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Exxon Valdez Oil Spill
Restoration Project Final Report

Long-term Monitoring Program -
Evaluating Chronic Exposure of Harlequin Ducks and Sea Otters
to Lingering Exxon Valdez Oil in Western Prince William Sound

Project 14120114-Q

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Study History: Harlequin ducks and sea otters have been studied extensively in Prince William Sound 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. For harlequin ducks, 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 2011, 22 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 the timeline for 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 2011 has led to continued monitoring to evaluate the timeline of exposure. Studies in 2013 indicated that P4501A levels were, for the first time since the spill, similar between ducks in oiled and unoiled areas. The current work was designed to determine whether that result was replicated in 2014, to evaluate whether cessation of oil exposure in harlequin ducks was indicated by additional data. Information on sea otters is described in previous final reports under the 120114-Q series; no sea otter work was conducted during the activities reported herein.

Abstract: We found that average cytochrome P4501A induction (as measured by EROD activity) during March 2014 was not elevated in wintering harlequin ducks captured in areas of Prince William Sound oiled by the 1989 Exxon Valdez oil spill, relative to those captured in unoiled areas. This result is consistent with findings from March 2013. We interpret these findings to indicate that exposure of harlequin ducks to residual Exxon Valdez oil abated within 24 years after the original spill. Results from preceding sampling in 2011 indicated that EROD activity was elevated in harlequin ducks in oiled relative to unoiled areas, although the magnitude of elevation was lower than in previous years (1998-2009), suggesting that the rate or intensity of exposure was diminishing by 2011. The data presented in this report add to a growing body of literature indicating 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, lingering oil, oil exposure, Prince William Sound, recovery.

**Project Data:** Data will be kept in digital format (csv files with metadata) at the U.S. Geological Survey, Alaska Science Center, Anchorage, Alaska. Data custodian – Daniel Esler, Research Wildlife Biologist and Project Leader, Nearshore Marine Ecosystem Research program, U.S. Geological Survey, Alaska Science Center, Anchorage, Alaska. Project data and associated metadata files also are served online on the Alaska Ocean Observing System Workspace as part of the Gulf Watch Alaska Data Management program (portal.aoos.org/gulf-of-alaska.php; EVOS Restoration Project 14120114).

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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 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 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 the same approach used in earlier Exxon Valdez studies and in similar studies of harlequin ducks and other sea ducks elsewhere. During March 2014, we captured 25 harlequin ducks in oiled areas of Prince William Sound and 25 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 harlequin duck CYP1A induction in 2014 was not related to area, with average (pmol/min/mg ± SE) EROD activity of 41.9 (± 7.1) in oiled areas and 28.3 (± 8.7) in unoiled areas. This is consistent with findings from sampling in March 2013, which was the first occasion since sampling was initiated in 1998 that CYP1A induction was not higher in birds from oiled areas than unoiled areas. The 2013 and 2014 findings differ from results in 2011, at which time CYP1A induction was higher on oiled areas, although the magnitude of the difference in 2011 was reduced relative to previous years (1998 to 2009). As in previous years, we found that attributes of individuals (age, sex, and mass) were not related to variation in EROD.

We interpret these results to indicate that harlequin ducks were no longer exposed to residual Exxon Valdez oil at detectable levels as of March 2013, 24 years after the spill. The consistent findings in 2013 and 2014 provide validation of this conclusion.

This work adds to the body of literature evaluating cytochrome P4501A induction in several nearshore vertebrates in Prince William Sound, and defines the timeline over which exposure to lingering oil was evident for a species particularly vulnerable to long-term exposure.
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 (PCDDs; 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), it does 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; Flint et al. 2012). CYP1A is routinely measured by 7-ethoxyresorufin-o-deethylase (EROD) activity, which is a catalytic function principally of hydrocarbon inducible CYP1A enzymes. In studies of captive harlequin ducks (Histrionicus histrionicus), 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).

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, Bodkin et al. 2012).

Harlequin ducks are one of the species that has had 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 (Robertson and Goudie 1999). They are common in Prince William Sound during the nonbreeding season (average of around 15,000 individuals between 1990 and 2010; Cushing et al. 2012), 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. Esler et al. (2002) concluded that continued exposure to lingering oil was likely a constraint on population recovery. A population model was used to estimate the timeline to numeric population recovery, which was estimated to be 24 years after the 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, 2006), and vulnerability of harlequin ducks to effects of oil exposure (Esler et al. 2002), the present study was conducted to reassess levels of CYP1A in this species. In previous studies, Trust et al. (2000) and Esler et al. (2010) found that average 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. In 2011, average EROD activity was higher in harlequin ducks from oiled areas than in those from unoiled areas, although the magnitude of the difference was smaller than during previous sample years (Esler 2011). In 2013, harlequin duck EROD activity was similar between oiled and unoiled areas (Esler 2013). The primary objective for the present study was to add samples from 2014 to the monitoring timeline and evaluate whether the lack of differences in EROD activity observed in 2013 persisted between birds from oiled and unoiled areas.
In addition to assessment of temporal and spatial 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 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 2014. Birds were captured in a number of areas oiled during the Exxon Valdez spill (Figure 1), 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), Lower Passage (60.5º N, 147.7º 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 (Figure 1). 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 assumed 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 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, induced by 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 -80º C freezer until they were shipped to the lab in liquid nitrogen.

Laboratory Analyses

CYP1A induction was determined by measuring hepatic EROD activity, following 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. 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; 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 2014 was not strongly associated with any of the explanatory variables. The best supported model was the null ($w = 0.55$; Table 2), which indicated that none of the explanatory variables explained meaningful variation in EROD activity. The next best supported model ($w = 0.37$) included only the parameter indicating whether harlequin ducks were captured from oiled or unoiled areas. Average EROD activity ($\pm$ SE) was 41.9 ($\pm$ 7.1) in birds from oiled areas and 28.3 ($\pm$ 8.7) in those from unoiled areas (Figure 2); although the direction of differences in point estimates was that expected under a hypothesis of continued oil exposure, these were not statistically different and the difference was much lower than
observed in earlier years. The group of individual attribute variables did not explain meaningful variation in EROD, as both models including individual attributes had small $w$ and received much less support than the null model (i.e., had larger AICc values; Table 2).

Parameter likelihood values also supported the inference that none of the variables had strong value for explaining variation in March 2014 EROD activity. The area parameter was poorly supported, with a parameter likelihood of 0.40 (Table 3). However, the weighted parameter estimate indicated that EROD activity was slightly higher on oiled areas than on unoiled areas (Figure 2), by an average of 5.4 pmol/min/mg protein (Table 3). The corresponding unconditional standard error for the area variable (8.8; Table 3) was larger than the parameter estimate, further indicating the lack of strong support for an area effect. Parameter likelihood values for individual attributes were small, and the weighted parameter estimates were smaller than the corresponding unconditional standard errors (Table 3), indicating that they did not have strong explanatory value.

**DISCUSSION**

We found that hepatic CYP1A levels in harlequin ducks captured in March 2014, based on EROD activity, were similar between areas that were oiled during the *Exxon Valdez* spill and nearby unoiled areas. This finding is consistent with that from March 2013, which constituted the first time since initiation of harlequin duck CYP1A sampling in 1998 that EROD activity was not higher in oiled areas than in unoiled areas of Prince William Sound (Figure 2). We interpret this to indicate that harlequin ducks are no longer exposed to residual oil from the 1989 *Exxon Valdez* spill. The timeline over which the observed return to baseline has occurred (24 years) is longer than anticipated at the time of the spill, given conventional assumptions at that time about duration of bioavailability of spilled oil (Peterson et al. 2003). Abatement of exposure to lingering oil implies that any potential direct, deleterious effects on individuals or populations also must have ceased. We recognize that evidence of exposure through 2011 could not necessarily be inferred to indicate ongoing damage (Lee and Anderson 2005), but absence of exposure in 2013 and 2014 assumes that any remaining damage is due to demographic or toxicological effects of previous exposure.

The observation of similar average EROD activity between oiled and unoiled areas in 2013 and 2014 follows observations in 2011 of a reduction in average CYP1A induction of harlequin ducks in oiled areas, compared to previous years. This suggests that the degree of exposure was declining at that time. This pattern of declines in exposure also was observed in Barrow’s goldeneyes (*Buchephalus islandica*), another nearshore-dwelling sea duck, although evidence of abated exposure occurred earlier in this species, by 2009 (Esler et al. 2011).

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 (masked greenlings [*Hexagrammos octogrammus*] and crescent gunnels [*Pholis laeta*]; Jewett et al. 2002). This body of evidence strongly 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. It also demonstrates that the timeline of exposure varies across species, with harlequin ducks being one of the last to show cessation of exposure, likely due to natural history characteristics that enhanced risk of exposure (Esler et al. 2002).

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, as demonstrated when comparing samples from ducks from oiled and unoiled areas. Also, other studies have indicated that PAHs in the areas where elevated CYP1A was observed in vertebrates were predominately from the *Exxon Valdez* spill, based on oil fingerprinting (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 and subsequent return to baseline levels of CYP1A induction in both harlequin ducks and Barrow’s goldeneyes 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 intertidal and shallow subtidal environments, particularly those that consume benthic organisms, were most likely to have prolonged elevation of CYP1A (Esler et al. 2002). This is presumably due in part to the 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, Michel et al. 2010). Also, because certain molluscan invertebrates have a limited capacity to metabolize PAHs (e.g., Chaty et al. 2004) and are known to ingest and accumulate 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 (Bodkin et al. 2012).

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, Esler et al. 2010, Esler et al. 2011), 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 impacts 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 by Esler et al. (2010), oil-induced effects on demographic rates were diminishing. Given observed demographic rates, Iverson and Esler (2010) projected numeric population recovery would occur by approximately 2013.

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, and mixed-function oxygenase activity in several avian taxa (Hoffman 1979, Naf et al. 1992, Peakall et al. 1980, Peakall et al. 1981). However, given the lack of CYP1A induction observed for harlequin ducks in 2013 and 2014, both lethal and sublethal direct effects of oil exposure can be considered to have ceased.

In summary, the EROD levels reported here provide evidence that since 2013 CYP1A induction is similar between harlequin ducks from oiled and unoiled areas, which we conclude is due to lack of continued exposure to residual Exxon Valdez oil. This suggests the period of exposure of this species to lingering oil was between 22 and 24 years. 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 duration of presence of residual oil and associated effects is
limited to a few years should be abandoned and replaced with the recognition that oil may persist and effects may occur over decades in certain, vulnerable species.

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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 2014. Numbers are listed by sex and age class cohort, and capture area (oiled during Exxon Valdez oil spill versus unoiled).

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<tr>
<td>AHY F</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>HY F</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

*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 hepatic 7-ethoxyresorufin-\(O\)-deethylase (EROD) activity of harlequin ducks (\(n = 50\)) captured in Prince William Sound, Alaska, during March 2014.

<table>
<thead>
<tr>
<th>Model</th>
<th>(K^a)</th>
<th>AIC(_c^b)</th>
<th>(\Delta\text{AIC}_c^c)</th>
<th>(w^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EROD = null</td>
<td>2</td>
<td>371.9</td>
<td>0.0</td>
<td>0.55</td>
</tr>
<tr>
<td>EROD = Area(^e)</td>
<td>3</td>
<td>372.7</td>
<td>0.8</td>
<td>0.37</td>
</tr>
<tr>
<td>EROD = Individual(^f)</td>
<td>5</td>
<td>376.6</td>
<td>4.7</td>
<td>0.05</td>
</tr>
<tr>
<td>EROD = Area + Individual</td>
<td>6</td>
<td>377.9</td>
<td>6.0</td>
<td>0.03</td>
</tr>
</tbody>
</table>

\(^aK = \) number of estimated parameters in the model.
\(^b\text{AIC}\_c = \) Akaike’s Information Criterion, corrected for small sample size.
\(^c\Delta\text{AIC}\_c = \) difference in \(\text{AIC}\_c\) from the best supported model.
\(^d w = \) \(\text{AIC}\_c\) weight.
\(^e\text{Area} = \) categorical variable indicating areas either oiled during the Exxon Valdez spill or unoiled.
\(^f\text{Individual} = \) 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 hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity (pmol/min/mg protein) of harlequin ducks captured in Prince William Sound, Alaska, during March 2014.

<table>
<thead>
<tr>
<th>P.L.</th>
<th>Estimate ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.00 26.31 ± 19.25</td>
</tr>
<tr>
<td>Areaa</td>
<td>0.40 5.39 ± 8.77</td>
</tr>
<tr>
<td>Sexb</td>
<td>0.08 -0.21 ± 1.71</td>
</tr>
<tr>
<td>Agec</td>
<td>0.08 -1.80 ± 3.99</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>0.08 0.01 ± 0.03</td>
</tr>
</tbody>
</table>

*aArea = categorical variable indicating areas either oiled during the Exxon Valdez spill or unoiled, with unoiled as the reference value.

*bSex = categorical variable (male versus female), with male as the reference value.

*cAge = categorical variable (hatch-year versus after-hatch-year), with hatch-year as the reference value.
Figure 1. Map of Prince William Sound, indicating the extent of the 1989 Exxon Valdez oil spill, place names mentioned in the text, and sites where harlequin ducks were sampled in March 2014 for biomarker indicators of induction of cytochrome P4501A as an measure of oil exposure.
Figure 2. Average (± SE) hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity (pmol/min/mg protein) of harlequin ducks (n = 50) captured in Prince William Sound, Alaska, in March 2014, contrasted with results from previous years (Esler et al. 2010, Esler 2011, Esler 2013). Sampling periods with an asterisk indicate significant differences in EROD activity between birds from oiled areas and those from unoiled areas.