser, and K. Wright. Veterinary expertise during field work was provided by Drs. D. Heard, M. McAdie, G. Myers, and J. Proudfoot. We thank D. Rand and his crew of the motor vessel *Discovery* for safe and comfortable passage. We also appreciate the institutional support provided by D. Derksen, D. Bohn, R. Ydenberg, A. Patterson, and M. Court. We thank J. Short and K. Springman for review of the manuscript.

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## TABLES AND FIGURES

Table 1.1. Sample sizes of harlequin ducks captured in Prince William Sound, Alaska for analyses of cytochrome P4501A induction. Numbers are listed by sampling period, sex and age class cohort, and capture area (oiled during *Exxon Valdez* oil spill versus unoiled).

Cohort <sup>a</sup>	March 2005		November 2006		March/April 2007		March 2009	
	Oiled	Unoiled	Oiled	Unoiled	Oiled	Unoiled	Oiled	Unoiled
AHY M	13	11	14	9	11	10	14	13
HY M	1	1	0	2	2	0	0	2
AHY F	5	3	9	8	7	9	4	4
HY F	1	1	2	1	5	1	1	1
TOTAL	20	20	25	20	25	20	19	20

<sup>a</sup>Cohort consists of an age class designation (HY = hatch-year, i.e., within one year of hatching; AHY = after-hatch-year) and sex (M = male; F = female).



Table 1.2. Results of information-theoretic analyses using general linear models to evaluate variation in hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity of harlequin ducks (n = 40) captured in Prince William Sound, Alaska during March 2005. Results are reported from two laboratories, University of California Davis (UCD) and Woods Hole Oceanographic Institution (WHOI), that independently analyzed paired samples from each individual.

Model	K <sup>a</sup>	UCD			w <sup>d</sup>	WHOI	
		AIC <sub>c</sub> <sup>b</sup>	ΔAIC <sub>c</sub> <sup>c</sup>			AIC <sub>c</sub>	ΔAIC <sub>c</sub>
EROD = Area <sup>e</sup>	3	391.1	0.0	0.87	377.3	0.0	0.91
EROD = null	2	395.7	4.6	0.09	383.2	5.9	0.05
EROD = Area + Individual <sup>f</sup>	6	397.4	6.3	0.04	383.6	6.5	0.04
EROD = Individual	5	401.5	10.4	0.00	388.6	11.3	0.00

<sup>a</sup>K = number of estimated parameters in the model.

<sup>b</sup>AIC<sub>c</sub> = Akaike's Information Criterion, corrected for small sample size.

<sup>c</sup>ΔAIC<sub>c</sub> = difference in AIC<sub>c</sub> from the best supported model.

<sup>d</sup>w = AIC<sub>c</sub> weight.

<sup>e</sup>Area = categorical variable indicating areas either oiled during the *Exxon Valdez* spill or unoiled.

<sup>f</sup>Individual = a grouping of variables describing attributes of individuals (age, sex, and mass).

Table 1.3. Parameter likelihoods (P.L.), weighted parameter estimates, and unconditional standard errors (SE) derived from information-theoretic analyses using general linear models to evaluate variation in hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/min/mg protein) of harlequin ducks captured in Prince William Sound, Alaska, USA during March 2005, winter 2006 to 07 (including samples from November 2006 and March and April 2007), and March 2009. Results from March 2005 samples are presented for data reported from two laboratories, University of California Davis (UCD) and Woods Hole Oceanographic Institution (WHOI), that independently analyzed paired samples from each individual.

Parameter	WHOI – March 2005		UCD – March 2005		UCD – 2006 and 2007		UCD – March 2009	
	P.L.	Estimate ± SE	P.L.	Estimate ± SE	P.L.	Estimate ± SE	P.L.	Estimate ± SE
Intercept	1.00	115.0 ± 51.7	1.00	70.4 ± 55.4	1.00	22.9 ± 24.1	1.00	50.37 ± 62.87
Area <sup>a</sup>	0.95	93.1 ± 32.0	0.91	96.0 ± 37.1	1.00	68.7 ± 13.4	0.76	50.23 ± 37.85
Sex <sup>b</sup>	0.04	-2.3 ± 3.1	0.04	-0.7 ± 3.0	0.09	-0.0 ± 2.1	0.06	-4.43 ± 9.46
Age <sup>c</sup>	0.04	-0.2 ± 2.4	0.04	-2.9 ± 4.2	0.09	2.2 ± 4.7	0.06	3.24 ± 7.87
Mass	0.04	-0.02 ± 0.04	0.04	-0.01 ± 0.04	0.09	0.00 ± 0.02	0.06	-0.04 ± 0.09
Season <sup>d</sup>	---	---	---	---	0.44	-7.7 ± 11.7	---	---

<sup>a</sup>Area = categorical variable indicating areas either oiled during the *Exxon Valdez* spill or unoiled, with unoiled as the reference value.

<sup>b</sup>Sex = categorical variable (male versus female), with male as the reference value.

<sup>c</sup>Age = categorical variable (hatch-year versus after-hatch-year), with hatch-year as the reference value.

<sup>d</sup>Season = categorical variable (November versus March and April), with November as the reference value.

Table 1.3. Results of information-theoretic analyses using general linear models to evaluate variation in hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity of harlequin ducks (n = 90) captured in Prince William Sound, Alaska during winter 2006-2007.

Model	K <sup>a</sup>	AIC <sub>c</sub> <sup>b</sup>	ΔAIC <sub>c</sub> <sup>c</sup>	w <sup>d</sup>
EROD = Area <sup>e</sup>	3	750.4	0.0	0.51
EROD = Area + Season <sup>f</sup>	4	750.9	0.4	0.41
EROD = Area + Individual <sup>g</sup>	6	755.0	4.5	0.05
EROD = Area + Season + Individual	7	755.9	5.4	0.03
EROD = null	2	771.7	21.3	0.00
EROD = Season	3	772.5	22.1	0.00
EROD = Individual	5	777.7	27.2	0.00
EROD = Season + Individual	6	778.5	28.1	0.00

<sup>a</sup>K = number of estimated parameters in the model.

<sup>b</sup>AIC<sub>c</sub> = Akaike's Information Criterion, corrected for small sample size.

<sup>c</sup>ΔAIC<sub>c</sub> = difference in AIC<sub>c</sub> from the best supported model.

<sup>d</sup>w = AIC<sub>c</sub> weight.

<sup>e</sup>Area = categorical variable indicating areas either oiled during the *Exxon Valdez* spill or unoiled.

<sup>f</sup>Season = categorical variable indicating period of sample collection (November versus March and April)

<sup>g</sup>Individual = a grouping of variables describing attributes of individuals (age, sex, and mass).

Table 1.5. Results of information-theoretic analyses using general linear models to evaluate variation in hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity of harlequin ducks ( $n = 39$ ) captured in Prince William Sound, Alaska during March 2009.

Model	K <sup>a</sup>	AIC <sub>c</sub> <sup>b</sup>	ΔAIC <sub>c</sub> <sup>c</sup>	w <sup>d</sup>
EROD = Area <sup>e</sup>	3	358.3	0.0	0.73
EROD = null	2	360.9	2.5	0.21
EROD = Individual	5	364.6	6.3	0.03
EROD = Area + Individual <sup>f</sup>	6	364.7	6.3	0.03

<sup>a</sup>K = number of estimated parameters in the model.

<sup>b</sup>AIC<sub>c</sub> = Akaike's Information Criterion, corrected for small sample size.

<sup>c</sup>ΔAIC<sub>c</sub> = difference in AIC<sub>c</sub> from the best supported model.

<sup>d</sup>w = AIC<sub>c</sub> weight.

<sup>e</sup>Area = categorical variable indicating areas either oiled during the *Exxon Valdez* spill or unoiled.

<sup>f</sup>Individual = a grouping of variables describing attributes of individuals (age, sex, and mass).

Figure 1.1. Scatterplot of hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity (pmol/min/mg protein) contrasting results from two labs (WHOI = Woods Hole Oceanographic Institution; UCD = University of California Davis) that independently analyzed subsamples of the same livers collected from harlequin ducks (n = 40) in Prince William Sound in March 2005.

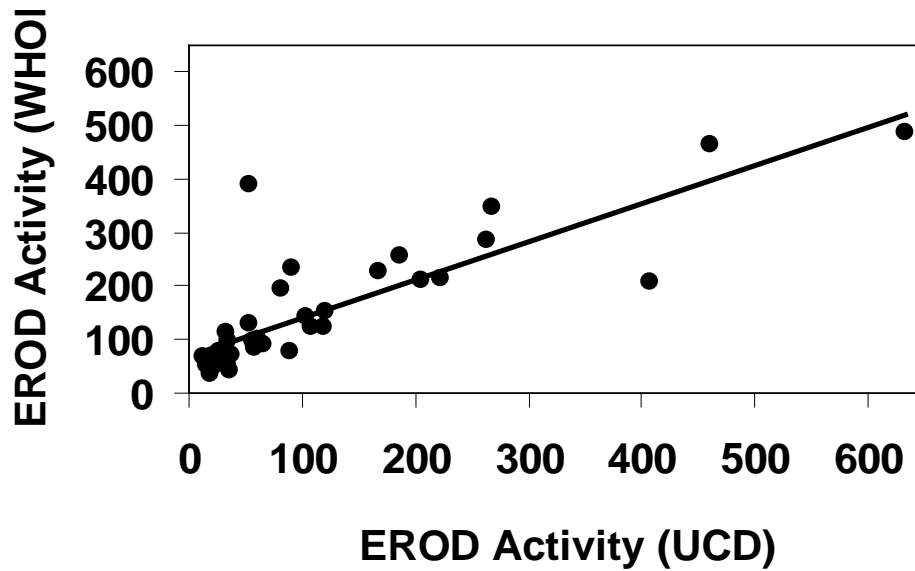


Figure 1.2. Average ( $\pm$  SE) hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity of harlequin ducks ( $n = 40$ ) captured in March 2005 in areas of Prince William Sound, Alaska oiled during the Exxon Valdez spill and nearby unoiled areas. Results are presented for two labs (WHOI = Woods Hole Oceanographic Institution; UCD = University of California Davis) that independently analyzed subsamples of the same livers.

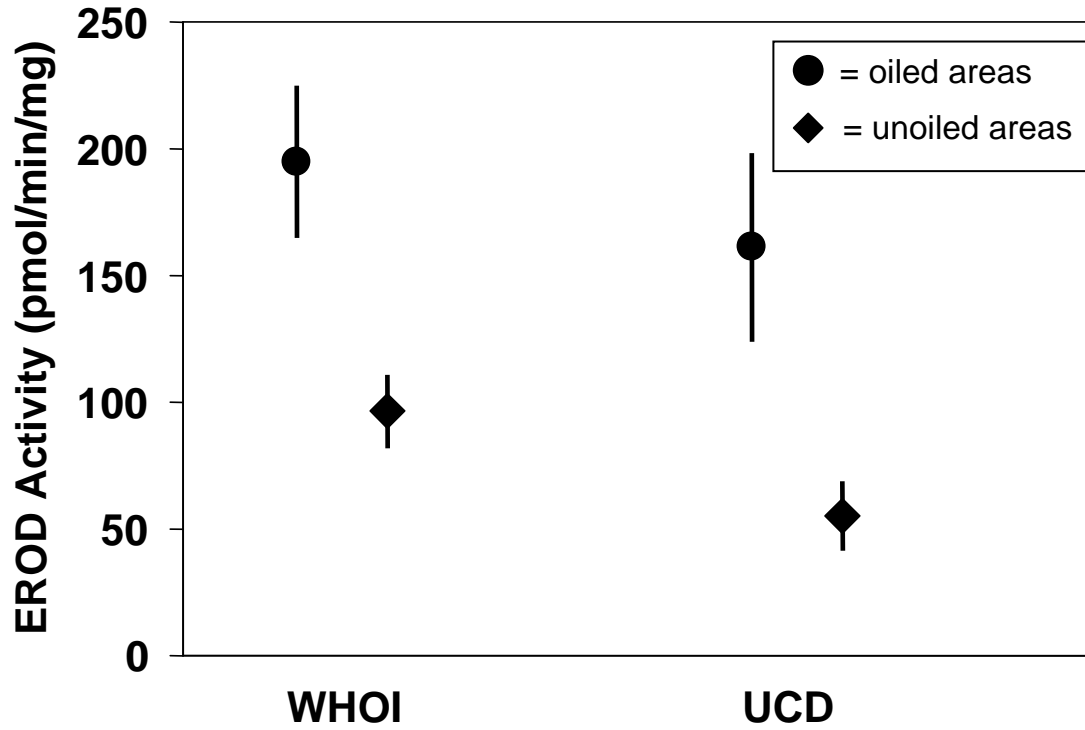
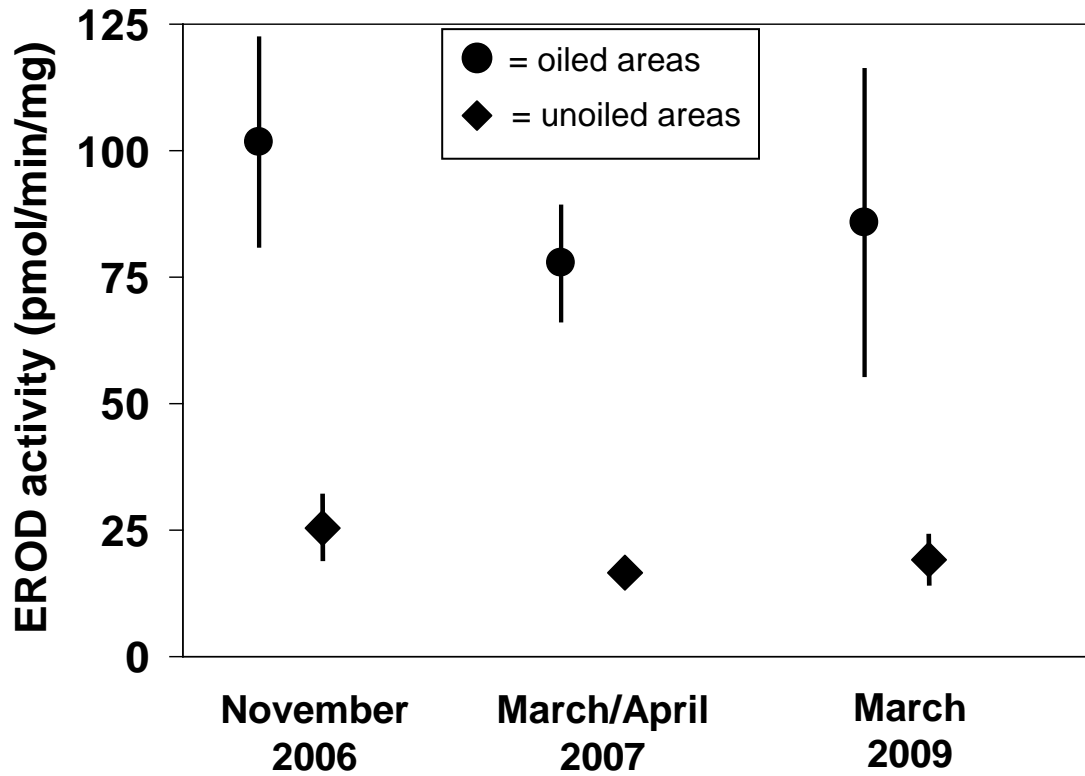


Figure 1.3. Average ( $\pm$  SE) hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity of harlequin ducks captured during winter 2006-2007 ( $n = 90$ ) and March 2009 ( $n = 39$ ) in areas of Prince William Sound, Alaska oiled during the Exxon Valdez spill and nearby unoiled areas. Samples were analyzed concurrently at the University of California Davis and results are presented separately for two capture seasons.



## 2. HARLEQUIN DUCK POPULATION DYNAMICS FOLLOWING THE 1989 *EXXON VALDEZ* OIL SPILL: ASSESSING INJURY AND PROJECTING A TIMELINE TO RECOVERY

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### ABSTRACT

The 1989 *Exxon Valdez* Oil Spill caused significant injury to wildlife populations in Prince William Sound, Alaska. Harlequin ducks (*Histrionicus histrionicus*) were particularly vulnerable to the spill and have been studied extensively since, leading to one of the most thorough considerations of the consequences of a major oil spill ever undertaken. We compiled demographic and survey data collected since the spill to evaluate the timing and extent of mortality using a population model. During the immediate aftermath of the spill, we estimated a 25% decrease in harlequin duck numbers in oiled areas. Survival rates remained depressed in oiled areas 6-9 years after the spill and did not equal those from unoiled areas until at least 11-14 years later. Despite a high degree of site fidelity to wintering sites, immigration was important for recovery dynamics, as the relatively large number of birds from habitats outside the spill zone provided a pool of individuals to facilitate numerical increases. On the basis of these model inputs and assumptions about fecundity rates for the species, we projected a timeline to recovery of 24 years under the most-likely combination of variables, with a range of 16 to 32 years for the best-case and worst-case scenarios, respectively. Our results corroborate assertions from other studies that the effects of spilled oil on wildlife can be expressed over much longer time frames than previously assumed and that the cumulative mortality associated with chronic exposure to residual oil may actually exceed acute mortality, which has been the primary concern following most oil spills.

### INTRODUCTION

Oil spills can have dramatic effects on wildlife and marine ecosystems. The *Exxon Valdez* Oil Spill (EVOS), which occurred on 24 March 1989 and resulted in the release of approximately 42 million litres of crude oil into Prince William Sound, Alaska (Galt et al. 1991, Wolfe et al. 1994; Fig. 1), is among the best known, most damaging, and well studied oil spills in history (Laughlin 1994, Wells et al. 1995, Paine et al. 1996, Rice et al. 1996, Peterson et al. 2003). In the immediate aftermath of the EVOS most attention by scientists and the public focused on assessing the extent of shoreline contamination and quantifying the magnitude of acute injury to wildlife resources. Indeed, hundreds of thousands of marine bird and mammal deaths were attributed to direct contact with floating oil on the sea surface during the immediate aftermath of the spill (Piatt et al. 1990, Garrott et al. 1993). However, long-term effects also were evident.



Residual oil remained trapped in intertidal and subtidal sediment in some areas (Wolfe et al. 1994, Short and Babcock 1996, Hayes et al. 1999, Short et al. 2004, 2006) and negative physiological and demographic effects were associated with chronic exposure to residual oil by wildlife (Bodkin et al. 2002, Golet et al. 2002, Esler et al. 2002, Bowyer et al. 2003).

Harlequin ducks (*Histrionicus histrionicus*) were among the species that experienced significant injury as a result of the EVOS (Esler et al. 2002). Several aspects of harlequin duck ecology rendered their population particularly vulnerable to the spill, including the species close association with marine intertidal habitats (Roberson and Goudie 1999) and a diet that consists primarily of benthic invertebrates in which oil constituents bioaccumulate (Fukuyama et al. 2000, Peterson 2001). Moreover, Prince William Sound is at the northern extent of the species range, where the additive effects of harsh winter weather, coupled with the high metabolic costs of dive-foraging, are thought to reduce their resilience to perturbation (Goudie and Ankney 1986). In addition, harlequin duck life histories are characterized by high site fidelity, low annual productivity and high adult survival (Goudie et al. 1994). These are characteristics that typically evolve in stable environments and can lead to delayed recovery dynamics even after constraints on population growth are removed.

Using data from carcass recoveries (Piatt et al. 1990, Piatt and Ford 1996) and population surveys (Lance et al. 1999), Esler et al. (2002) estimated that approximately 7% of the harlequin ducks present in Prince William Sound at the time of the spill died as a result of acute exposure to spilled oil. Hydrocarbon metabolites were found in a further 74% of live harlequin ducks collected from oiled areas during 1989 and 1990, implying potential for further lethal or sublethal damages (Patten et al. 2000). Monitoring studies initiated during the mid-1990s to measure physiological evidence for exposure to residual oil found elevated cytochrome P450 (CYP1A) induction in harlequin ducks from oiled areas compared to unoiled reference sites (Trust et al. 2000) and these findings were corroborated by studies on other near-shore marine vertebrates, including Barrow's goldeneyes (*Bucephala islandica*; Trust et al. 2000), pigeon guillemots (*Cepphus columba*; Golet et al. 2002), river otters (*Lontra canadensis*; Bowyer et al. 2003), masked greenlings (*Hexagrammos octogrammus*; Jewett et al. 2002), and crescent gunnels (*Pholis laeta*; Jewett et al. 2002). Concurrent with this physiological evidence, population surveys indicated numerical declines for harlequin ducks in oiled areas (Rosenberg and Petrula 1998), densities that were lower than expected after considering variation in habitat attributes (Esler et al. 2000a), and lower winter survival rates (Esler et al. 2000b). More recent studies suggest that differences in survival between oiled and unoiled areas largely disappeared by the early 2000s (Esler and Iverson 2010); however residual oil persists in some locations (Short et al. 2006) and CYP1A induction by harlequin ducks in oiled areas remains elevated (Esler et al. 2010).

Despite the fact that a range of population parameters have been estimated for harlequin ducks in the wake of the EVOS, these data have never been used to quantify the extent of mortality related to the spill. In this paper, we compiled demographic and survey data collected during the last 20 years to construct a set of projection matrix population models and explore post-spill recovery dynamics. Similar models have been used in wildlife management decision making (e.g., Hitchcock and Gratto-Trevor 1997, Beissinger and Westphal 1998, Flint et al. 2006) and have the advantage over approaches that rely solely on numerical endpoints in that they allow

testable predictions to be made about the factors underlying population growth rates (Gauthier et al. 2007). Our specific objectives were to (1) compare the relative magnitudes of acute versus chronic oil spill mortality, (2) determine the importance of various demographic constraints on recovery (i.e., reduced survival, low productivity, and low dispersal), (3) assess current recovery status, and (4) project a timeline to recovery for harlequin ducks and thereby provide scientific insight into the factors regulating post-spill population dynamics.

## METHODS

### *Modeling approach*

We used an age-structured demographic projection matrix model to assess population growth rates (Caswell 2001, Morris and Doak 2002). In our model we evaluated female vital rates because for waterfowl with male-biased sex ratios females are the limiting sex from a population growth perspective (Johnson et al. 1992). We employed a birth-pulse projection to reflect the synchronous breeding pattern of northern hemisphere migratory birds and used a post-breeding census formulation for the state transition when individuals advance an age class. We divided the life cycle into juvenile and adult classes, with separate estimates of annual survival for each. We defined the juvenile period as that extending from when young birds first arrive on coastal areas in year  $t$  (at the age of ~50 days or ~0.1 years) until the start of the next non-breeding season in year  $t+1$  (~1.1 years of age). We assumed that only adults breed, thus the reproduction term in our projection matrix, fecundity ( $F$ ), represents the number of juvenile females that each adult female in the current census will contribute to the next census. To make such a contribution, the breeding female itself must survive and reproduce; making  $F$  the product of adult survival ( $s_{2+}$ ) and fertility ( $f$ ; Fig. 2).

The EVOS was a one-time perturbation without replication and for which little pre-spill data are available for comparison (Paine et al. 1996). As such, we relied on several simplifying assumptions, the most important of which was that in the absence of oil spill effects demographic rates should be similar across habitats. This assumption has been subject to interpretation (Wiens and Parker 1995, Wiens et al. 2001); however, it is well supported by the available data within the range of habitats and spatial scales under consideration (Esler et al. 2002). We defined recovery as a return to the long-term average from survey counts and used a deterministic formulation because we were not able to assess the effect of environmental variation on recovery rates. Finally, in instances where empirical estimates were not available for certain demographic parameters, we evaluated alternative scenarios using plausible values garnered from the literature to place confidence bounds on estimates.

### *Data sources*

Several studies were relevant for our quantitative assessment of post-spill population dynamics. Our uses of these data are summarized below. Briefly,

*Abundance* – U.S. Fish and Wildlife Service (Irons et al. 1988, Klosiewski and Laing 1994, Agler et al. 1994, Agler and Kendall 1997, Lance et al. 1999, Sullivan et al. 2004, McKnight et al. 2006), Alaska Department of Fish and Game (Rosenberg and Petrusla 1998, Rosenberg et al.

2005), and Exxon Corporation (Wiens et al. 1996, Day et al. 1997, Murphy et al. 1997) all sponsored and/or collected data to describe trends in marine bird abundance after the EVOS. There has been some disagreement over interpretation of survey results (see Irons et al. 2000, Wiens et al. 2001, Irons et al. 2001), most of which stems from methodological differences and difficulties inherent in separating oil spill effects from natural environmental variation. For our purposes, Alaska Department of Fish and Game estimates of population sex ratios (Rosenberg et al. 2005) and U.S. Fish and Wildlife Service estimates of total abundance (McKnight et al. 2006) were the most useful for parameterizing our model (Table 1).

*Survival* – Survival estimates came from several sources. We derived an estimate for acute mortality by converting the estimate for total number of harlequin duck mortalities in Prince William Sound (Esler et al. 2002) by the number of individuals at risk within oiled areas. Survival rates during the recovery period were estimated using radio telemetry data collected during two intervals. The first was during the winters of 1995-1996 through 1997-1998 (hereafter 1995 to 1998; Esler et al. 2000b) and the second during the winters of 2000-2001 through 2002-2003 (hereafter 2000 to 2003; Esler and Iverson 2010). In these studies, female harlequin ducks were tracked in previously oiled areas around Green Island, Knight Island and the Kenai Peninsula, and in unoiled areas around Montague Island. We combined these estimates with survival rates measured during other annual cycle stages, including the breeding season at several locations in the Pacific Flyway (Bond et al. 2009) and during the post-breeding period in Prince William Sound (Iverson and Esler 2007), to infer annual survival (Table 2).

*Reproduction* – Breeding parameters were investigated for a small number of harlequin ducks using inland habitats adjacent to Prince William Sound during the summers of 1990 and 1991 (Crowley and Patten 1996). However, these data were limited. Therefore, we also consulted published estimates for studies conducted in Alberta during the mid-1990s (Smith et al. 2000) and Iceland (Gardarsson and Einarsson 2008), as well as estimates for other sea duck species evaluated in long term studies (Swennen 1991, Kremetz et al. 1997) to parameterize our model.

*Dispersal* – Harlequin duck movements in Prince William Sound were evaluated using mark-recapture (Iverson et al. 2004) and radio telemetry (Iverson and Esler 2006). We used these estimates to derive transition probabilities between oiled and unoiled areas, as well as the probability of emigration from Prince William Sound as a whole during the 1995-1997 and 2000-2003 monitoring periods (Table 3).

### *Model development*

We conducted our analyses using the R package popbio (Stubben and Milligan 2007), which is a statistical modeling platform that consists primarily of translated MATLAB code (The MathWorks, Inc. 2007) for models developed by Caswell (2001) and Morris and Doak (2002). We began with models for which our primary purpose was diagnostic and included the effects of reduced survival, variable productivity, and dispersal in a step-wise manner. We then proceeded to evaluate a series of fully parameterized models representing the worst-case, best-case, and most likely combinations of input data (Table 4).

*Null model*—For our most basic modeling formulation we used demographic estimates from unoiled areas to characterize the population. In our null model:

$$\mathbf{n}(t+1) = \mathbf{A}(t) \mathbf{n}(t) \quad (\text{equation 1a})$$

population size was included in vector  $\mathbf{n}$  and vital rates in matrix  $\mathbf{A}$

$$\mathbf{n}(t) = \begin{bmatrix} n_1 \\ n_{2+} \end{bmatrix} \quad \text{and} \quad \mathbf{A}(t) = \begin{bmatrix} 0 & F_{2+} \\ s_1 & s_{2+} \end{bmatrix} \quad (\text{equation 1b})$$

We used our null model as a basis for comparison and to determine the stable age distribution of the population ( $\mathbf{w}$ ), as well as the sensitivity ( $S_{ij}$ ) and elasticity ( $E_{ij}$ ) of the growth rate ( $\lambda$ ) to variation in the underlying matrix elements. To parameterize matrix  $\mathbf{A}$ , we used mean survival rates in unoiled areas ( $\text{SURV}_0$ ). For juvenile birds, we assumed that survival during the breeding season was proportional to the rate measured during the non-breeding season (Iverson and Esler 2010). For the fecundity term, we assumed a constant population size ( $\lambda=1$ ) and calculated the rate that would be necessary to maintain numbers at equilibrium. We defined the corresponding fertility rate as  $f_{\text{base}}$ , and used it for the null fertility parameterization ( $\text{FERT}_0$ ). We parameterized the population vector using long-term averages from survey counts adjusted for the number of females and projected forward 25 years to assure numerical convergence.

*Survival probability in relation to oiling history* – Our next step was to incorporate survival probability in relation to oiling history ( $\text{SURV}_1$ ). For modeling simplicity, we began by assuming no immigration and modeled the population growth rate in oiled areas using matrix  $\mathbf{B}$ . We retained the null fertility parameterization ( $\text{FERT}_0$ ), making our estimate one of gross mortality (i.e., the total number of deaths in the absence of compensatory reproduction).

To derive estimates for harlequin duck survival rates during years in which empirical data were not collected, we fit the available data to an exponential rise to maximum equation using a least squares model fitting approach (Systat Software Inc., Richmond, CA, USA) for the equation:

$$s_i \text{ winter}(t) = s_0 \text{ winter} + a(1 - b^{yr(t)}) \quad (\text{equation 2})$$

where parameters  $a$  and  $b$  are constants and winter survival estimates were input for intervals  $t=1$  (the year that the spill occurred),  $t=8$  (the rate during 1995 to 1998),  $t=13$  (the rate during 2000 to 2003), and  $t=15$  (the baseline rate from unoiled areas; Fig. 3). We then compared projections for the null model [ $\text{SURV}_0 + \text{FERT}_0$ ] to those made when estimates from oiled areas were used [ $\text{SURV}_1 + \text{FERT}_0$ ].

*Reproduction and population recovery*—To explore the effects of compensatory reproduction on recovery rate we evaluated three scenarios for post-spill productivity: low, moderate, and high fertility. We assumed that in the absence of perturbation abundance should be maintained at a maximum level ( $K$ ), which we set just above the long-term average from U.S. Fish and Wildlife Service survey counts. We assumed that when numbers dropped fertility rates increase and estimated a value by either (1) applying  $f_{\text{base}}$  in situations where  $N_i \geq K$ , (2) applying the maximum fertility rate ( $f_{\text{max}}$ ) when  $N_i \leq$  minimum from FWS counts, or (3) calculating for intermediate values using the 3 parameter sigmoidal function:

$$f_i(t) = \frac{a}{1 + e^{\frac{N_i(t) - N_0}{b}}} \quad (\text{equation 3})$$

where  $a$ ,  $b$ , and  $N_0$  and are constants used to ensure model fit (Fig. 4).

Our low fertility parameterization (FERT<sub>1</sub>) assumed that individual productivity could increase by as much as 5% when densities were reduced ( $f_{max} = 0.610$  fledgling females per adult female). The moderate fertility formulation assumed a 10% increase was possible (FERT<sub>2</sub>;  $f_{max} = 0.639$ ) and the high fertility formulation a 25% increase (FERT<sub>3</sub>;  $f_{max} = 0.726$ ). The low and moderate portion of the range were approximated values for harlequin ducks in Alaska (0.6-1.1 total fledglings per female; Crowley and Patten 1996), Alberta (1.2 total fledglings per female; Smith et al. 2000) and Iceland ( $0.8 \pm 0.2$  total fledglings per female; Gardarsson and Einarsson 2008), while the upper end of the range was covered by estimates for other sea duck species during periods of numerical increase [white-winged scoter (*Melanitta fusca*): 0.2-1.6 young per adult in the fall flight, Kremetz et al. 1997; common eiders (*Somateria mollissima*): 0.0-1.5 total fledglings per female, Swennen 1991).

We also included in our analysis a formulation to consider the implications of sublethal effects of oil contamination on harlequin duck productivity. In this model we allowed the fertility rate to vary as a function of winter survival such that:

$$f'_i(t) = f_i(t) \sqrt{s_{2+}(t)/\hat{s}_{2+}} \quad (\text{equation 4})$$

where  $f'_i$  is the correlated fertility rate and  $\hat{s}_{2+}$  is the survival rate of adult females in unoiled areas.

Growth projections were then made for 3 models in which fertility was not correlated with survival and oil exposure: [SURV<sub>1</sub> + FERT<sub>1</sub>], [SURV<sub>1</sub> + FERT<sub>2</sub>] and [SURV<sub>1</sub> + FERT<sub>3</sub>] and a fourth model in which correlated fertility was assumed [SURV<sub>1</sub> + FERT<sub>2</sub>].

*Movement between oiled and unoiled areas* – As a final step in model development, we incorporated dispersal between oiled and unoiled areas. Our interest was to determine the degree to which movement influenced recovery dynamics, including the possibility of demographic rescue by immigrating females. The incorporation of movement required several simplifying assumptions that previous studies indicated were well supported (Iverson and Esler 2006). First, we treated the aggregation of birds wintering in Prince William Sound as a closed population and treated oiled and unoiled areas as discrete population segments. Second, we assumed that when dispersal events did occur they happened immediately after the post-breeding period. Finally, we assumed that movement probabilities were equivalent for juvenile and adult age classes.

For models that considered movement between areas we combined matrices **A** and **B** into a single grand matrix **G** (Morris and Doak 2002):

$$\mathbf{n}(t+1) = \mathbf{G}(t) \mathbf{n}(t) \quad (\text{equation 5a})$$

where,

$$\mathbf{n}(t) = \begin{bmatrix} n_{1A} \\ n_{2+A} \\ n_{1B} \\ n_{2+B} \end{bmatrix} \text{ and } \mathbf{G}(t) = \begin{bmatrix} 0 & F_{2+A} & 0 & F_{2+B} \\ s_{1A}(1-m_{AB}) & s_{2+A}(1-m_{AB}) & s_{1B}m_{AB} & s_{2+B}m_{AB} \\ 0 & F_{2+A} & 0 & F_{2+B} \\ s_{1A}m_{BA} & s_{2+A}m_{BA} & s_{1B}(1-m_{BA}) & s_{2+B}(1-m_{BA}) \end{bmatrix} \quad (\text{equation 5b})$$

In matrix  $\mathbf{G}$  the population is divided into four categories based on movement history: females that remained site faithful to unoiled areas (upper left:  $1 - m_{AB}$ ), females that immigrated to oiled areas from unoiled areas (upper right:  $m_{AB}$ ), females that immigrated to unoiled areas from oiled areas (lower left:  $m_{BA}$ ), and females that remained site faithful to the oiled areas (lower right:  $1 - m_{BA}$ ).

We parameterized matrix  $\mathbf{G}$  using two alternative formulations. The first assumed that the movement rates we documented during the 1995 to 1998 and 2000 to 2003 study periods were constant over time ( $\text{MOVE}_1$ ). Under this formulation the dispersal rate was higher for females moving from oiled to unoiled areas than in the reverse ( $m_{AB} = 0.014$ ;  $m_{BA} = 0.026$ ; Table 3); however, because the total number of ducks was larger in unoiled areas this parameterization resulted in a net flow of females from unoiled to oiled areas over time. The second formulation ( $\text{MOVE}_2$ ) explored the possibility of an adaptive behavioral response by individuals during the immediate aftermath of the EVOS (and before telemetry studies were initiated). For this scenario we assumed that a higher proportion of females moved out of oiled areas during the first winter after the spill when the habitat was most disturbed and clean up efforts were most intense ( $m_{AB} = 0.005$ ;  $m_{BA} = 0.150$ ).

For the models that considered movement we applied a cap to immigration when matrix projections indicated that  $N_i \geq K$  and compared two formulations [ $\text{SURV}_1 + \text{FERT}_2 + \text{MOVE}_1$ ] and [ $\text{SURV}_1 + \text{FERT}_2 + \text{MOVE}_2$ ].

*Evaluation models* – To assess the full suite of interacting variables that influence population dynamics we compared the results of three models representing the worst-case, best-case, and what we believed to be the most likely-case scenarios of model input data. In our worst-case scenario model [ $\text{SURV}_1 + \text{FERT}_1 + \text{MOVE}_1$ ], we assumed a low potential for population growth through increased fertility, fertility rates that were negatively correlated with oil exposure, and constant movement rates over time. In our best-case scenario model [ $\text{SURV}_1 + \text{FERT}_3 + \text{MOVE}_2$ ], we assumed a high capacity for increased fertility, no correlation between survival and fertility, and avoidance behavior during the first winter in the spill zone. These models were used to generate confidence bounds around estimates for our most likely recovery scenario: [ $\text{SURV}_1 + \text{FERT}_2 + \text{MOVE}_2$ ], in which we assumed a moderate capacity for population increase through reproduction, a negative relationship between oil exposure and fertility; and avoidance behavior immediately after the spill. We based these assumptions on our knowledge of the reproductive ecology of harlequin ducks, documented reductions in breeding performance by other marine bird species affected by the EVOS in Prince William Sound (Golet et al. 2002), and the considerable disturbance created by cleanup efforts.

## RESULTS

### *Null model*

For the null model [SURV<sub>0</sub> + FERT<sub>0</sub>], matrix **A** was parameterized using the estimates:

$$\mathbf{A}(t) = \begin{bmatrix} 0 & 0.430 \\ 0.605 & 0.740 \end{bmatrix}$$

where  $F_{2+}$  was a calculated term representing the productivity necessary to maintain a stable population. This rate of productivity corresponds to a baseline fertility rate of  $f_{base} = 0.581$ , which was used to calculate subsequent fertility scenarios. Matrix calculations indicated that at the stable age distribution (**w**) the population was comprised of 30% juvenile and 70% adult females. The damping ratio was estimated at 3.8, which suggests that equilibrium is attained fairly rapidly compared to species in which reproduction is more tightly regulated by age class.

As expected, our sensitivity and elasticity analysis indicated that  $\lambda$  was most responsive to changes adult female survival rates. The sensitivity matrix was estimated as:

$$[S_{ij}] = \begin{bmatrix} - & 0.480 \\ 0.341 & 0.794 \end{bmatrix}$$

and the elasticity matrix, which gives the sensitivity measured in terms of proportional changes as:

$$[E_{ij}] = \begin{bmatrix} - & 0.206 \\ 0.206 & 0.587 \end{bmatrix}$$

Thus, adult female survival exerted approximately 2-3 times the effect on  $\lambda$  as the other variables under consideration.

### *Survival probability in relation to oiling history*

Under the model [SURV<sub>1</sub> + FERT<sub>0</sub>], we estimated a gross decline in female harlequin duck numbers within oiled areas of 25.0% during the acute phase of exposure. This corresponded to approximately 400 female deaths (Fig. 5). A further loss of 772 females was indicated during the chronic exposure phase. Thus, a total of 1173 female deaths (or 73.4% of the starting population) was estimated as a direct result of the EVOS. After rescaling to include males, our model projected 2860 total mortalities.

### *Reproduction and population recovery*

Even under the most optimistic of scenarios for fecundity our models projected a prolonged recovery period through *in situ* reproduction alone (Fig. 6). Our low fertility model [SURV<sub>1</sub> + FERT<sub>1</sub>] projected a timeline of >100, compared to projections of 63 years under the moderate fertility model [SURV<sub>1</sub> + FERT<sub>2</sub>] and 27 years under the high fertility model [SURV<sub>1</sub> + FERT<sub>3</sub>]. Models assuming lower fertility also predicted deeper reductions in numbers and longer intervals before declining trends could be reversed and for numerical recovery to began. With respect to potential sublethal effects on productivity, the model [SURV<sub>1</sub> + FERT<sub>2</sub>] projected a 70 year timeline to recovery or approximately 10% longer than the similarly parameterized uncorrelated fertility model [SURV<sub>1</sub> + FERT<sub>2</sub>].

### *Movement between sites*

In our model [SURV<sub>1</sub> + FERT<sub>2</sub> + MOVE<sub>1</sub>], we assumed that the rates of movement between oiled and unoiled reference areas were constant and projected a timeline to recovery of 21 years for Prince William Sound as a whole and 24 years within oiled areas (Fig. 7). Projections for the adaptive movement model [SURV<sub>1</sub> + FERT<sub>2</sub> + MOVE<sub>2</sub>] were nearly identical, estimated at 22 years for Prince William Sound as a whole and 25 years within oiled areas. The primary differences between the projections of the two models was that in the adaptive movement model lower numbers were expected in oiled areas immediately after the spill as a result of emigration. However, the key finding was that under both models the projected recovery timeline was approximately a third as long as when no movement was assumed.

### *Evaluation models*

After combining variables into a worst-case, best-case, and most likely-case scenario we projected a 5.6% reduction (range: 4.2%-6.5%) in female harlequin numbers during the acute phase of oil exposure for Prince William Sound as a whole and a 24.9% reduction (range: 25.5%-32.4%) within oiled areas (Fig. 8). A declining population trend was predicted to persist for 6 years [range: 5-8] after the spill across Prince William Sound as a whole, with a mean population growth rate of  $\lambda = 0.976$  during this period. At their lowest point, female numbers were predicted to be reduced 14.7% below the pre-spill abundance and by 55.3% below pre-spill abundance in oiled areas. Once the declining trend was reversed a population growth rate of 1.008 was estimated with numeric recovery predicted in 24 years [range: 16-32].

## **DISCUSSION**

A primary objective of our modeling exercise was to evaluate the relative magnitudes of acute and chronic injury to harlequin duck populations resulting from the *Exxon Valdez* spill. Contrary to assertions that negative effects on wildlife were short-lived (Wiens et al. 1996, Day et al. 1997, Murphy et al. 1997) model projections from demographic data for harlequin ducks suggest long-term impact with mortality resulting from chronic exposure to residual oil exceeding acute phase mortality. This finding rests on empirical estimates of reduced survival nearly a decade after the spill (Esler et al. 2000b, Esler and Iverson 2010) and supports a growing body of evidence that major spills like the EVOS can have significant long-term consequences for marine vertebrate populations (Peterson et al. 2003). Another related objective of our analyses was to compare the effects of various demographic constraints on population recovery. Our sensitivity analyses confirmed that adult female survival exerts the greatest influence on harlequin duck population growth rates and even under the most optimistic assumptions about fecundity declining population trends were projected to persist in oiled areas for several years after the spill. Moreover, complete recovery was projected to be delayed until after survival rates in oiled areas converged with those in unoiled areas. Prolonged reductions in adult survival within oiled areas were the primary driver of these projections and the most important predictor of recovery rate.

Unfortunately, empirical estimates of fecundity were not available with that same specificity as those for survival and dispersal and probably never will given the difficulties of working with



nesting birds and applying information from breeding grounds to a wintering population. Although the estimates that we used in our model were in line with those from long term studies (Swennen 1991, Kremetz et al. 1997, Gardarsson and Einarsson 2008) their correspondence to the actual rates realized by harlequin ducks in Prince William Sound after the EVOS cannot be known. This is an important issue because assumptions about the capacity for compensatory reproduction strongly influenced confidence bounds around our estimates for recovery. All models indicated a prolonged period would be necessary, but our best and worst-case scenarios ranged from 16 to 32 years. Also uncertain was the extent to which oil contamination indirectly affected harlequin duck productivity after the spill. Delays in egg laying, reductions in the proportion of adults laying eggs, lower hatching success, lower fledging success and lower return rates of adults to breeding locations have all been documented for marine bird species exposed to petroleum hydrocarbons (Ainley et al. 1981, Fry et al. 1986, Butler et al. 1988, Walton et al. 1997). Although it is not possible to relate oil exposure by harlequin ducks to their breeding performance Rizzolo et al. (2004) determined that harlequin ducks dosed externally with oil had increased energy intake, increased oxygen consumption and decreased body mass in comparison to control birds in a laboratory setting. This observation, combined with observations by Regehr (2003a) that young accompany their mothers to non-breeding areas, would suggest a potential mechanism for localized effects similar to that identified by Golet et al. (2002) for pigeon guillemots.

With respect to dispersal, previous studies have indicated high site fidelity by harlequin ducks to non-breeding areas in Prince William Sound (Iverson et al. 2004, Iverson and Esler 2006) and elsewhere in their Pacific coast range (Cooke et al. 2000, Robertson et al. 2000, Regehr 2003). On this basis we expected recovery of local population segments to occur largely by recruitment (Esler et al. 2002); however our model projections emphasized the importance of dispersal on recovery even when site fidelity exceeded 95% in both oiled and unoiled areas. This dynamic was a result of the comparatively large number of birds from outside the spill zone available to facilitate numerical increases within oiled areas and was projected to occur despite higher dispersal rates from oiled areas to unoiled areas in telemetry studies conducted during the mid 1990s and early 2000s.

Concordance between our projections and estimates derived from survey counts was strong during the first decade after the EVOS, when the effects were most pronounced; however, some discrepancies were evident. Among the various surveys, U.S. Fish and Wildlife Service counts have been the longest-term, most geographically extensive, and most useful for inferring trends (Lance et al. 2001). These surveys were initiated immediately after the spill and are conducted during late winter, which is a period when harlequin ducks are present in large numbers on non-breeding areas. Although the confidence intervals associated U.S. Fish and Wildlife Service survey estimates for harlequin ducks preclude fine-scale comparison, they indicate a substantial and prolonged period of increases in oiled areas from 1990 to 2000 (McKnight et al. 2006). Unlike our projections, in which a low point in numbers was predicted to occur several years after the EVOS, the survey data suggest the population bottomed out immediately after the spill during the winter of 1990. Surveys conducted after 2000 suggest a leveling off or slight decrease in numbers in oiled areas, whereas our population model projected sustained growth. One prediction from our model was that numbers were projected to increase in unoiled areas of Prince William Sound during the years immediately after the spill as a result of emigration from

oil contaminated areas and then decrease as dispersal patterns favored the repopulation of previously oiled areas. This prediction appeared borne out by U.S. Fish and Wildlife Service data, in which numbers in unoiled areas peaked during 1993 and 1994 and gradually declined thereafter.

Alaska Department of Fish and Game also conducted surveys in Prince William Sound during 1995 to 2005 (Rosenberg and Petrula 1998, Rosenberg et al. 2005). These surveys focused exclusively on coastal waterfowl and yielded very precise estimates abundance, as well as sex and age ratios for the harlequin duck population. They also had very high degree of spatial and temporal correspondence to the studies in which we estimated demographic rates. Similar to our model projections, the Alaska Department of Fish and Game surveys indicated delayed population declines in oiled areas through 1997. Statistically significant trends were not apparent from 2000-2005 in oiled areas, nor were they apparent during the study period as a whole in unoiled areas. Finally, our projections showed little correspondence to estimates described in Exxon Corporation sponsored studies (Wiens et al. 1996, Day et al. 1997, Murphy et al. 1997). The Exxon sponsored studies used data collected across a range of oil contamination levels and concluded that the negative effects associated with the EVOS were short-lived (<2.5 years) for most wildlife species. These conclusions were based on estimates of species richness, habitat use, and summer abundance. Unfortunately, the data are of limited utility for evaluating harlequin duck population trends because of their timing (nearly all adult harlequin ducks migrate to inland breeding areas during the summer) and their duration (the surveys were conducted from 1989 to 1991 only).

A persistent point of contention concerning interpretation of post-EVOS survey data has been the difficulty in discerning oil spill effects from environmental variation. This is exacerbated by the paucity of pre-spill data with which to judge post-spill dynamics. These topics have received considerable attention in the scientific literature (Irons et al. 2001, Lance et al. 2001, Wiens et al. 2001, 2004) and although our analyses cannot resolve these disagreements, they do provide new insights into the processes regulating population dynamics. A growing body of evidence suggests that exposure to residual *Exxon Valdez* oil has been responsible for physiological challenges affecting the demographic properties of harlequin ducks (Trust et al. 2000, Esler et al. 2002) and other marine vertebrate species (Bodkin et al. 2002, Jewett et al. 2002, Bowyer et al. 2003). Although exposure does not necessarily imply negative effects on individuals or populations (Underwood and Peterson 1988) our research is unique in that demographic rates were empirically estimated in relation to oiling history allowing consideration of population-level effects.

As with any model, the strength of our conclusions is highly dependent on the validity of the underlying assumptions and when developing our model we made several important assumptions that require consideration. First, we assumed that in the absence of oil spill effects, harlequin duck demographic rates should be similar across habitats within Prince William Sound. Supporting this assumption for harlequin ducks was the finding that winter survival rates in unoiled areas remained constant over time (Esler and Iverson 2010); whereas survival in oiled areas was depressed after the spill (Esler et al. 2000b, Esler et al. 2002) but eventually increased converged to the level documented in unoiled areas over time (Esler and Iverson 2010). This is not to suggest habitat related differences do not exist. Esler et al. 2000a evaluated harlequin duck

densities in relation to habitat characteristics, including substrate, exposure to wind and waves, distance to stream mouths and offshore reefs, intertidal slope, prey biomass and history of contamination by the EVOS. After accounting for habitat relationships it was concluded that oiling history was negatively related to harlequin duck densities; however densities within unoiled habitats tended to be higher overall, particularly in relation to intertidal slope and distance to stream mouths and offshore reefs. Similarly, the extent of oil contamination and rate of dissipation within the spill zone has not been uniform over time. Some areas were more heavily oiled than others and fine scale population dynamics have almost certainly more complex than described in our models.

A second assumption of our model was that the aggregation of harlequin ducks wintering in Prince William Sound could be modeled as two discrete population segments. Previous studies have demonstrated that fewer than 2% of female harlequin ducks marked with radio transmitters disperse from Prince William Sound each year (Iverson and Esler 2006) and that annual return rates exceed 95% (Iverson et al. 2004). Within Prince William Sound movements between oiled and unoiled areas were studied in detail during the chronic phase of oil exposure, but it is possible that dispersal patterns differed during the years immediately after the spill and from 2004 onward, when no measurements were taken. We evaluated a scenario for adaptive movements away from oiled areas during the first winter when contamination was most severe and cleanup efforts most intense, but this scenario was not empirically derived. Nonetheless, the projected timeline to recovery differed little between models that assumed constant rates of movement and those in which it varied during the immediate post-spill period.

We also assumed that environmental variation did not affect harlequin duck demographic rates and that the fecundity of individual females was density-dependent. These assumptions were made for practical reasons and we acknowledge that they are a simplification of biological reality. The effect of environmental stochasticity on wildlife population dynamics is an important topic that has received considerable attention in the literature. As was evident from survey data in Prince William Sound (Rosenberg et al. 2005, McKnight et al. 2006) annual variations in harlequin duck numbers were significant and at times obscured population trends. Because our projections were based on demographic rates averaged over a multiple years they tended to smooth predictions about change over time. With respect to density dependent population regulation, fecundity of female harlequin ducks likely operates through complex interactions on reproductive output and/or post-fledging juvenile survival rates. Our model accommodates these mechanisms the same way, so which of those that is operating is not necessary to know. Available data suggest that feeding by pre-laying female harlequin ducks is limited (Goudie and Jones 2005), implying that they are not nutrient limited in producing clutches. Moreover, the species nests at such low densities there would appear to be little density related regulation of nesting success or hatching rates. Evidence for density dependent breeding propensity and/or post-fledging survival has been suggested for other sea ducks (Mehl 2004) and may operate for harlequin ducks.

It has been suggested that structured waterfowl populations are often characterized by non-linear dynamics following perturbation such that they exhibit significant momentum before population trends can be reversed (Hauser et al. 2006). Our findings confirm assertions that effects of oil spills on wildlife populations are expressed over much longer time frames than previously

assumed and that chronic exposure to spilled oil can have far reaching population-level consequences. Our study also defines the duration over which reduced demographic performance may be expected in areas contaminated during a major oil spill. This understanding of duration and mechanism is critical when applying management following large spills, for example considering the costs and benefits of removal of residual oil, and when applying risk assessment under scenarios of catastrophic contaminant releases. It also demonstrates the sensitivity of benthic-foraging vertebrates to residual oil sequestered in sediments and indicates that species with natural history and life history traits similar to those of harlequin ducks might be particularly vulnerable. We recommend continued monitoring of harlequin duck survival and movement rates in Prince William Sound to accompany ongoing survey and habitat monitoring efforts. We believe that it is only through a multifaceted approach that relies on different sources of data, including the physiological and mechanistic factors influencing individual survival and behavior that a complete picture of the population recovery process can be attained.

## ACKNOWLEDGEMENTS

This research was supported primarily by the *Exxon Valdez* Oil Spill Trustee Council. However, the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council. Although this effort was largely a desk-top exercise, the data used to build the models was collected by a small army of field workers, who exerted a huge effort under frequently difficult winter conditions. For their efforts, we thank B. Baetsle, R. Ballas, B. Benter, T. Bowman, K. Charleton, J. DeGroot, M. Evans, T. Fondell, T. Fontaine, D. Mather, D. Monson, J. Morse, D. Ruthrauff, D. Safine, R. Sargent, J. Stout, K. Trust, and C. Van Hemert. Veterinary assistance during field work was provided by Drs. Dan Mulcahy, K. Burek, D. Schaeffer, and M. Stoskopf. We thank the crews of the motor vessels *Auklet*, *Julia Breeze*, *Kittiwake II*, *Waters*, *Discovery*, and *Babkin* and the pilots and staff of Cordova Air and Fishing and Flying for support and service. We also acknowledge the institutional support provided by Dirk Derksen, Dede Bohn, Ron Ydenberg, Judy Higham, and Monica Court. Finally, we thank Drs. Risa Sargent and Eric Reed for their reviews of the manuscript.

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## TABLES AND FIGURES

Table 2.1. Harlequin ducks abundance in Prince William Sound, Alaska estimated from U.S. Fish and Wildlife Service surveys conducted during 1990-2000, with averages for oiled and unoiled areas after adjusted for the proportion female.

Year	Number of females	
	Oiled areas ( $\pm$ SE)	Unoiled estimate ( $\pm$ SE)
1990	2738.6 $\pm$ 972.5	7881.0 $\pm$ 2320.2
1991	2831.8 $\pm$ 987.0	8326.7 $\pm$ 2676.1
1993	3315.2 $\pm$ 1254.4	15303.9 $\pm$ 7232.2
1994	4418.9 $\pm$ 1456.5	14785.3 $\pm$ 4302.9
1996	4954.5 $\pm$ 1799.0	12196.6 $\pm$ 3589.4
1998	4016.2 $\pm$ 1274.0	10604.9 $\pm$ 3267.1
2000	4934.9 $\pm$ 1755.6	9940.9 $\pm$ 2807.2

Long-term average for the number of females in oiled areas: 1593.7  
Long-term average for the number of females in unoiled areas: 4629.4

Table 2.2. Annual cycle stage-specific cumulative survival rate (CSR) estimates for female harlequin ducks.

Stage	Year	Location	Age class	CSR ( $\pm$ SE)	Source
Acute exposure phase (24 Mar – 14 Aug)	1989	PWS (Oiled)	All	0.748 <sup>†</sup>	1,2
Post-breeding (15 Aug – 6 Oct)	1995 to 1998	PWS: Green Island, Knight Island, Kenai Peninsula (Oiled); Montague Island (Unoiled)	Adult	0.999 $\pm$ 0.002	3
Winter (7 Oct – 14 Apr)	1995 to 1998	PWS: Green Island, Knight Island, Kenai Peninsula (Oiled)	Adult	0.780 $\pm$ 0.033	4
	1995 to 1998	PWS: Montague Island (Unoiled)	Adult	0.837 $\pm$ 0.029	4
	2000 to 2003	PWS: Green Island, Knight Island, Kenai Peninsula (Oiled)	Adult	0.834 $\pm$ 0.065	5
	2000 to 2003	PWS: Montague Island (Unoiled)	Adult	0.837 $\pm$ 0.064	5
	2000 to 2003	PWS: Green Island, Knight Island, Kenai Peninsula (Oiled)	Juvenile	0.766 $\pm$ 0.138	5
	2000 to 2003	PWS: Montague Island (Unoiled)	Juvenile	0.758 $\pm$ 0.152	5
	1994 to 2004	Cascade Mountains, Oregon; Coast Mountains, British Columbia	Adult	0.885 $\pm$ 0.077	6

<sup>†</sup> Estimated as  $1 - d/r$ , where  $d$  is the estimated number of harlequin duck deaths (980) and  $r$  is the estimated number of individuals at risk within oiled areas taken from U.S. Fish and Wildlife Service survey estimates (3887.2).

<sup>1</sup> Esler et al. 2002; <sup>2</sup> McKnight et al. 2006; <sup>3</sup> Iverson and Esler 2007; <sup>4</sup> Esler et al. 2000a; <sup>5</sup> Esler and Iverson *in press*; <sup>6</sup> Bond et al. 2009.

Table 2.3. Probability of dispersal between oiled and unoiled areas of Prince William Sound, Alaska by female harlequin ducks during 1995-1997 and 2000-2003.

Description of the transition	Probability ( $\pm$ SE) of movement <sup>†</sup>
Oiled areas to unoiled areas	0.026 ( $\pm$ 0.003)
Unoiled areas to oiled areas	0.014 ( $\pm$ 0.002)

<sup>†</sup> On average, >98% of all marked females remained within the 4500 km<sup>2</sup> study area in Prince William Sound.

Table 2.4. Models used to evaluate harlequin duck population recovery. Variables include survival in relation to oiling history (SURV), fertility (FERT), and movement probability between oiled and unoiled areas (MOVE).

Model	Notation	Parameterization		
		SURV	FERT	MOVE
<i>Diagnostic models</i>				
Null model	$SURV_0 + FERT_0$	Unoiled area	Baseline rate	None
Gross mortality	$SURV_1 + FERT_0$	Oiled area	Base rate	None
Low fertility	$SURV_1 + FERT_1$	Oiled area	Low	None
Moderate fertility	$SURV_1 + FERT_2$	Oiled area	Moderate	None
High fertility	$SURV_1 + FERT_3$	Oiled area	High	None
Correlated reproduction	$SURV_1 + FERT_2'$	Oiled area	Correlated/Moderate	None
Observed movement	$SURV_1 + FERT_2 + MOVE_1$	Oiled and unoiled areas	Moderate	Constant
Avoidance behavior	$SURV_1 + FERT_2 + MOVE_2$	Oiled and unoiled areas	Moderate	Variable
<i>Evaluation models</i>				
Worst case model	$SURV_1 + FERT_1' + MOVE_1$	Oiled and unoiled areas	Correlated/Low	Constant
Best case model	$SURV_1 + FERT_3 + MOVE_2$	Oiled and unoiled areas	High	Variable
Most likely model	$SURV_1 + FERT_2' + MOVE_2$	Oiled and unoiled areas	Correlated/Moderate	Variable

Figure 2.1. Map of Prince William Sound (PWS), Alaska showing areas affected by the 1989 *Exxon Valdez* Oil Spill.

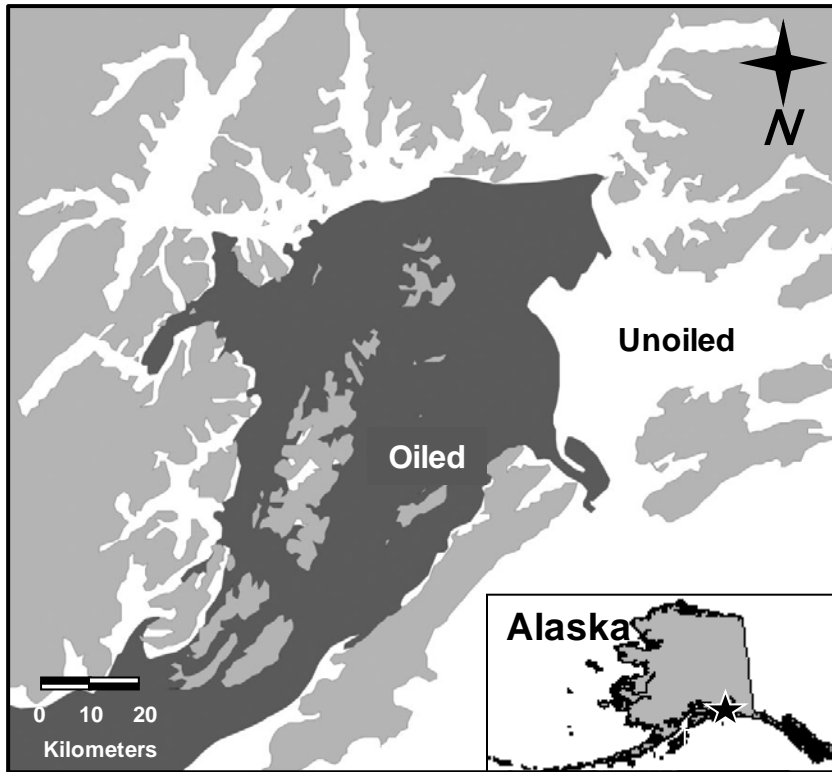




Figure 2.2. Life cycle diagram for female harlequin ducks. Two reproductive stages are denoted, which correspond to juvenile and adult age classes. In the model, a post-breeding census formulation was used, making fecundity ( $F_{2+}$ ) the product of adult survival ( $s_{2+}$ ) and fertility ( $f$ ).

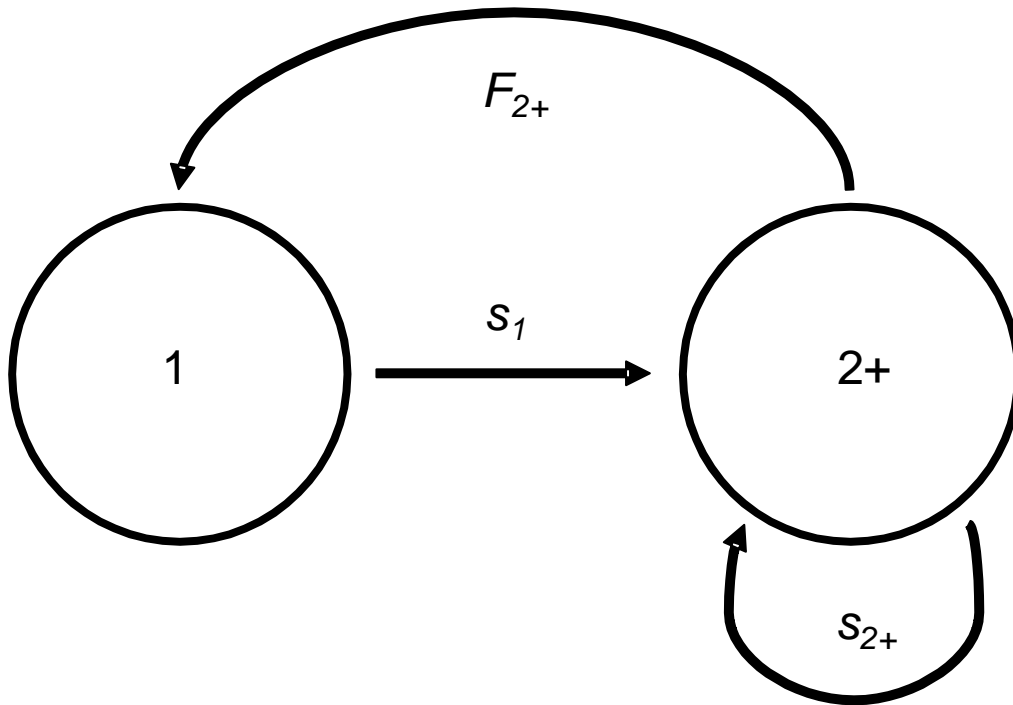


Figure 2.3. Estimated winter survival rate of (a) juvenile and (b) adult female harlequin ducks inferred from empirical estimates (circles) collected in Prince William Sound, Alaska following the 1989 *Exxon Valdez* Oil Spill.

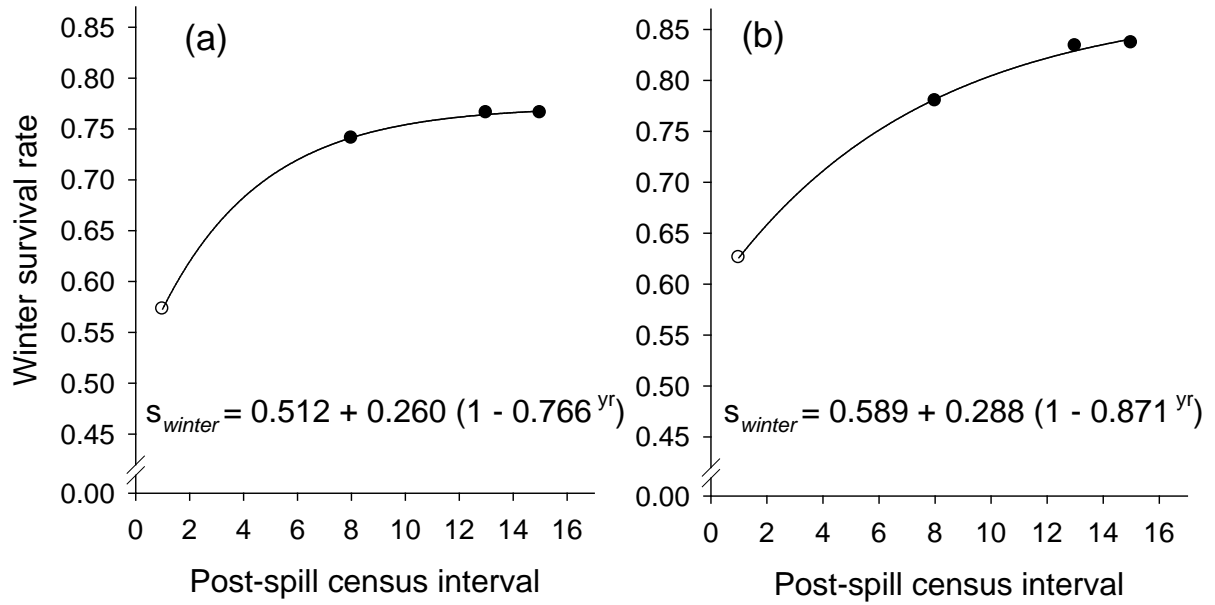


Figure 2.4. Fertility estimates used to infer the relationship between population size ( $N_i$ ) and fecundity in Prince William Sound, Alaska following the 1989 *Exxon Valdez* Oil Spill.

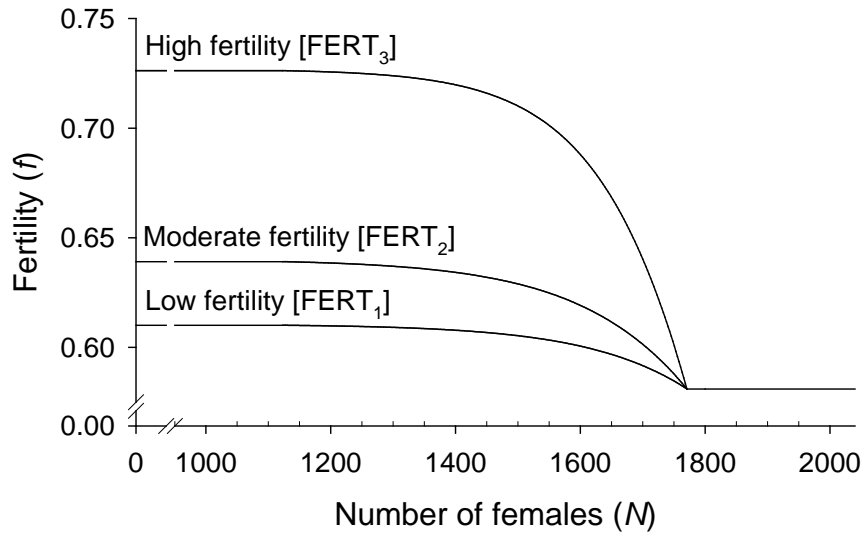


Figure 2.5. Projected mortality of female harlequin ducks in the absence of compensatory reproduction in Prince William Sound, Alaska following the 1989 *Exxon Valdez* Oil Spill. Mortality is divided into that occurring during the acute (1989) and chronic (1990 – 2004) phases of oil exposure.

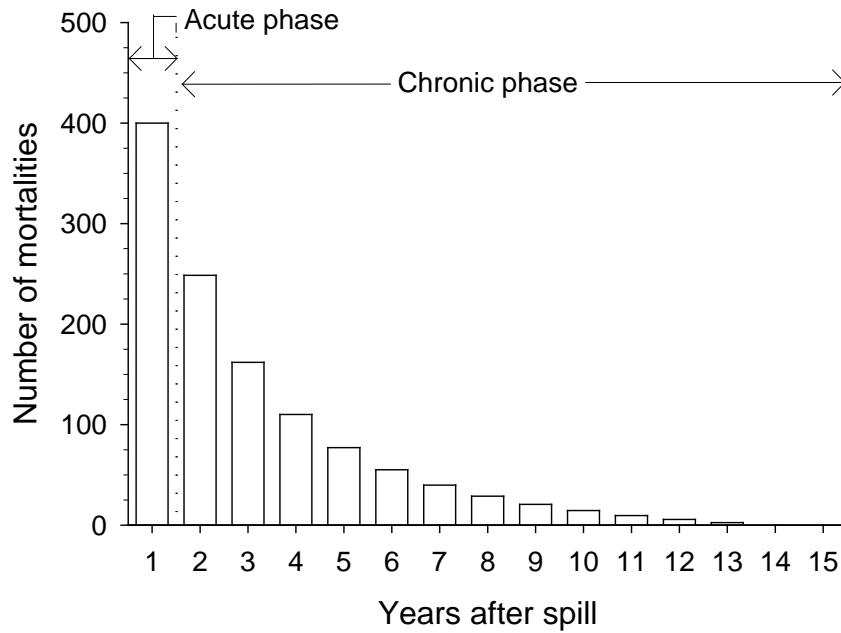


Figure 2.6. Projected recovery rate for harlequin ducks under low  $[SURV_1 + FERT_1]$ , moderate  $[SURV_1 + FERT_2]$ , and high  $[SURV_1 + FERT_3]$  fertility scenarios when assuming no immigration. Dashed line indicates the pre-spill parameterization for the population and level at which recovery is considered complete.

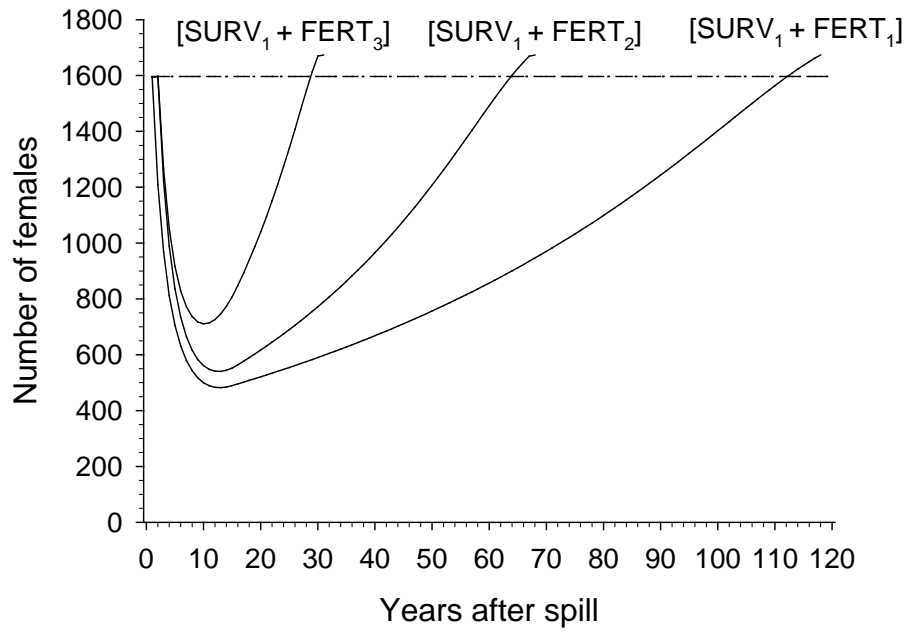


Figure 2.7. Projected recovery rate for harlequin ducks under observed [ $SURV_1 + FERT_2 + MOVE_1$ ] and adaptive [ $SURV_1 + FERT_2 + MOVE_2$ ] movement scenarios. Dashed line indicates the pre-spill parameterization for the population and level at which recovery is considered complete.

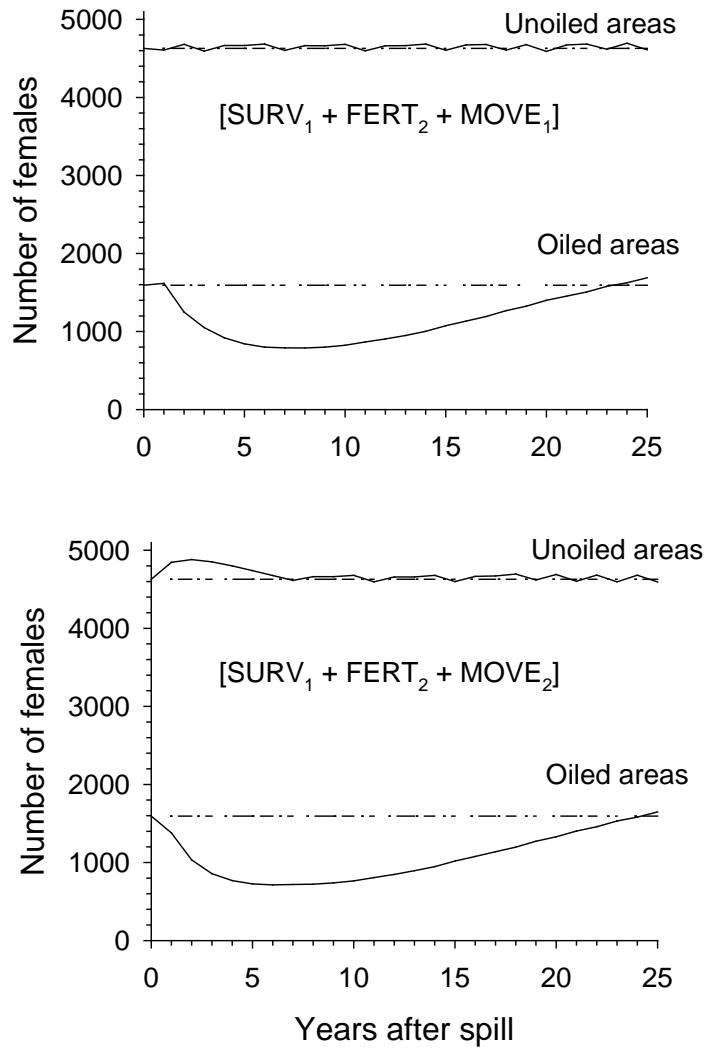


Figure 2.8. Projected recovery rate for harlequin ducks in Prince William Sound, Alaska following the 1989 *Exxon Valdez* Oil Spill. Estimates were derived using the most likely combination of model inputs (solid line) with confidence intervals derived using worst- and best-case scenario models (dotted lines).

