Exxon Valdez Oil Spill
Restoration Project Final Report

Nearshore Synthesis: Sea Otters and Sea Ducks (amendment)

Restoration Project 11100808
Final Report

Daniel Esler

Centre for Wildlife Ecology
Department of Biological Sciences
Simon Fraser University
5421 Robertson Road
Delta, British Columbia V4K 3N2 Canada

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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 2009, 20 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 direct population injury persisted 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 had abated. Using demographic data, a population model was constructed to estimate timeline until recovery of numbers to pre-spill levels, which was projected to be 24 years post-spill or 2013. However, persistence of oil in the environment and evidence of exposure of harlequin ducks to that oil through 2009 has led to continued monitoring to evaluate the timeline of exposure. The current work was designed as another data point in that time series for 2011.

Abstract: We found that average cytochrome P4501A induction (as measured by EROD activity) during March 2011 was significantly higher in wintering harlequin ducks captured in areas of Prince William Sound oiled by the 1989 *Exxon Valdez* spill, relative to unoiled areas. EROD activity did not vary in relation to age, sex, or mass of individuals. We interpret these results to indicate that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil up to 22 years after the original spill. However, 2011 results also indicate improving conditions, i.e., reductions in exposure, and hence progress towards recovery. First, average EROD activity in birds from oiled areas was approximately 2 times that in birds from unoiled areas in 2011; this represents an improvement over observations from 2005 to 2009, in which EROD activity was 3 to 5 times higher in oiled areas. Also, the incidence of elevated EROD activity was 24% of individuals from oiled areas, which was similar to results from 2009 (21%) but markedly lower than values from 2005 to 2007 (45% to 64%). The data presented in this report add to a growing body of literature suggesting that persistence of oil in the environment, and exposure of wildlife to that oil, can occur over much longer time frames than previously assumed.

Key Words: biomarker, cytochrome P4501A, *Exxon Valdez* oil spill, harlequin ducks, *Histrionicus histrionicus*, oil exposure, 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.

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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.

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 Va*ldez 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 March 2011, we captured 25 harlequin ducks in oiled areas of Prince William Sound and 20 in unoiled areas. Small liver biopsies were surgically removed from each individual, frozen immediately in liquid nitrogen, and subsequently shipped to 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 ± SE) EROD activity of 41.5 (± 6.4) in oiled areas and 20.9 (± 5.7) in unoiled areas. Our observation of roughly 2 times higher average EROD activity in oiled areas represents improvement over the 2005 to 2009 period, in which EROD in oiled areas ranged from roughly 3 to 5 times higher in oiled areas than unoiled areas. 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. We also considered the incidence of elevated exposure (defined as the number of individuals with EROD activity $\geq$ 2 times the average on unoiled areas for that year); for 2011 samples, we found that 24% of individuals captured in oiled areas had elevated EROD, compared to 10% in unoiled areas. These results were similar to those from 2009 (% elevated = 21% from oiled areas), but markedly lower that those from previous years (45 to 64% in oiled areas during 2005 to 2007).

We interpret these results as strong evidence that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil, as much as 22 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. Evidence of declines in average EROD activity and incidence of elevated EROD activity in oiled areas are encouraging signs that the degree of exposure is declining and population recovery is proceeding. These same signals were observed in Barrow's

goldeneye CYP1A induction, prior to convergence of values between oiled and unoiled areas, supporting the interpretation that these may be signs of recovery progress in harlequin ducks. 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 foreseen at the time of the spill.

INTRODUCTION

Effects of the 1989 *Exxon Valdez* oil spill on wildlife populations and communities in Prince William Sound, Alaska have been intensively studied, to document the process and timeline of population and ecosystem recovery. As part of that research, 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 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; Esler et al. 2010; Esler et al. 2011).

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; Esler et al. 2010; Esler et al. 2011). 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; Esler et al. 2010). 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). A population model built with available demographic information was used to estimate the timeline to numeric population recovery, which was estimated to be 24 years after the *Exxon Valdez* spill, or the year 2013 (Iverson and Esler 2010).

Because of the history of elevated indicators of CYP1A induction (Trust et al. 2000; Esler et al. 2010), 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 research describing elevated biomarkers of CYP1A in this species. In previous studies, Trust et al. (2000) and Esler et al. 2010 found that average (± 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 through 2009. The primary objective for the present study was to add to the monitoring timeline during 2011, 22 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) 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 previous work (Trust et al. 2000; Esler et al. 2010). We captured wintering harlequin ducks using a modified floating mist net (Kaiser et al. 1995) during March 2011. Birds were captured in a number of areas oiled during the *Exxon Valdez* spill, including Crafton Island (60.5º N, 147.9º W), Green Island (60.3º N, 147.4º W), Foul Pass (60.5º N, 147.6º W), and Herring Bay (60.5º N, 147.7º W). Also, birds were captured on nearby northwestern Montague Island (60.3º N, 147.3º 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 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.

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. 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 Miles et al. (2007). The measure of EROD activity is expressed in picomoles per minute per milligram of protein, i.e., pmol/min/mg protein.

Statistical Analyses

Variation in EROD activity was analyzed in relation to capture location and individual attributes for birds captures during March 2009. 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.

RESULTS

Variation in EROD activity of harlequin ducks captured in March 2011 was strongly associated with whether they were from oiled or unoiled areas. The model with area as the only explanatory variable received nearly five times the support of any other model,

with a *w* of 0.77 (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).

Parameter likelihood values also supported the importance of area for explaining variation in March 2011 EROD activity. The area parameter was strongly supported, with a parameter likelihood of 0.83 (Table 3). Also, the weighted parameter estimate indicated that areas differed by an average of 17.1 pmol/min/mg protein, with EROD activity higher in oiled areas (Figure 1). 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.

Several measures of CYP1A induction suggested that the degree and incidence of oil exposure were lower in 2011 than in previous years. First, average (pmol/min/mg ± SE) EROD activity on oiled areas was 41.5 (± 6.4) in 2011, compared to point estimates > 75 pmol/min/mg in the previous 3 sampling periods, despite very similar estimates of EROD activity in unoiled areas (20.9 ± 5.7 in 2011; Figure 1). Similarly, when data were scaled relative to the reference values from birds captured on unoiled areas, which accounted for interannual variability in results (Esler 2008), findings from 2011 stood out as indicating a lower degree of EROD activity on oiled areas relative to previous years (Figure 2). Average EROD activity in birds from oiled areas was approximately 2 times that in birds from unoiled areas in 2011, compared to 3 to 5 times higher from 2005 to 2009 (Figure 2). Finally, the incidence of elevated EROD activity was 24% of individuals from oiled areas (Figure 3), which was similar to results from 2009 (21%) but markedly lower than values from 2005 to 2007 (45% to 64%).

DISCUSSION

We found that hepatic CYP1A levels in harlequin ducks captured in March 2011, based on EROD activity, were significantly higher in areas oiled during the *Exxon Valdez* spill than in nearby unoiled areas. Our results are consistent with findings of Trust et al. (2000) and Esler et al. (2010) from previous years indicating that harlequin ducks were exposed to CYP1A inducers more frequently or in higher concentrations at oiled areas relative to unoiled areas through 2009. 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 2011, 22 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).

Despite evidence of continued, elevated EROD activity indicative of exposure to lingering *Exxon Valdez* oil through 2011, several lines of evidence suggest that incidence

and degree of exposure are declining, which in turn suggests that population recovery is progressing. In Barrow's goldeneyes (*Buchephala islandica*), another nearshore-dwelling sea duck, similar declines in average EROD activity and incidence of elevated CYP1A were observed in years preceding the eventual return of these metrics to background or reference levels (Esler et al. 2011). Observation of similar patterns in harlequin ducks offer hope that this species will cease to be exposed to residual oil within the near future.

Differential CYP1A induction between oiled and unoiled areas has been described for other vertebrates in Prince William Sound, including Barrow's goldeneyes (Trust et al. 2000; Esler et al. 2011), adult pigeon guillemots (*Cepphus 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*). 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) and Ricca et al. (2010) 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. Finally, our results indicating declines in CYP1A induction in both harlequin ducks and Barrow's goldeneye over time were consistent with exposure to a source declining in availability over time, as would be expected with *Exxon Valdez* oil, rather than compounds predicted to be constant over time such as atmospheric PCBs or oil from natural seeps.

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. Given observed demographic rates, Iverson and Esler (2010) projected numeric population recovery would occur by approximately 2013, although this projection assumes that continued exposure to residual oil does not affect vital rates beyond gross winter survival.

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 22 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.

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TABLES AND FIGURES

Table 1. Sample sizes of harlequin ducks captured in Prince William Sound, Alaska for analyses of cytochrome P4501A induction in March 2011. Numbers are listed by sex and age class cohort, and capture area (oiled during *Exxon Valdez* oil spill versus unoiled).

Cohort[a]	Oiled	Unoiled
AHY M	15	12
HY M	2	0
AHY F	7	7
HY F	1	1
TOTAL	25	20

[a]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 2. Results of information-theoretic analyses using general linear models to evaluate variation in hepatic7-ethoxyresorufin-*O*-deethylase (EROD) activity of harlequin ducks (n = 45) captured in Prince William Sound, Alaska during March 2011.

Model	K[a]	AIC_c[b]	ΔAIC_c[c]	w[d]
EROD = Area[e]	3	308.7	0.0	0.77
EROD = null	2	311.8	3.1	0.16
EROD = Area + Individual[f]	6	314.0	5.3	0.06
EROD = Individual	5	317.7	9.0	0.01

[a]K = number of estimated parameters in the model.
[b]AIC_c = Akaike's Information Criterion, corrected for small sample size.
[c]ΔAIC_c = difference in AIC_c from the best supported model.
[d]w = AIC_c weight.
[e]Area = categorical variable indicating areas either oiled during the *Exxon Valdez* spill or unoiled.
[f]Individual = a grouping of variables describing attributes of individuals (age, sex, and mass).

Table 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 hepatic7-ethoxyresorufin-*O*-deethylase (EROD) activity (pmol/min/mg protein) of harlequin ducks captured in Prince William Sound, Alaska during March 2011.

	P.L.	Estimate ± SE
Intercept	1.00	15.38 ± 18.55
Area[a]	0.83	17.13 ± 10.85
Sex[b]	0.06	1.39 ± 2.95
Age[c]	0.06	-0.14 ± 1.21
Mass	0.06	0.01 ± 0.03

[a]Area = categorical variable indicating areas either oiled during the *Exxon Valdez* spill or unoiled, with unoiled as the reference value.
[b]Sex = categorical variable (male versus female), with male as the reference value.
[c]Age = categorical variable (hatch-year versus after-hatch-year), with hatch-year as the reference value.

Figure 1. Average (± SE) hepatic7- ethoxyresorufin-O-deethylase (EROD) activity (pmol/min/mg protein) of harlequin ducks (n =45) captured in Prince William Sound, Alaska in March 2011, contrasted with results from previous years (Esler et al. 2010).

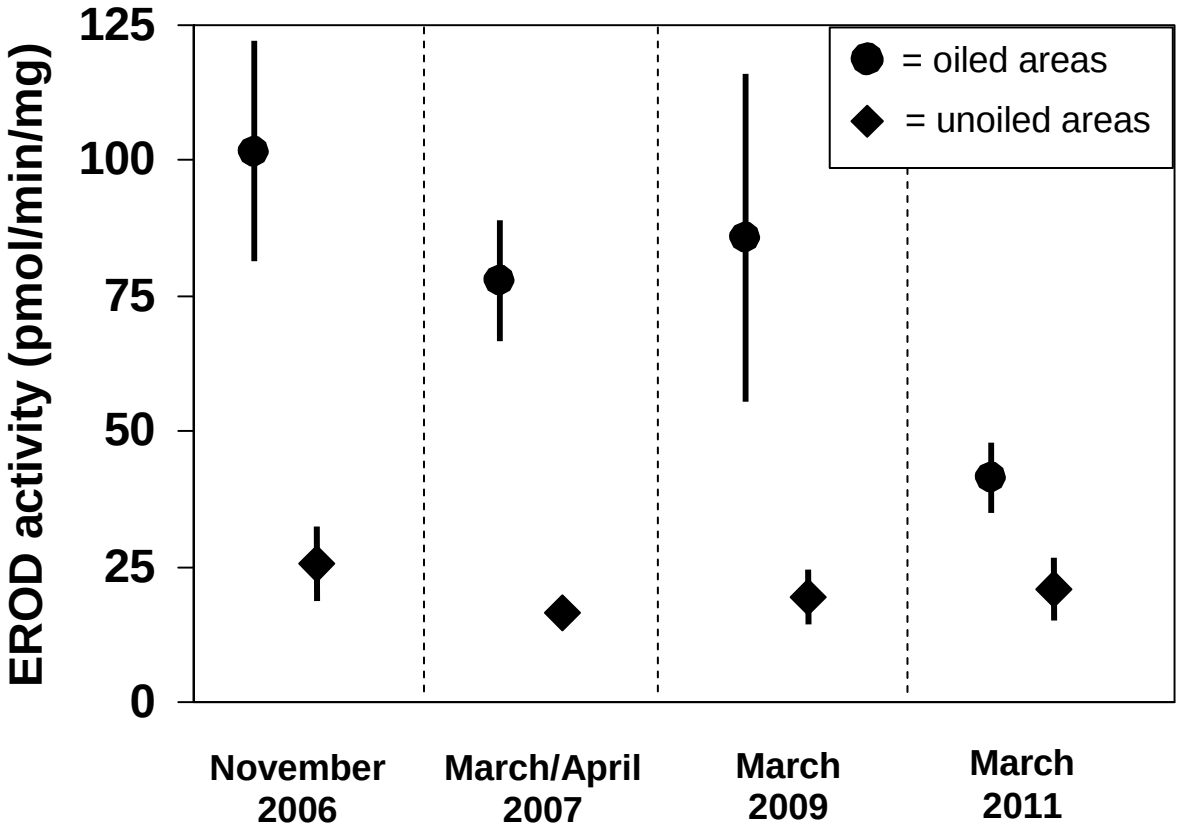

Figure 2. Average (± SE) scaled hepatic7-ethoxyresorufin-O-deethylase (EROD) activity of harlequin ducks (n = 45) captured in March 2011 in areas of Prince William Sound, Alaska oiled during the Exxon Valdez spill relative to nearby unoiled areas, contrasted with results from previous years (Esler et al. 2010). Results are scaled such that the average for each year is set to 1; therefore, the data point for each year represents the multiplicative degree to which EROD is elevated on oiled areas (e.g., in 2011, EROD activity was approximately 2 times higher on oiled areas than on unoiled areas).

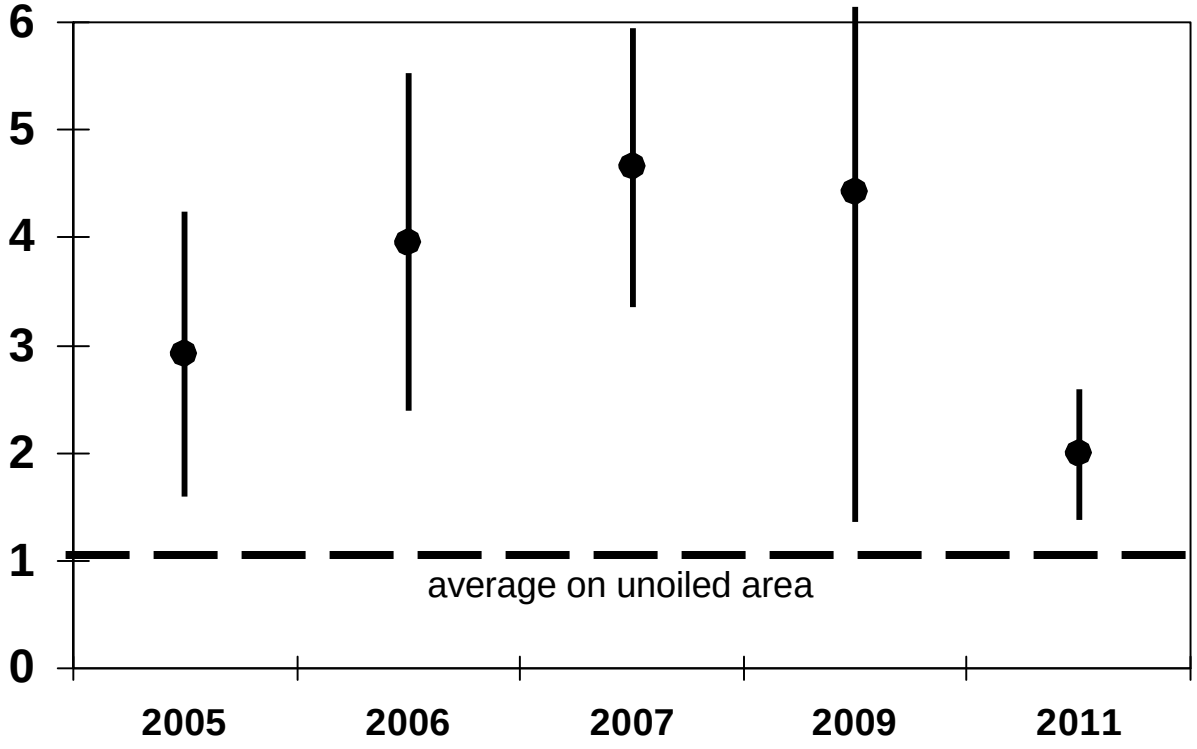

Figure 3. Proportion (y-axis) of captured harlequin ducks with elevated hepatic7-ethoxyresorufin-O-deethylase (EROD) activity, defined as 2 times the average among birds from unoiled areas. Data include results from this study (March 2011) contrasted against findings from previous studies (Esler et al. 2010).