Marine Pollution Bulletin 62 (2011) 609–614

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Cytochrome P4501A biomarker indication of the timeline of chronic exposure of Barrow's goldeneyes to residual *Exxon Valdez* oil

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ARTICLE INFO

Keywords:
Alaska
Bucephala islandica
CYP1A
Exposure
Exxon Valdez
Oil

ABSTRACT

We examined hepatic EROD activity, as an indicator of CYP1A induction, in Barrow's goldeneyes captured in areas oiled during the 1989 *Exxon Valdez* spill and those from nearby unoiled areas. We found that average EROD activity differed between areas during 2005, although the magnitude of the difference was reduced relative to a previous study from 1996/1997, and we found that areas did not differ by 2009. Similarly, we found that the proportion of individuals captured from oiled areas with elevated EROD activity ($\geqslant$2 times unoiled average) declined from 41% in winter 1996/1997 to 10% in 2005 and 15% in 2009. This work adds to a body of literature describing the timelines over which vertebrates were exposed to residual *Exxon Valdez* oil and indicates that, for Barrow's goldeneyes in Prince William Sound, exposure persisted for many years with evidence of substantially reduced exposure by 2 decades after the spill.

1. Introduction

Some of the oil released during the 1989 *Exxon Valdez* oil spill has remained in intertidal sediments of Prince William Sound, Alaska over the subsequent two decades (Boehm et al., 2008; Short et al., 2004, 2006). Estimates of the quantity remaining (Boehm et al., 2008; Short et al., 2004) represent a small fraction of the oil that originally settled on Prince William Sound beaches following the spill (Wolfe et al., 1994), with residual oil in intertidal areas estimated to exceed 50,000 kg in 2001 (Short et al., 2004). Concern and debate persist over whether vertebrates continue to be exposed to the remaining oil and, if so, whether that exposure negatively affects individuals or populations.

Exposure of vertebrates to residual *Exxon Valdez* oil has been assessed by the use of indicators of induction of specific members of the cytochrome P4501 gene family. Members of the cytochrome P4501A (CYP1A) gene subfamily in vertebrates are induced by polycyclic aromatic hydrocarbons (PAH) found in crude oil, as well as certain halogenated aromatic hydrocarbons including planar polychlorinated biphenyls (PCB) and dioxins (Goksøyr, 1995; Payne et al., 1987; Whitlock, 1999; Woodin et al., 1997). Because CYP1As are induced by a limited number of compounds, measures of induction of CYP1A mRNA, protein, or resulting enzyme activity are particularly useful biomarkers for evaluating exposure to those contaminants (Whyte et al., 2000). Elevated enzyme activity does not directly indicate compromised survival or health of individuals (Lee and Anderson, 2005); however, it does indicate exposure to inducing compounds at levels sufficient to elicit a biochemical response and, thus, at least the potential for associated toxic consequences.

Measurement of hepatic 7-ethoxyresorufin-*O*-deethylase (EROD) activity, a catalytic function of hydrocarbon – inducible CYP1 enzymes, is a widely used and recognized method for indicating CYP1 induction. There are multiple CYP1 genes in vertebrates. Two CYP1A genes (CYP1A4 and CYP1A5) have been identified in birds, both of which are inducible by aryl hydrocarbon receptor (AHR) agonists and both of which catalyze EROD activity (Kubota et al., 2009; Rifkind et al., 1994). EROD activity has been shown

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doi:10.1016/j.marpolbul.2010.11.015

to be a reliable indicator of oil exposure in birds generally and sea ducks specifically (Esler, 2008; Miles et al., 2007).

Elevated indicators of CYP1A induction have been documented in a number of vertebrate species sampled from areas of Prince William Sound, Alaska that received oil during the 1989 *Exxon Valdez* oil spill (Esler et al., 2010; Jewett et al., 2002; Trust et al., 2000). For example, harlequin ducks (*Histrionicus histrionicus*), which forage in the intertidal zone, were shown to have elevated EROD activity when collected from oiled areas of Prince William Sound relative to unoiled areas, during the years 1998–2009 (Esler et al., 2010; Trust et al., 2000). Evidence of PAH exposure coincided with evidence of injury to individuals and populations of some vertebrates for at least a decade following the *Exxon Valdez* spill (Bodkin et al., 2002; Esler et al., 2000, 2002), although evidence of exposure persisted in some vertebrates beyond the period when gross demographic injury was evident and populations were projected to be recovering (Esler and Iverson, 2010; Iverson and Esler, 2010). The timeline over which exposure and injury were documented was an unanticipated finding stemming from the large body of research following the *Exxon Valdez* spill (Peterson et al., 2003). For example, mortalities of harlequin ducks thought to be related to chronic exposure to oil were estimated to exceed the acute mortality that occurred in the weeks and months immediately following the spill (Iverson and Esler, 2010), which is commonly perceived as the period of highest damage.

Trust et al. (2000) found that Barrow's goldeneyes (*Bucephala islandica*), a common sea duck in the area contaminated by the 1989 *Exxon Valdez* oil spill, had elevated EROD activity during winter 1996–1997, which was interpreted as evidence of continued exposure to lingering oil. Wintering Barrow's goldeneyes in Prince William Sound inhabit nearshore areas and forage primarily on blue mussels (*Mytilus trossulus*) in the intertidal zone (Esler, 2000; Koehl et al., 1982; Vermeer, 1982) where much of the residual *Exxon Valdez* oil persisted (Short et al., 2004, 2006).

Because of the evidence of exposure to oil nearly a decade after the spill (Trust et al., 2000) and their potential vulnerability for exposure as long as oil remains in intertidal sediments, we compared EROD activity in Barrow's goldeneyes captured in oiled and unoiled areas of Prince William Sound, Alaska during March 2005 and March 2009. Sea ducks generally have high levels of winter site fidelity (Iverson and Esler, 2006) and thus we assumed that movements of birds between oiled and unoiled areas were unlikely and therefore would not influence our inferences about differences in EROD activity in birds from different areas. Satellite telemetry data from adult male Barrow's goldeneye marked in Prince William Sound confirm high winter site fidelity (W.S. Boyd, Environment Canada, unpublished data). We also accounted for variation related to individual attributes, in case these confounded interpretation of area effects (Lee and Anderson, 2005).

2. Materials and methods

Evidence of CYP1A induction in wintering Barrow's goldeneyes in Prince William Sound, Alaska, was evaluated by measuring hepatic EROD activity, which required capture of wintering Barrow's goldeneyes, surgery to nonlethally remove a liver biopsy, and subsequent laboratory analysis. To facilitate comparisons to previous work on Barrow's goldeneyes, we designed the study and procedures per Trust et al. (2000).

2.1. Capture and sample collection

We captured Barrow's goldeneyes using floating mist nets in March 2005 and 2009. Barrow's goldeneyes winter in Prince William Sound between October and May. Our capture period in March was designed to capture individuals that had been on sampling sites all winter, but before pre-migratory movements, including local movements that some sea ducks exhibit in relation to Pacific herring (*Clupea pallasi*) spawn (Lok et al., 2008; Rodway et al., 2003). Barrow's goldeneyes were captured in a number of sites in Prince William Sound oiled during the *Exxon Valdez* spill, including Bay of Isles (60° 22′ N, 147° 40′ W), Herring Bay (60° 28′ N, 147° 44′ W), Crafton Island (60° 29′ N, 147° 57′ W), Falls Bay (60° 32′ N, 148° 01′ W), Green Island (60° 18′ N, 147° 24′ W), and Foul Pass (60° 29′ N, 147° 38′ W). These sites were documented as having received oil during the *Exxon Valdez* spill; contemporary occurrence or distribution of residual oil was not known for each specific sampling site within the oiled area. Also, birds were captured at unoiled reference sites on nearby northwestern Montague Island (60° 15′ N, 147° 12′ W) and Culross Passage (60° 43′ N, 148° 15′ W), which were reported to have not received oil during the *Exxon Valdez* spill. Sex of each bird was determined by plumage and cloacal characteristics, and age class was determined by the depth of the bursa of Fabricius (Mather and Esler, 1999) for females and bursal depth and plumage characteristics for males. Age class was summarized as either hatch-year (HY, i.e., hatched the previous breeding season) or after-hatch-year (AHY).

A liver biopsy (<0.5 g and <5 mm diameter) was surgically removed by a veterinarian from each anesthetized Barrow's goldeneye. The biopsy was then placed into a labeled cryovial and flash frozen in liquid nitrogen. All samples were maintained at −80 °C until laboratory analysis. The birds were held until full recovery and then released near the location of capture.

2.2. Laboratory analyses

EROD activity was measured at 2 laboratories: 2005 samples were analyzed at Woods Hole Oceanographic Institution and 2009 samples at the University of California Davis using methods described in detail by Trust et al. (2000) and Miles et al. (2007), respectively. Paired liver samples of harlequin ducks captured in 2005 were analyzed in both labs and comparisons confirmed that the labs gave consistent results and inferences (Esler et al., 2010). All lab analyses were conducted without *a priori* knowledge of the areas from which the samples were collected. The measure of EROD activity is expressed in picomoles per minute per milligram of protein, i.e., pmol/min/mg protein.

2.3. Statistical analyses

We evaluated sources of variation in hepatic EROD activity of Barrow's goldeneyes. We analyzed data from each winter (2005 and 2009) separately because of the potential for variation between laboratory runs (Esler, 2008). Within each year, our analysis was designed to determine whether area (oiled versus unoiled) explained variation in EROD activity, after accounting for any effects of age class, sex, and body mass. We used least squares general linear models (GLM) using SAS 9.1 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 to model selection and inference in which support for various model configurations was contrasted using Akaike's Information Criterion (AIC; Burnham and Anderson, 2002). We included or excluded age, sex, and body mass variables (which we termed "individual attributes") 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