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CESSATION OF OIL EXPOSURE IN HARLEQUIN DUCKS AFTER THE *EXXON VALDEZ* OIL SPILL: CYTOCHROME P4501A BIOMARKER EVIDENCE

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Abstract: The authors quantified hepatic hydrocarbon-inducible cytochrome P4501A (CYP1A) expression, as ethoxyresorufin-O-deethylase (EROD) activity, in wintering harlequin ducks (*Histrionicus histrionicus*) captured in Prince William Sound, Alaska (USA), during 2011, 2013, and 2014 (22–25 yr following the 1989 *Exxon Valdez* oil spill). Average EROD activity was compared between birds from areas oiled by the spill and those from nearby unoiled areas. The present study replicated studies conducted from 1998 to 2009 demonstrating that harlequin ducks using areas oiled in 1989 had elevated EROD activity, indicative of oil exposure, up to 2 decades post spill. In the present study, it was found that average EROD activity during March 2011 was significantly higher in wintering harlequin ducks captured in oiled areas relative to unoiled areas, which the authors interpret to indicate that harlequin ducks continued to be exposed to residual *Exxon Valdez* oil up to 22 yr after the original spill. However, the 2011 results also indicated reductions in exposure relative to previous years. Average EROD activity in birds from oiled areas was approximately 2 times that in birds from unoiled areas in 2011, compared with observations from 2005 to 2009, in which EROD activity was 3 to 5 times higher in oiled areas. It was also found that average EROD activity during March 2013 and March 2014 was not elevated in wintering harlequin ducks from oiled areas. The authors interpret these findings to indicate that exposure of harlequin ducks to residual *Exxon Valdez* oil abated within 24 yr after the original spill. The present study finalizes a timeline of exposure, extending over 2 decades, for a bird species thought to be particularly vulnerable to oil contamination in marine environments. *Environ Toxicol Chem* 2016;9999:1–7. Published 2016 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

Keywords: Biomarkers Cytochrome P450 *Exxon Valdez* oil spill Harlequin duck Oil spills

INTRODUCTION

A large body of research has evaluated effects of the 1989 *Exxon Valdez* oil spill on wildlife populations, documenting the processes and timelines of injury and recovery. This research showed that the *Exxon Valdez* spill affected wildlife in many ways, including direct and indirect effects, over immediate and decadal time scales [1]. Direct, chronic effects of the spill occurred for a longer duration than expected, as a result of persistence of oil in subsurface sediments of some intertidal areas [2–4], exposure of animals that utilize such habitats to residual oil [5,6], and deleterious consequences of that exposure [7–10].

Fish and wildlife exposure to lingering *Exxon Valdez* oil has been assessed using indicators of induction of cytochrome P450 1A (CYP1A). In vertebrates, CYP1A genes are induced by a limited number of compounds, including larger polycyclic aromatic hydrocarbons (PAHs) such as those found in crude oil, and halogenated aromatic hydrocarbons, including planar polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins and difurans [11,12]. Because of its specificity, CYP1A is useful as a biomarker for evaluating exposure to that limited suite of chemicals [13].

Indicators of induction of CYP1A messenger RNA, protein, or activity have been used routinely to evaluate exposure to PAHs, PCBs, and dioxins in fish [12,14–18]. Although less common for birds and mammals, indicators of CYP1A induction have been used successfully as biomarkers of exposure of these taxa to inducing compounds, including PAHs [5,19–24].

Following the *Exxon Valdez* oil spill, indicators of CYP1A induction were used to document exposure to lingering oil for a number of fish and wildlife species within Prince William Sound (Alaska, USA), the site of the 1989 spill [5,17,23,25]. These studies demonstrated that CYP1A expression in several species was higher in areas oiled by the *Exxon Valdez* spill relative to nearby unoiled areas a decade or more after the spill. The implication of this finding was that oil remaining in the environment, particularly in intertidal areas, was encountered and ingested by some nearshore vertebrate species. This conclusion is consistent with documentation of the occurrence of residual *Exxon Valdez* oil in intertidal sediments of Prince William Sound during the same period in which elevated CYP1A was documented [2,3], as well as calculations that intertidal-foraging vertebrates were likely to encounter lingering oil on multiple occasions over the course of a year [6,26].

Harlequin ducks (*Histrionicus histrionicus*) are 1 of the species that had elevated CYP1A induction in oiled areas of Prince William Sound relative to unoiled areas [5,25]. As a sea duck, harlequin ducks spend much of the year in intertidal and shallow subtidal habitats of temperate and subarctic marine areas. Harlequin ducks are common and widespread in Prince William Sound during the nonbreeding season (average of ∼15 000 individuals between 1990 and 2010 [27]), and are at

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higher risk of exposure to lingering *Exxon Valdez* oil than many other seabirds, given their close association with nearshore habitats, where a disproportionate amount of oil was deposited [28] and where lingering oil has remained [2,4].

In addition to higher likelihood of exposure to residual oil than many other species, harlequin ducks also have a number of traits that make them particularly vulnerable to oil pollution [8]. These include a life history strategy requiring high adult survival rates, nearly exclusive consumption of benthic invertebrates that live on or in nearshore sediments, and a small body size, relative to other sea ducks, that may constrain their flexibility when confronted with additive energetic demands. As might be expected given their sensitivities to effects of oil contamination, harlequin ducks were documented to exhibit demographic impacts in oiled areas of Prince William Sound following the *Exxon Valdez* spill. Observed impacts included declining population trends [29], reduced densities [30], and poorer female survival [31] in oiled areas relative to unoiled areas. Based on demographic data, a population model was used to estimate the timeline to numeric population recovery, which was estimated to be 24 yr after the spill, or 2013 [9].

Because of previous studies describing elevated indicators of CYP1A induction [5,25], continued occurrence of lingering oil in intertidal habitats where harlequin ducks forage [4], and the characteristics of harlequin ducks that make them vulnerable to effects of oil exposure [8], concerns remained about harlequin duck exposure to lingering *Exxon Valdez* oil. Therefore, the present study was conducted to reevaluate bioindicators of CYP1A in harlequin ducks inhabiting Prince William Sound. In past studies, Trust et al. [25] and Esler et al. [5] documented that average CYP1A expression levels, measured by 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 of the present study was to collect and analyze samples from 2011 to 2014 to determine whether evidence of oil exposure persisted.

In addition to assessment of temporal and spatial variation in CYP1A induction, potential effects of individual attributes (age, sex, and body mass) were also considered. Age, sex, and condition have been shown to affect CYP1A induction in some fish [13,32], and thus these factors should be accounted for when variation in CYP1A induction is evaluated [33].

METHODS

Capture and sample collection

To allow direct comparisons with previous works, the present study closely followed the design and procedures used by Trust et al. [25] and Esler et al. [5]. Wintering harlequin ducks were captured using a floating mist net [34] during March 2011, 2013, and 2014. Captures occurred at several sites oiled during the *Exxon Valdez* spill, including Crafton Island (60.5° N, 147.9° W), Green Island (60.3° N, 147.4° W), Foul Pass (60.5° N, 147.6° W), Lower Passage (60.5° N, 147.7° W), and Herring Bay (60.5° N, 147.7° W). Birds also were captured at several places on nearby northwestern Montague Island (60.3° N, 147.3° W), which was not oiled (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 [35]. 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 US 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 [36,37]. Age class was summarized as either hatch-year, that is, hatched the previous breeding season, or after-hatch-year. Numbers of individuals used in analyses of CYP1A induction are indicated in Table 1, by age class, sex, and area (oiled vs unoiled).

Small (<0.5 g) liver biopsies were surgically removed from each harlequin duck 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 while in the field or during shipping and otherwise were kept in a –80 °C freezer until they were processed. Birds were held until fully recovered from anesthetic effects and then released near their capture sites.

Laboratory analyses

Induction of CYP1A was determined by measuring hepatic EROD activity, following standard methods used in previous studies, described in detail by Miles et al. [21]. Although species vary in their sensitivity to PAH induction of CYP1A, in studies of captive harlequin ducks, EROD activity was confirmed to be significantly higher in birds chronically ingesting weathered Prudhoe Bay crude oil, compared with controls [38]. Similarly, oil-dosed Steller's eiders (*Polysticta stelleri*), another sea duck, had roughly 4-fold increased EROD activity compared with controls [21].

Samples were maintained in –80 °C freezers or liquid nitrogen from the time of capture until microsome preparation, which was approximately 3 mo, 2 mo, and 4.5 mo for 2011, 2013, and 2014 samples, respectively. Microsomes were frozen at –80 °C after preparation, and all assays for a given year were performed on the same day, ranging from 5 d to 17 d after preparation. Activity of EROD is expressed in pmol/min/mg protein. Precision and sensitivity of EROD assays were evaluated and found to be within acceptable bounds.

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 vs unoiled) explained variation in EROD activity, after accounting for any effects of age class, sex, and body mass. Least squares general linear models 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 [39] 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 variables, resulting in a candidate model set consisting of: 1) EROD = area; 2) EROD = individual attributes; and

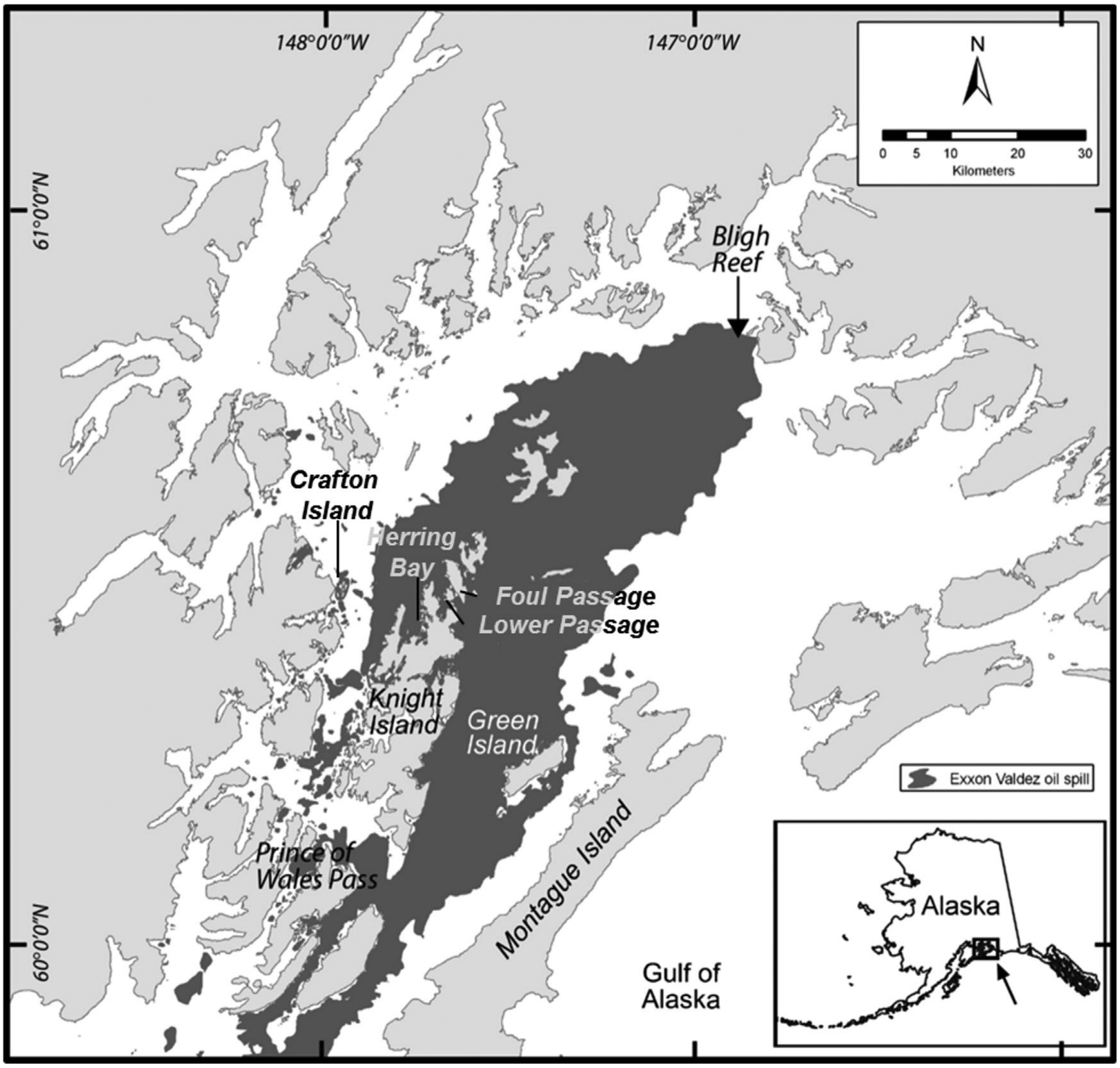

Figure 1. Map of Prince William Sound, Alaska, USA indicating the extent of the 1989 *Exxon Valdez* oil spill, place names mentioned in the text, and sites where harlequin ducks were sampled in March 2011, 2013, and 2014, for biomarker indicators of induction of cytochrome P4501A as a measure of oil exposure.

3) EROD = area + individual attributes. We also included a null model, which consisted of estimates of a mean and variance across all the data; support for the null model would indicate that variables considered in other candidate models did not explain important variation in the response. Analyses were run independently for each year, in recognition of the fact that variability can occur among laboratory runs [38], potentially related to sample degradation during storage [13]; this does not affect contrasts between areas, because all samples within a year were run concurrently.

Table 1. Sample sizes of harlequin ducks captured in Prince William Sound, Alaska (USA), for analyses of cytochrome P4501A induction in March 2011, 2013, and 2014[a]

	March 2011		March 2013		March 2014	
Cohort[b]	Oiled	Unoiled	Oiled	Unoiled	Oiled	Unoiled
AHY M	15	12	18	15	15	14
HY M	2	0	0	2	0	1
AHY F	7	7	7	7	6	8
HY F	1	1	0	1	4	2
Total	25	20	25	25	25	25

[a]Numbers are listed by sex and age class cohort, and capture area (oiled during *Exxon Valdez* oil spill vs unoiled).
[b]Cohort consists of an age class designation (HY = hatch-year, i.e., within 1 yr of hatching; AHY = after-hatch-year) and sex (M = male; F = female).

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, 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 AIC_c weight 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

We found that EROD activity of harlequin ducks captured in March 2011 was higher for birds from oiled areas compared with unoiled areas (Figure 2), indicating that they continued to be exposed to residual *Exxon Valdez* oil. The model with area as the only explanatory variable received nearly 5 times the support of any other model, with an AIC_c weight of 0.77 (Table 2). Parameter likelihood values also supported the importance of area, with a parameter likelihood of 0.83 (Table 3). Also, the weighted parameter estimate indicated that EROD activity in oiled areas was higher than in unoiled areas by an average of 17.1 pmol/min/mg protein in 2011 (Figure 2).

Although areas differed in EROD activity, the results suggested that the degree and incidence of oil exposure were lower in 2011 than in previous years. Average (pmol/min/mg $\pm$ standard error [SE]) EROD activity on oiled areas was 41.5 ($\pm$ 6.4) in 2011, compared with point estimates >75 pmol/min/mg in the previous 3 sampling periods, despite very similar estimates of EROD activity in unoiled areas (20.9 $\pm$ 5.7 in 2011; Figure 2).

In contrast with 2011, EROD activity of harlequin ducks captured in March 2013 and 2014 did not indicate higher EROD activity in oiled areas. For 2013, the best supported model included only the parameter indicating whether harlequin ducks were captured from oiled or unoiled areas (AIC_c weight = 0.43; Table 2). However, support for that model was virtually indistinguishable from the null model (AIC_c weight = 0.43), which indicated that none of the explanatory variables was strongly supported. In addition, average EROD activity was lower in oiled areas than in unoiled areas (Table 3 and Figure 2); therefore, the moderate support for an area effect was in the opposite direction than expected under a hypothesis of continued oil exposure.

Parameter likelihood values also supported the inference that none of the variables was strongly related to March 2013 EROD activity. The area parameter was moderately supported, with a parameter likelihood of 0.49 (Table 3). However, the weighted parameter estimate indicated that EROD activity was slightly higher in unoiled areas than in oiled areas (Figure 2), by an average of 4.8 pmol/min/mg protein (Table 3). The corresponding unconditional standard error for the area variable (6.5; Table 3) was larger than the parameter estimate, further indicating the lack of strong support for an area effect.

The EROD activity of harlequin ducks captured in March 2014 was not associated with any of the explanatory

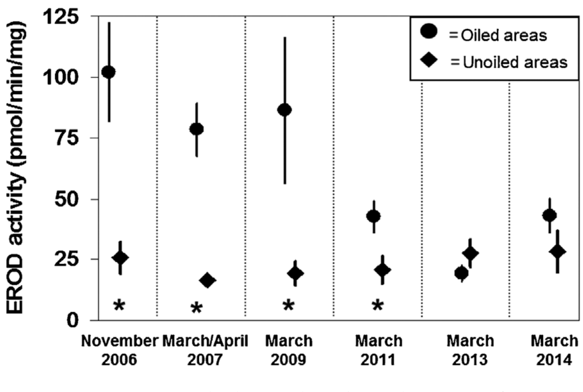

Figure 2. Average ($\pm$ standard error) hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity (pmol/min/mg protein) of harlequin ducks captured in Prince William Sound, Alaska, USA, in March 2011, 2013, and 2014, contrasted with results from previous years shown by Esler et al. in 2010 [5]. Sampling periods with an asterisk indicate statistical differences in EROD activity between birds from oiled areas and those from unoiled areas.

variables. The null model was best supported (AIC_c weight = 0.55; Table 2), which indicated that none of the variables influenced EROD activity. The next best supported model (AIC_c weight = 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, estimates were not statistically different and the difference between point estimates was much lower than observed in earlier years (Figure 2).

Parameter likelihood values supported the conclusion that none of the variables were related to variation in March 2014 EROD activity. The area parameter was not strongly supported, with a parameter likelihood of 0.40 (Table 3). However, the weighted parameter estimate indicated that EROD activity was slightly higher in oiled areas than in unoiled areas (Figure 2), by 5.4 pmol/min/mg protein (Table 3). The unconditional standard error for the area variable (8.8; Table 3) was larger than the parameter estimate, confirming the lack of evidence of an effect of area.

In all years, the group of individual attribute variables was not related to EROD, because models including individual attributes had small AIC_c weights and received much less support than the null model (i.e., had larger AIC_c values; Table 2). Also, in all years, parameter likelihood values for individual attributes were small, and the weighted parameter estimates were smaller than the corresponding unconditional standard errors (Table 3), confirming that they did not have strong explanatory value.

DISCUSSION

We found that harlequin ducks had elevated EROD activity in areas of Prince William Sound oiled by the *Exxon Valdez* spill through 2011, 22 yr after the spill occurred. This suggests that this species was exposed to lingering oil in intertidal sediments for over 2 decades. The data from 2011 show a moderated CYP1A response relative to previous years (Figure 2), suggesting declining exposure. By 2013 and 2014 (24 yr and 25 yr post spill, respectively), there was no statistical difference in EROD activity between oiled and unoiled areas, indicating that harlequin ducks likely were no longer being measurably exposed to *Exxon Valdez* oil. 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 does not necessarily indicate ongoing population or individual-level damage [33], although both overt demographic effects [31] and subtle effects on individual health [18] are known to result from exposure. However, absence of evidence of exposure in 2013 and 2014 implies that any remaining population damage or individual effects would be the result of demographic or toxicological effects of previous oil exposure. The estimated duration of residual oil exposure in this species is much longer than initial assumptions about duration of bioavailability of oil from the *Exxon Valdez* spill [1]. The present study adds to the unprecedented timeline evaluating oil exposure in a wildlife species following a major oil spill, extending over a quarter century.

Differential CYP1A induction between oiled and unoiled areas has been described for other vertebrates in Prince William Sound, including Barrow's goldeneyes (*Bucephala*

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 captured in Prince William Sound, Alaska, (USA), during March 2011, 2013, and 2014

		March 2011			March 2013			March 2014		
Model	K	AIC_c	ΔAIC_c	AIC_c weight	AIC_c	ΔAIC_c	AIC_c weight	AIC_c	ΔAIC_c	AIC_c weight
EROD = null	2	311.8	3.1	0.16	319.9	0.0	0.43	371.9	0.0	0.55
EROD = area	3	308.7	0.0	0.77	319.9	0.0	0.43	372.7	0.8	0.37
EROD = individual	5	317.7	9.0	0.01	323.3	3.4	0.08	376.6	4.7	0.05
EROD = area + individual	6	314.0	5.3	0.06	324.1	4.2	0.05	377.9	6.0	0.03

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

islandica) [23,25], adult pigeon guillemots (*Cepphus columba*) [40], river otters (*Lontra canadensis*) [41], and 2 demersal fishes (masked greenlings [*Hexagrammos octogrammus*] and crescent gunnels [*Pholis laeta*] [17]). Taken together, these findings strongly indicate 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 varied across species, with harlequin ducks being the last to show cessation of exposure, likely because of natural history characteristics that enhanced exposure risk [8]. Of the other taxa studied in Prince William Sound after the oil spill, the species most similar to harlequin ducks, Barrow's goldeneyes, another nearshore-dwelling sea duck, showed similar protracted exposure and subsequent return in average EROD activity to reference levels by 2009 [23].

The source of CYP1A-inducing compounds in Prince William Sound has been debated [42], because there may be multiple sources of inducing compounds within any given area [33]. Some studies [42–45] have suggested that non-*Exxon Valdez* sources of PAHs are more likely to have resulted in observed CYP1A induction than residual *Exxon Valdez* oil. However, the spatial correspondence between elevated CYP1A induction and history of contamination during the *Exxon Valdez* oil spill provides strong evidence for causation, as illustrated by the contrasts of samples from ducks from oiled and unoiled areas. In addition, higher variation observed in oiled areas when EROD was elevated (Figure 2) is consistent with a patchily distributed and intermittently encountered inducing agent, as would be the case with lingering subsurface *Exxon Valdez* oil. Also, other studies have indicated that in the areas where elevated CYP1A was observed in vertebrates, PAHs were predominantly from the *Exxon Valdez* spill, based on oil fingerprinting [2], supporting the inference that *Exxon Valdez* oil was the inducing agent. Additional studies have indicated that sites with residual *Exxon Valdez* oil had bioavailable PAHs that elicited CYP1A induction when experimentally injected into fish [46].

Other potential CYP1A inducers in Prince William Sound, specifically PCBs, were very low and below concentrations that would induce CYP1A induction, consistent with broad-scale atmospheric deposition [47]. Trust et al. [25] and Ricca et al. [48] 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. Also, Short et al. [26] 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, results indicating declines and subsequent return to baseline levels of CYP1A induction in both harlequin ducks and Barrow's goldeneyes over time are 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 more constant over time such as atmospheric PCBs or oil from natural seeps.

In summary, the EROD levels reported in the present study provide evidence that 2013 CYP1A induction was similar between harlequin ducks from oiled and unoiled areas, which we conclude is the result of lack of measurable exposure to residual *Exxon Valdez* oil. This suggests that the period of exposure of this species to lingering oil was between 22 yr and 24 yr. Given the lack of CYP1A induction observed for harlequin ducks in 2013 and 2014, it is assumed that oil exposure was no longer occurring at that time and thus any potential lethal or sublethal direct effects of oil exposure can be considered to have ceased. We note that oil from other contamination events also has been reported to persist over long

Table 3. Parameter likelihoods (PL), 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 2011, 2013, and 2014

	March 2011		March 2013		March 2014	
	PL	Estimate ± SE	PL	Estimate ± SE	PL	Estimate ± SE
Intercept	1.00	15.38 ± 18.55	1.00	25.99 ± 13.25	1.00	26.31 ± 19.25
Area	0.83	17.13 ± 10.85	0.49	−4.76 ± 6.50	0.40	5.39 ± 8.77
Sex	0.06	1.39 ± 2.95	0.13	1.57 ± 3.42	0.08	−0.21 ± 1.71
Age	0.06	−0.14 ± 1.21	0.13	−1.12 ± 3.31	0.08	−1.80 ± 3.99
Mass (g)	0.06	0.01 ± 0.03	0.13	−0.00 ± 0.02	0.08	0.01 ± 0.03

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

periods [49,50]. We agree with Peterson et al. [1] that the conventional paradigm that duration of presence of residual oil, and associated exposure and potential effects, is limited to a few years should be abandoned and replaced with the recognition that oil may persist and exposure may occur over decades in certain, vulnerable species.

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Data Availability—Data are available from the senior author on request (desler@usgs.gov). Data are also available from the US Geological Survey at http://dx.doi.org/10.5066/F7FN1498.

REFERENCES

1. Peterson CH, Rice SD, Short JW, Esler D, Bodkin JL, Ballachey BE, Irons DB. 2003. Long-term ecosystem response to the *Exxon Valdez* oil spill. *Science* 302:2082–2086.
2. Short JW, Lindeberg MR, Harris PM, Maselko JM, Pella JJ, Rice SD. 2004. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the *Exxon Valdez* oil spill. *Environ Sci Technol* 38:19–25.
3. Li HL, Boufadel MC. 2010. Long-term persistence of oil from the *Exxon Valdez* spill in two-layer beaches. *Nat Geosci* 3:96–99.
4. Carls M, Fugate C. 2016. 25 year retention of PAHs and alkanes in sediment oiled by the *Exxon Valdez*. Report to the Exxon Valdez Oil Spill Trustee Council. US National Oceanic and Atmospheric Administration, Juneau, AK, USA.
5. Esler D, Trust KA, Ballachey BE, Iverson SA, Lewis TL, Rizzolo DJ, Mulcahy DM, Miles AK, Woodin BR, Stegeman JJ, Henderson JD, Wilson BD. 2010. Cytochrome P4501A biomarker indication of oil exposure in harlequin ducks up to 20 years after the *Exxon Valdez* oil spill. *Environ Toxicol Chem* 29:1138–1145.
6. Bodkin JL, Ballachey BE, Coletti HA, Esslinger GG, Kloecker KA, Rice SD, Reed JA, Monson DH. 2012. Long-term effects of the *Exxon Valdez* oil spill: Sea otter foraging in the intertidal as a pathway of exposure to lingering oil. *Mar Ecol Prog Ser* 447:273–287.
7. Bodkin JL, Ballachey BE, Dean TA, Fukuyama AK, Jewett SC, McDonald L, Monson DH, O'Clair CE, VanBlaricom GR. 2002. Sea otter population status and the process of recovery from the 1989 *Exxon Valdez* oil spill. *Mar Ecol Prog Ser* 241:237–253.
8. Esler D, Bowman TD, Trust K, Ballachey BE, Dean TA, Jewett SC, O'Clair CE. 2002. Harlequin duck population recovery following the *Exxon Valdez* oil spill: Progress, process, and constraints. *Mar Ecol Prog Ser* 241:271–286.
9. Iverson SA, Esler D. 2010. Harlequin duck population dynamics following the 1989 *Exxon Valdez* oil spill: Assessing injury and projecting a timeline to recovery. *Ecol Appl* 20:1993–2006.
10. Monson DH, Doak DF, Ballachey BE, Bodkin JL. 2011. Could residual oil from the *Exxon Valdez* spill create a long-term population "sink" for sea otters in Alaska? *Ecol Appl* 21:2917–2932.
11. Payne JF, Fancey LL, Rahimtula AD, Porter EL. 1987. Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. *Comp Biochem Physiol* 86:233–245.
12. Goksøyr A. 1995. Use of cytochrome P450 1A (CYP1A) in fish as a biomarker of aquatic pollution. *Arch Toxicol Suppl* 17:80–95.
13. Whyte JJ, Jung RE, Schmitt JE, Tillitt DE. 2000. Ethoxyresorufin-*O*-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit Rev Toxicol* 30:347–570.
14. Stegeman JJ, Kloepper-Sams PJ, Farrington JW. 1986. Monooxygenase induction and chlorobiphenyls in the deep-sea fish *Coryphaenoides armatus*. *Science* 231:1287–1289.
15. Marty GD, Short JW, Dambach DM, Willits NH, Heintz RA, Rice SD, Stegeman JJ, Hinton DE. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Can J Zool* 75:989–1007.
16. Woodin BR, Smolowitz RM, Stegeman JJ. 1997. Induction of cytochrome P4501A in the intertidal fish (*Anoplarchus purpurescens*) by Prudhoe Bay crude oil and environmental induction in fish from Prince William Sound. *Environ Sci Technol* 31:1198–1205.
17. Jewett SC, Dean TA, Woodin BR, Hoberg MK, Stegeman JJ. 2002. Exposure to hydrocarbons ten years after the *Exxon Valdez*: Evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. *Mar Environ Res* 54:21–48.
18. Carls MG, Heintz RA, Marty GD, Rice SD. 2005. Cytochrome P4501A induction in oil-exposed pink salmon *Oncorhynchus gorbuscha* embryos predicts reduced survival potential. *Mar Ecol Prog Ser* 301:253–265.
19. Peakall DB, Norstrom RJ, Jeffrey DA, Leighton FA. 1989. Induction of hepatic mixed function oxidases in the herring gull (*Larus argentatus*) by Prudhoe Bay crude oil and its fractions. *Comp Biochem Physiol C* 94:461–463.
20. Ben-David M, Kondratyuk T, Woodin BR, Snyder PW, Stegeman JJ. 2001. Induction of cytochrome P4501A1 expression in captive river otters fed Prudhoe Bay crude oil: Evaluation by immunohistochemistry and quantitative RT-PCR. *Biomarkers* 6:218–235.
21. Miles AK, Flint PL, Trust KA, Ricca MA, Spring SE, Arietta DE, Hollmén T, Wilson BW. 2007. Polycyclic aromatic hydrocarbon exposure in Steller's eiders (*Polysticta stelleri*) and harlequin ducks (*Histrionicus histrionicus*) in the eastern Aleutian Islands, Alaska. *Environ Toxicol Chem* 26:2694–2703.
22. Velando A, Munilla I, López-Alonso M, Friere J, Pérez C. 2010. EROD activity and stable isotopes in seabirds to disentangle marine food web contamination after the *Prestige* oil spill. *Environ Pollut* 158:1275–1280.
23. Esler D, Ballachey BE, Trust KA, Iverson SA, Reed JA, Miles AK, Henderson JD, Wilson BW, Woodin BR, Stegeman JJ, McAdie M, Mulcahy DM. 2011. Cytochrome P4501A biomarker indication of the timeline of chronic exposure of Barrow's goldeneye to residual *Exxon Valdez* oil. *Mar Pollut Bull* 62:609–614.
24. Flint PL, Schamber JL, Trust KA, Miles AK, Henderson JD, Wilson BW. 2012. Chronic hydrocarbon exposure of harlequin ducks in areas affected by the *Selendang Ayu* oil spill at Unalaska Island, Alaska. *Environ Toxicol Chem* 31:2828–2831.
25. Trust KA, Esler D, Woodin BR, Stegeman JJ. 2000. Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. *Mar Pollut Bull* 40:397–403.
26. Short JW, Maselko JM, Lindeberg MR, Harris PM, Rice SD. 2006. Vertical distribution and probability of encountering intertidal *Exxon Valdez* oil on shorelines of three embayments within Prince William Sound. *Environ Sci Technol* 40:3723–3729.
27. Cushing DA, McKnight A, Irons DB, Kuletz KJ, Howlin S. 2012. Prince William Sound marine bird surveys, synthesis and restoration. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 10100751). US Fish and Wildlife Service, Anchorage, AK.
28. Wolfe DA, Hameedi MJ, Galt JA, Watabayashi G, Short J, O'Claire C, Rice S, Michel J, Payne JR, Braddock J, Hanna S, Sale D. 1994. The fate of the oil spilled from the *Exxon Valdez*. *Environ Sci Technol* 28:561–568.
29. Rosenberg DH, Petrula MJ. 1998. Status of harlequin ducks in Prince William Sound, Alaska, after the *Exxon Valdez* oil spill, 1995-1997. *Exxon Valdez* Oil Spill Restoration Project Final Report 97427. Alaska Department of Fish and Game, Division of Wildlife Conservation, Anchorage, AK, USA.
30. Esler D, Bowman TD, Dean TA, O'Clair CE, Jewett SC, McDonald LL. 2000. Correlates of harlequin duck densities during winter in Prince William Sound, Alaska. *Condor* 102:920–926.
31. Esler D, Schmutz JA, Jarvis RL, Mulcahy DM. 2000. Winter survival of adult female harlequin ducks in relation to history of contamination by the Exxon Valdez oil spill. *J Wildl Manag* 64:839–847.
32. Sleiderink HM, Oostingh I, Goksøyr A, Boon JP. 1995. Sensitivity of cytochrome P450 1A induction in dab (*Limanda limanda*) of different age and sex as a biomarker for environmental contaminants in the southern North Sea. *Arch Environ Contam Toxicol* 28:423–430.
33. Lee RF, Anderson JW. 2005. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. *Mar Pollut Bull* 50:705–723.

34. Kaiser, GW, Derocher AE, Crawford SC, Gill MJ, Manley IA. 1995. A capture technique for marbled murrelets in coastal inlets. *J Field Ornithol* 66:321–333.
35. Iverson SA, Esler D. 2006. Site fidelity and the demographic implications of winter movements by a migratory bird, the harlequin duck. *J Avian Biol* 37:219–228.
36. Mather DD, Esler D. 1999. Evaluation of bursal depth as an indicator of age class of harlequin ducks. *J Field Ornithol* 70:200–205.
37. Smith CM, Goudie RI, Cooke F. 1998. Winter age ratios and the assessment of recruitment of harlequin ducks. *Waterbirds* 24:39–44.
38. Esler D. 2008. Quantifying temporal variation in harlequin duck cytochrome P4501A induction. *Exxon Valdez* Oil Spill Trustee Council Gulf Ecosystem Monitoring and Research Project Final Report (GEM Project 050777). Centre for Wildlife Ecology, Simon Fraser University, Delta, BC, Canada.
39. Burnham KP, Anderson DR. 2002. *Model Selection and Multimodel Inference: A Practical Information Theoretic Approach*, 2nd ed. Springer-Verlag, New York, NY, USA.
40. Golet GH, Seiser PE, McGuire AD, Roby DD, Fischer JB, Kuletz KJ, Irons DB, Dean TA, Jewett SC, Newman SH. 2002. Long-term direct and indirect effects of the *Exxon Valdez* oil spill on pigeon guillemots in Prince William Sound, Alaska. *Mar Ecol Prog Ser* 241:287–304.
41. Bowyer RT, Blundell GM, Ben-David M, Jewett SC, Dean TA, Duffy LK. 2003. Effects of the *Exxon Valdez* oil spill on river otters: Injury and recovery of a sentinel species. *Wildl Monogr* 153:1–53.
42. Harwell MA, Gentile JH. 2006. Ecological significance of residual exposures and effects from the *Exxon Valdez* oil spill. *Integr Environ Assess Manage* 2:204–246.
43. Page DS, Boehm PD, Douglas GS, Bence AE, Burns WA, Mankiewicz PJ. 1996. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska, USA. *Environ Toxicol Chem* 15:1266–1281.
44. Page DS, Boehm PD, Douglas GS, Bence AE, Burns WA, Mankiewicz PJ. 1997. An estimate of the annual input of natural petroleum hydrocarbons to seafloor sediments of Prince William Sound, Alaska. *Mar Pollut Bull* 34:744–749.
45. Boehm PD, Page DS, Burns WA, Bence AE, Mankiewicz PJ, Brown JS. 2001. Resolving the origin of the petrogenic hydrocarbon background in Prince William Sound, Alaska. *Environ Sci Technol* 35:471–479.
46. Springman KR, Short JW, Lindeberg MR, Maselko JM, Khan C, Hodson PV, Rice SD. 2008. Semipermeable membrane devices link site-specific contaminants to effects: Part I—Induction of CYP1A in rainbow trout from contaminants in Prince William Sound, Alaska. *Mar Environ Res* 66:477–486.
47. Short JW, Springman KR, Lindeberg MR, Holland LG, Larsen ML, Sloan CA, Khan C, Hodson PV, Rice SD. 2008. Semipermeable membrane devices link site-specific contaminants to effects: Part II—A comparison of lingering *Exxon Valdez* oil with other potential sources of CYP1A inducers in Prince William Sound, Alaska. *Mar Environ Res* 66:487–498.
48. Ricca MA, Miles AK, Ballachey BE, Bodkin JL, Esler D, Trust KA. 2010. PCB exposure in sea otters and harlequin ducks in relation to history of contamination by the *Exxon Valdez* oil spill. *Mar Pollut Bull* 60:861–872.
49. Vandermeulen JH, Singh JG. 1994. ARROW oil spill, 1970-1990: Persistence of 20-yr weathered Bunker C fuel oil. *Can J Fish Aquat Sci* 51:845–855.
50. Peacock EE, Nelson RK, Solow AR, Warren JD, Baker JL, Reddy CM. 2005. The West Falmouth oil spill: 100 kg of oil persists in marsh sediments. *Environ Forensics* 6:273–281.