Exxon Valdez Oil Spill Trustee Council
Restoration Project Final Report

Quantifying Temporal Variation in Harlequin Duck Cytochrome P4501A Induction

Restoration Project 050777
Final Report

Dan Esler

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

March 2008

Exxon Valdez Oil Spill Trustee Council
Restoration Project Final Report

Quantifying Temporal Variation in Harlequin Duck Cytochrome P4501A Induction

Restoration Project 050777
Final Report

Dan Esler

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

March 2008

Quantifying Temporal Variation in Harlequin Duck Cytochrome P4501A Induction

Restoration Project 050777
Final Report

Study History: Cytochrome P4501A (CYP1A) has proven to be useful for evaluating exposure to hydrocarbons for harlequin ducks (*Histrionicus histrionicus*) and other vertebrates following the *Exxon Valdez* oil spill. Liver biopsies of harlequin ducks were taken for CYP1A analysis (via 7-ethoxyresorufin-O-deethylase [EROD] activity) in March/April 1998, November 2000, November 2001, November 2002, and March 2005. One of the important uses for these data is evaluation of changes in hydrocarbon exposure over time; however, the ability to interpret and quantitatively contrast CYP1A data across years has been eroded by vastly different magnitudes of CYP1A values reported from the lab from year to year. The interannual variation in the reported EROD activity results far exceeded the degree of variation that could be explained by any reasonable biological process. Therefore, this project was undertaken to try and understand the underlying explanation for this inter-annual variation, and to consider laboratory and data transformation methods that would allow for interannual comparisons.

Abstract: CYP1A has been used widely as an indicator of exposure of vertebrates to polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), and the use of EROD activity as a measure of CYP1A has been validated and accepted as a standard method. EROD activity has been used since 1998 as an indicator of the degree of exposure of harlequin ducks to residual *Exxon Valdez* oil in Prince William Sound, Alaska. These data have proven to be extremely valuable for understanding the processes by which population recovery of harlequin ducks has been constrained following the *Exxon Valdez* oil spill. EROD activity data were collected in 1998, 2000, 2001, 2002, and 2005 from wintering harlequin ducks captured in Prince William Sound; one reason for collecting these data was to provide a time series with which changes in exposure could be tracked over years. However, the data reported from the laboratory varied dramatically in magnitude from year to year, to a degree far beyond what could be expected due to normal biological variation. Similar variation was observed across years in harlequin ducks held at the Alaska SeaLife Center under strictly controlled circumstances. Therefore, this project was initiated to determine the explanation for this interannual variation, and to apply laboratory analyses and data standardization to allow valid interannual comparisons. Additional data analysis of EROD activity of wintering harlequin ducks captured in Prince William Sound in 2005 through 2007 confirmed that individual attributes (age, sex, and mass) and season of capture did not explain observed variation in EROD activity. In review of the originally reported EROD values, it was discovered that some data were reported in incorrect units. However, even after these were corrected, the large interannual discrepancies were not completely resolved. Other potential factors, such as low protein yields, were considered; exclusion of samples with low protein values, along with application of appropriate units, considerably reduced interannual variation, although differences were still apparent that reduced confidence in interannual comparisons. EROD reanalysis of old samples resulted in data that were correlated ($R^2 = 0.53$) with original data, but values were considerably different in

magnitude, possibly as a result of sample degradation or annually varying lab techniques or equipment. Also, not all individuals had adequate sample volume remaining for reanalysis, reducing the power for detecting differences across areas and years if only reanalyzed data were used. In consultation with the laboratory, it was concluded that changes in analysis approaches and equipment may have led to the observed interannual differences and that, although within-year comparisons between areas were valid, between-year comparisons were not appropriate without corrections. Therefore, a method indexing CYP1A values was created that allows for interannual contrasts, in which average EROD activity for harlequin ducks captured at our unoiled/control area (Montague Island) is set to 1 for each year and all values are adjusted accordingly within the same sample year. Therefore, for each year, the average indexed EROD activity for birds from oiled areas is a ratio of oiled to unoiled averages. The average indexed values can be compared across years to consider annual changes in oil exposure, under the assumption that oil exposure at unoiled Montague Island was the same across years. Further, the indexed values can be calculated for each individual, which allows consideration of relationships between individual variation in oil exposure and variation in survival.

Key Words: biomarker, cytochrome P4501A, *Exxon Valdez* oil spill, harlequin ducks, *Histrionicus histrionicus*, oil exposure, Prince William Sound, variability

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

Citation: Esler, D. 2008. Quantifying temporal variation in Harlequin Duck cytochrome P4501A induction. *Exxon Valdez* Oil Spill Trustee Council Restoration Project Final Report (Restoration Project 050777), Centre for Wildlife Ecology, Simon Fraser University, Delta, British Columbia, Canada.

TABLE OF CONTENTS:

LIST OF TABLES

LIST OF FIGURES

Figure 1. Scatterplot of EROD activity (pmol/min/mg) of female harlequin ducks captured during November 2001 in Prince William Sound, Alaska. On the Y-axis are values from original analyses (with low protein data points removed) and on the X-axis are values from analyses of archived microsomes of the same samples run in 2005. Statistical description of this relationship is provided in the text.

Figure 2. Scatterplot of EROD activity (pmol/min/mg) of captive female harlequin ducks held at the Alaska SeaLife Center during winter 2001-02. On the Y-axis are values from original analyses (with low protein data points removed) and on the X-axis are values from analyses of archived microsomes of the same samples run in 2005. Statistical description of this relationship is provided in the text.

Figure 3. Scatterplot of harlequin duck EROD activity contrasting 2 labs that measured subsamples of the same livers collected from harlequin ducks in Prince William Sound in March 2005.

Figure 4. Variation in P450 Index (y-axis; the ratio of EROD in oiled areas: EROD in unoiled areas ± 95% CI) across years.

INTRODUCTION

The degree of induction of cytochrome P4501A (CYP1A) has been used to evaluate exposure to contaminants for a wide range of vertebrates (Lee et al. 1985, Peakall et al. 1989, Rattner et al. 1993, Trust et al. 1994, Goksøyr 1995, Whyte et al. 2000, Ben-David et al. 2001). Because CYP1A is induced by a limited number of compounds, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), it is a specific and hence particularly useful biomarker (Whyte et al. 2000).

Although there are questions regarding the ability to infer deleterious effects on individuals or populations based on evidence of exposure to contaminants through CYP1A induction (Lee and Anderson 2005), elevated CYP1A unequivocally indicates exposure to inducing compounds and, hence, at least the potential for associated toxic consequences. Therefore, measurements of CYP1A have been part of many assessments of effects of environmental contamination, including the case of the *Exxon Valdez* oil spill.

The *Exxon Valdez* ran aground in Prince William Sound (PWS), Alaska in March 1989, spilling an estimated 42 million L of North Slope crude oil. As much as 40% of this oil was estimated to have been deposited on the beaches of PWS (Galt et al. 1991, Wolfe et al. 1994), leading to concerns about acute and chronic effects on wildlife populations and the habitats and communities in which they occur (Peterson 2001, Peterson et al. 2003). As part of the large body of research addressing the occurrence, mechanisms, and importance of effects of the Exxon Valdez oil spill on wildlife populations in PWS, CYP1A induction has been used to evaluate the spatial and temporal extent of exposure to oil in a wide range of vertebrates (Trust et al. 2000, Jewett et al. 2002, Bodkin et al. 2002, Ballachey et al. 2006). These studies have demonstrated that CYP1A was higher in areas oiled by the Exxon Valdez spill relative to unoiled areas in many of the species examined.

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). However, the literature indicates that PAHs in the areas where elevated CYP1A was observed in vertebrates are predominately from the *Exxon Valdez* (Short et al. 2004), supporting the inference that *Exxon Valdez* oil is the inducing agent. Also, Trust et al. (2000) considered the potential role of PCBs in observed CYP1A indication in sea ducks in PWS and found that concentrations were very low and were generally not related to CYP1A induction. 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 *Exxon Valdez* oil was the inducing compound.

Harlequin ducks (*Histrionicus histrionicus*) have been the subject of a series of investigations following the *Exxon Valdez* oil spill. The process of population recovery of this species has been monitored and studied intensively, and it is one of the few species (along with sea otters) for which information is available that quantifies the

demographic factors leading to population change. The Nearshore Vertebrate Predator project (NVP), funded by the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC), was initiated in 1995, and consisted of a suite of studies addressing constraints to population recovery for 4 vertebrates, including harlequin ducks. Results of the NVP included the unanticipated finding that harlequin ducks had elevated CYP1A induction in areas receiving oil from the Exxon Valdez spill (Trust et al. 2000), suggesting that they continued to be exposed to residual *Exxon Valdez* oil through at least 1998 (Ballachey et al. 2006). This duration of exposure was much longer than expected, based on conventional assumptions about persistence of spilled oil (Peterson et al. 2003). NVP studies also documented demographic problems that corresponded with continued exposure to lingering oil, including reduced survival of sea otters (Monson et al. 2000) and harlequin ducks (Esler et al. 2000). In fact, exposure to lingering oil was considered to be a primary factor constraining population recovery of sea otters (Bodkin et al. 2002) and harlequin ducks (Esler et al. 2002).

Based on NVP findings, the EVOSTC funded a new set of studies (//423), which included consideration of the relationship between continuing oil exposure and population demography of harlequin ducks, at both individual and population levels. This work led to the conclusion that differences in harlequin duck survival between oiled and unoiled areas diminished over time, and were equivalent by 2002 (Bodkin et al. 2003). This was accompanied by suggestions of corresponding diminishment of oil exposure over time (see below). Clearly, quantification of oil exposure is an important component of these findings. For harlequin ducks, along with other nearshore vertebrates, inferences about oil exposure have been drawn through quantification of CYP1A. In the case of harlequin ducks, liver samples were collected to assess CYP1A induction by measuring 7-ethoxyresorufin-O-deethylase (EROD) activity. EROD activity, which measures the catalytic function of hydrocarbon-inducible CYP1A, is a widely used and recognized method. However, interpretation of EROD data has been hampered by large interannual differences in values reported from the laboratory. Therefore, this project was initiated to consider causes of observed interannual differences and evaluate laboratory or mathematical methods that could improve interpretation of CYP1A induction across years.

In addition to concerns about interannual variation, CYP1A induction in a number of fish has been shown to vary in relation to age, sex, and season (Sleiderink et al. 1995, Goksøyr and Larsen 1991, Whyte et al. 2000, Kammann et al. 2005), and these factors should be accounted for when evaluating sources of variation in CYP1A induction (Lee and Anderson 2005).   Analyses of effects of age, sex, and season are included and referenced in this document to facilitate interpretation of observed interannual variation.

PROJECT OBJECTIVES

This project consisted of a single objective:

1. Thoroughly review harlequin duck EROD activity data to evaluate sources of interannual variation and apply recalculations to facilitate interannual comparisons and changes in CYP1A induction over time.

STUDY AREA

The samples used in these analyses were collected from a number of sites within Prince William Sound that were used consistently across years. These include areas that were oiled during the *Exxon Valdez* spill (Green Island, Bay of Isles, Lower Passage, Herring Bay, Crafton Island, Main Bay, and Foul Bay) and nearby unoiled sites on northwestern Montague Island (Stockdale Harbor, Port Chalmers, and Mooselips Bay). These are the same sites that have been used for harlequin duck research since the initiation of NVP studies in 1995.

Also, studies of captive harlequin ducks were conducted at the Alaska SeaLife Center in Seward. Adult females were captured during wing molt at study sites on northwestern Montague Island, and shipped to the Alaska SeaLife Center for experiments during winters 2000-01 and 2001-02.

METHODS

Harlequin ducks were captured in 1998 (31 March – 9 April), 2000 (7 – 14 November), 2001 (31 October – 11 November), 2002 (17 November – 2 December), and 2005 (5 – 16 March) using a modified floating mist net (Kaiser et al. 1995). Upon capture, all birds were transported by skiff to a larger vessel where U.S. Fish and Wildlife Service bands were affixed, morphological measurements taken, and surgeries were conducted to take a small (<1g) liver biopsy for CYP1A analysis. Sex determinations were made based on plumage characteristics and cloacal examination and age class was determined by probing bursal depth (Mather and Esler 1999). During 1998 and 2005, all age and sex cohorts were sampled; in 2000, 2001, and 2002 only females (of all age classes) were sampled, as a key part of the study (EVOSTC Project //423) was to evaluate the relationship between oil exposure and female survival. Sample sizes by year and treatment are presented in Table 2.

When liver biopsies were surgically removed, they were immediately placed into a labeled cryovial and frozen in a liquid nitrogen shipper. All samples were maintained in liquid nitrogen or a -80° C freezer until they were shipped to the lab in liquid nitrogen. During 2005, two liver biopsies were taken from each individual to allow inter-laboratory comparisons. Each sample was placed in a separate, labeled cryovial before being placed in the liquid nitrogen shipper. One of the paired samples was sent to Wood's Hole Oceanographic Institute, the lab used for all pre-2005 analyses, and the other to the University of California Davis.

For experiments at the Alaska SeaLife Center, adult female harlequin ducks were captured during wing molt in early September. Twenty-five were transported by float plane to the Alaska SeaLife Center in each year of the study (2000 and 2001), where they were held in captivity for the duration of the winter and used as subjects in the experiments described below and in Bodkin et al. (2003).

Captive harlequin ducks were housed in a mesh-enclosed outdoor pen exposed to local climate and photoperiod conditions. The enclosure was partitioned into three 3.7 x 3.7 m sections, each with a flowing seawater pool (2000-01: diameter 1.8 m, depth 0.9 m; 2001-02: diameter 2.4 m, depth 0.6 m). The captive diet consisted of *ad libitum* commercially obtained Atlantic silversides (*Menidia menidia*) presented in trays on land during winter 2000-01, and an *ad libitum* mixed diet of Atlantic silversides and Antarctic krill (*Euphausia superba*) placed in pools during winter 2001-02. Freshwater was offered in each pen in heated plastic bowls to prevent freezing, along with a mixture of sand and crushed oyster shell for grit.

In both years of the oil ingestion experiment, birds were given 4 weeks to acclimate to the captive environment and diet before the start of oil-dosing. After acclimation, the 21 birds that had adjusted to captivity best, based on maintenance of body mass, were randomly assigned to 1 of 3 treatments (control, low-dose, high-dose), each with 7 birds. The birds assigned to each treatment group were kept together in one of the 3 partitions of the outdoor enclosure. Each bird was banded with a pair of colored plastic tarsus bands to allow identification of individuals. The average body mass (± 1 SD) of the harlequin ducks used in the study was 523.0 ± 25.8 g and was similar in both years.

Different dosing regimes were used in the 2 years of the oil ingestion experiment. In 2000, dosing was conducted twice weekly. Low-dose birds were fed 1 mL of oil per kg body mass at each dosing, high-dose birds were fed 10 mL of oil per kg body mass at each dosing, and controls were fed no oil, although they were handled identically to the dosed birds. Doses were administered via gavage with a syringe and feeding tube; control birds were gavaged with an empty feeding tube. Dosing was done in the morning before birds had fed. The dosing regime was changed in the second year of the study to better simulate low-level, intermittent ingestion of weathered crude oil. During winter 2001-02, dosing was conducted every other day in the evening, after birds had fed. Low-dose birds were fed 0.57 mL of oil per kg body mass, high-dose birds were fed 5.71 mL of oil per kg body mass, and control birds were handled identically, but were fed no oil. The cumulative dose over a 2-week period for each treatment was equivalent in both years of the study (low-dose 4 mL kg^{-1}, high-dose 40 mL kg^{-1}), but doses were smaller and more frequent in the second year. In both years, birds were held for 60-90 minutes after dosing in an indoor, mesh-bottomed pen before being returned to the outdoor enclosure to prevent contamination from oily excreta.

Birds were dosed with weathered crude oil from Prudhoe Bay, Alaska. To simulate weathering, crude oil was mixed with seawater and stirred continuously for 10 days at 25°C, after which it was separated from water and stored in air tight containers until dosing. Doses were based on body masses measured at the previous handling event.

Dosing occurred between 15 October 2000 and 20 February 2001 during the first year (total dosings = 37), and between 15 October 2001 and 10 February 2002 in the second year (total dosings = 60). Birds that became sick during the course of the experiment were treated by ASLC veterinary staff. If possible, oil-dosing was continued through the course of treatment; however no data were collected until sick birds returned to good health as judged by the veterinary staff. If the illness was severe, the sick bird was removed from the experiment. Mortalities unrelated to oil-dosing occurred in all 3 treatments during both winters of the experiment. The sample sizes for CYP1A analysis at the end of dosing were 17 in 2000-01 and 18 in 2001-02.

At the end of the oil dosing experiments, liver biopsies were taken from captive birds (23-24 February 2001 and 10 February 2002). Sample handling followed the same procedures as described above, and samples were sent to the laboratory for analysis at the same time as samples from wild-captured birds for each year.

Frozen liver samples from field and captive bird studies were shipped in liquid nitrogen to the laboratory of Dr. John Stegeman at the Wood's Hole Oceanographic Institute for preparation and analysis. Individual liver pieces were homogenized in 7 ml final volume homogenizing buffer (0.05 M Tris, 0.15 M KCl, pH 7.4), and microsomes sedimented by differential centrifugation. Samples for reanalysis, archived at Woods Hole as prepared microsomes, were resuspended in approximately 2 ml per g tissue with resuspension buffer (0.05 M Tris, 0.1 mM EDTA, 1 mM DTT, 20% v/v glycerol, pH 7.4). Protein was determined in a 96 well plate. 7-Ethoxyresorufin-O-deethylase (EROD), the catalytic function of hydrocarbon-inducible CYP1A, was measured using a kinetic modification of the plate-based assay. EROD activity was determined in duplicate in a 48 well plate at 20° C using a Cytofluor® fluorescent plate reader (Millipore, Bedford, MA). Each well contained 200 µl consisting of 1µl of microsomes (4-15 µg protein), 2 µM 7-ethoxy resorufin in 50 mM Tris buffer, 0.1 M NaCl, pH = 7.8. Catalytic activity was initiated by the addition of NADPH in buffer to a final 1.67 mM concentration. Fluorescence was determined at 1 min intervals over 6 min, and the linear slope (fluorescence per minute) was divided by the slope of the resorufin product standard curve (fluorescence per pmol) determined under the same conditions to yield pmol per minute per mg protein catalytic rates.

To consider potential confounding effects of age, sex, and body mass on interpretation of interannual variation, data from 2005 were used to analyze effects of these variables, along with area (oiled versus unoiled), on variation in reported EROD activity. Of the 20 birds captured in oiled areas, 5 were adult (after-hatch year) females, 1 was a juvenile (hatch year) female, 13 were adult males, and 1 was a juvenile male. In unoiled areas, the sample consisted of 7 adult females, 1 juvenile female, 11 adult males, and 1 juvenile male. Variation in EROD activity in relation to age, sex, body mass, and area was analyzed using general linear models in SAS. Model selection and inference was conducted in an information-theoretic context (Burnham and Anderson 2002), in which support for various model configurations is contrasted using Akaike's Information Criterion, corrected for small sample size (AIC_c). The candidate model set for this

analysis included all additive combinations of explanatory variables, as well as a null model. The model with the lowest AIC_c value is considered to have the strongest support from the data. Another metric, AIC_c weight (*w*), was calculated for each model; these sum to 1.0 across the entire model set and provide a clear 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.

The first step in the evaluation of the observed interannual variation in reported EROD activity data was a thorough data review, in which the Wood's Hole scientists double-checked the analysis results spreadsheets and we confirmed linkages to capture databases. A subset of archived samples from 2001 with adequate microsome volume was reanalyzed using EROD activity methods described above. A simple linear regression was used to evaluate the relationship between original and archived samples; a relationship with low unexplained variation (high R^2) and a slope near 1 would indicate that archived samples could be used to double-check questionable values originally reported by the lab.

In March 2005, as part of EVOSTC study //0774, liver subsamples from harlequin ducks were sent for EROD analysis at the laboratory of Barry Wilson and Keith Miles at the University of California Davis (UCDavis), as well as to Wood's Hole where all prior analyses had been conducted. The UCDavis laboratory methods followed standard procedures, detailed in Miles et al. (2007). This allowed for an inter-lab comparison of harlequin duck EROD activity. Both labs conducted analyses without a priori knowledge of the areas from which the samples were collected and neither lab was privy to the data from the other. Lab results were compared with a simple linear regression, with the expectation that there would be low unexplained variation around this relationship.

RESULTS

EROD Variation Related to Individual Attributes

When analyzing data to determine whether age, sex, body mass, or area explained variation in EROD activity of harlequin ducks captured in PWS during March 2005, the model including only area as an explanatory variable received the most support. This model had the lowest AIC_c value, and a *w* that was more that 3 times higher than that of any competing model (Table 1). In addition, the area term was included in all of the best supported models, and had a parameter likelihood of 0.95. In contrast, the sex, age, and mass variables were not well-supported for explaining variation in EROD. These did not occur in the best supported model and had parameter likelihoods < 0.28. These results strongly support the conclusion that, although EROD activity differed between oiled and unoiled areas, it did not vary significantly in relation to age, sex, or body mass. These results are corroborated by findings from samples collected during November 2006 and

March 2007 (D. Esler, unpublished data); in this case, EROD was not related to sex, age, mass, or season, after accounting for effects of area. Taken together, these findings indicate that differences in the individuals composing each sample over the years can not explain observed levels of interannual variation in EROD (see below).

Table 1. Results of information-theoretic model selection analysis of general linear models evaluating variation in hepatic CYP1A induction of harlequin ducks captured during March 2005 in Prince William Sound, Alaska. The response variable (EROD) is 7-ethoxyresorufin-O-deethylase activity, and explanatory variables include area (oiled versus unoiled), sex (male versus female), age (hatch year versus after hatch year), and mass (whole body mass). AIC_c is Akaike's Information Criterion corrected for small sample size and w is the AIC_c weight (see text for description and interpretation of these metrics).

Model Description	**AIC_c**	**W**
EROD = area	377.3	0.40
EROD = area + sex	379.5	0.13
EROD = area + age	379.5	0.13
EROD = area + mass	379.6	0.12
EROD = area + sex + mass	381.0	0.06
EROD = area + sex + age	381.8	0.04
EROD = area + age + mass	382.1	0.04
EROD = null	383.2	0.02
EROD = area + sex + age + mass	383.8	0.02
EROD = sex	384.9	0.01
EROD = age	385.3	0.01
EROD = mass	385.4	0.01
EROD = sex + mass	385.9	0.01
EROD = sex + age	387.2	0.00
EROD = age + mass	387.8	0.00
EROD = sex + age + mass	388.6	0.00

Originally Reported EROD Activity

Average EROD activity reported by the lab for harlequin ducks captured during winter in oiled areas of PWS ranged from 40.2 to 1981.8 pmol/min/mg across years and, for unoiled areas, the range was from 36.0 to 1187.9 pmol/min/mg (Table 2). These values were derived from the figures originally reported by the Wood's Hole Oceanographic Institute laboratory contracted to prepare and run the samples. The magnitude of differences among years is dramatic and raised questions about the utility of the data for comparing across years and evaluating temporal trends in CYP1A induction. The observed variation was the impetus for initiating this project to evaluate causes of reported interannual variation and to derive methods allowing comparisons across years.

Several observations are important when considering the originally reported data. In particular, one would expect that average EROD activity should remain stable over time in unoiled areas, in the absence of analytically-induced variation. In PWS, CYP1A inducing compounds in areas not oiled during the *Exxon Valdez* spill occur in low concentrations that would not be expected to vary dramatically across years (Trust et al. 2000, Short et al. 2004). Hence, the average degree of CYP1A induction in unoiled areas should be stable, in stark contrast to the multiple orders of magnitude interannual differences in the reported data (Table 2). Another feature of the originally reported data was that interannual differences were fairly consistent between areas when considered across years; e.g., for both areas, results were more than 30 times higher for winter 2001-02 than winter 2002-03. If this were due to variation in availability or concentrations of CYP1A inducing compounds, it would have had to have occurred at a very large geographic scale, i.e., that of the entirety of western PWS.

Table 2. Average hepatic EROD activity (± SE) originally reported from the laboratory (Wood's Hole Oceanographic Institute) from harlequin ducks captured in Prince William Sound (PWS) and captive harlequin ducks held at the Alaska SeaLife Center (ASLC).

Area - Treatment	1998	2000	2001	2002	2005
PWS - Oiled	204.6 (± 20.3) n = 19	777.6 (± 193.0) n = 25	1981.8 (± 661.7) n = 25[b]	40.2 (± 18.4) n = 21	194.9 (± 30.1) n = 20
PWS - Unoiled	70.7 (± 21.5) n = 18	146.6 (± 54.0) n = 8[a]	1187.9 (± 254.6) n = 23[b]	36.0 (± 14.3) n = 21[c]	96.6 (± 14.4) n = 20
ASLC – Oiled[d]		634.6 (± (163.7) n = 12	2239.4 (± 825.4) n = 11		
ASLC – Unoiled		86.7 (± 34.1) n = 5	235.3 (± 69.9) n = 7		

[a]17 additional samples were taken, but were lost when a liquid nitrogen shipper failed.
[b]Averages and sample sizes exclude 3 samples in each treatment that were below detection limits.
[c]Average and sample size exclude 1 sample below detection limit.
[d]Low and high dose treatments combined.

In addition, CYP1A results from studies of captive harlequin ducks at the ASLC showed considerable interannual variation in reported values (Table 2), despite the controlled conditions under which they were held. During 2 winters (2000-01 and 2001-02), female harlequin ducks were captured from an unoiled area and held from September to March in captivity. In each winter, ducks ingested oil in controlled amounts and their CYP1A response was measured at season's end. Despite similar, controlled handling and dosing of ducks between years, dramatically different results were reported in the 2 years. EROD activity of oiled birds averaged 634.6 and 2239.4 pmol/min/mg, respectively, in 2000-01 and 2001-02. More surprisingly, EROD activity of control birds averaged 86.7 and 235.3 pmol/min/mg in 2000-01 and 2001-02, respectively. The ratio of EROD activity for oiled:control birds was similar between years (7.3:1 and 9.5:1 in 2000-01 and

2001-02, respectively), suggesting that the magnitude of the differences between treatments was valid, but that the originally reported values could not be directly compared across years. Also, the results from the captive birds from the ASLC paralleled those from captured PWS harlequin ducks. For both groups, reported EROD activity increased dramatically from 2000 to 2001 in all treatments, despite very different origins and different conditions. The only commonality was that all of the 2000 data (ASLC and PWS) were run concurrently at Wood's Hole, as were all of the 2001 data a year later.

Review and Revision of Originally Reported Data

The first step in evaluation of the observed interannual variation reported in Table 2 was to review the data to determine whether they were error-free. This review revealed that data delivered from the lab for 2001 samples, for both wild-captured birds and captive birds at the Alaska SeaLife Center, were reported in incorrect units (pmol/min/ml instead of pmol/min/mg). In addition, a number of samples were determined to have low protein yields (< 1.0 mg/ml), including 8 samples from wild-caught birds in 2001, 2 captive birds in winter 2000-01, and 4 captive birds in winter 2001-02. Low protein yield may have been related to the small size of the liver biopsies taken, which was intended to minimize effects on the birds. As described by Dr. Bruce Woodin with the Wood's Hole lab, low protein yield can lead to unreliable EROD activity results. Therefore, average EROD activity was recalculated, using the appropriate units and excluding samples with low protein yields (Table 3). Note that these data are from the same samples reported in Table 2; however, Table 3 reports all the data in pmol/min/mg and samples with low protein yield are not included in calculations.

Table 3. Average hepatic EROD activity (± SE) of harlequin ducks captured in Prince William Sound (PWS) and captive harlequin ducks held at the Alaska SeaLife Center (ASLC), after standardizing all data to the same units (pmol/min/mg) and removing samples with low protein yield (< 1.0 mg/ml).

Area - Treatment	1998	2000	2001	2002	2005
PWS - Oiled	204.6 (± 20.3) n = 19	777.6 (± 193.0) n = 25	392.0 (± 94.9) n = 21[a]	40.2 (± 18.4) n = 21	194.9 (± 30.1) n = 20
PWS - Unoiled	70.7 (± 21.5) n = 18	146.6 (± 54.0) n = 8	218.3 (± 59.9) n = 20[b]	36.0 (± 14.3) n = 21[c]	96.6 (± 14.4) n = 20
ASLC – Oiled[d]		606.5 (± 177.2) n = 10	933.2 (± 397.5) n = 9		
ASLC – Unoiled		86.7 (± 34.1) n = 5	81.2 (± 18.4) n = 5		

[a]Average and sample size excludes 3 samples that were below detection limits and 5 that had low protein yields (one sample fell into both exclusion categories).
[b]Average and sample size excludes 3 samples below detection limits and 3 with low protein yield.
[c]Average and sample size excludes 1 sample below detection limit.
[d]Low and high dose treatments combined.

Correction of data units and deletion of samples deemed unreliable due to low protein yield resulted in a reduction in interannual variation (Table 2 versus Table 3). For example, recalculations reduced differences between years in the Alaska SeaLife Center data, after accounting for treatment, from nearly 4-fold differences in the originally reported data to very similar values, which would be expected given the very similar conditions during the 2 years of experiments. Also, the 2001 data from Prince William Sound, which were subject to both application of incorrect units and several low protein yields, were much closer to levels for other years following recalculation. However, the average for unoiled areas in 2001 was still considerably higher than other years. After data corrections (Table 3), 2002 stands out as an outlier with values lower than reported for other years.

Reanalysis of Archived Samples

To consider reliability of analyses and potential for using archived samples to reanalyze CYP1A induction in wild-caught and captive harlequin ducks, EROD analysis was conducted on leftover microsomes stored at Wood's Hole, using the procedures employed for analysis of 2005 data. Using available archived samples (n = 23) from birds captured during winter 2001 in Prince William Sound, EROD activity for original data (after corrections for inappropriate units and low protein yields) was significantly correlated with EROD activity in re-runs using a simple linear regression (P < 0.001; Figure 1). This was encouraging, as it indicated that the general patterns in the data held over time. However, nearly half of the variation in the data (R^2 = 0.53) was unexplained, indicating that predictive value of re-runs was poor. In addition, the magnitude of EROD activity values from re-runs averaged considerably lower than for the original data, as evidenced by the parameter estimate (slope) for the re-run variable of 2.6 (± 0.5 SE).

Similar patterns were observed for re-run data from captive birds subject to experiments at the Alaska SeaLife Center (Figure 2). Original data were positively correlated with re-runs, although the fit was poor (n = 9, P = 0.055, R^2 = 0.43) and re-runs averaged lower than originals by a factor of 1.5 (± 0.7) based on the parameter estimate for the re-run variable.
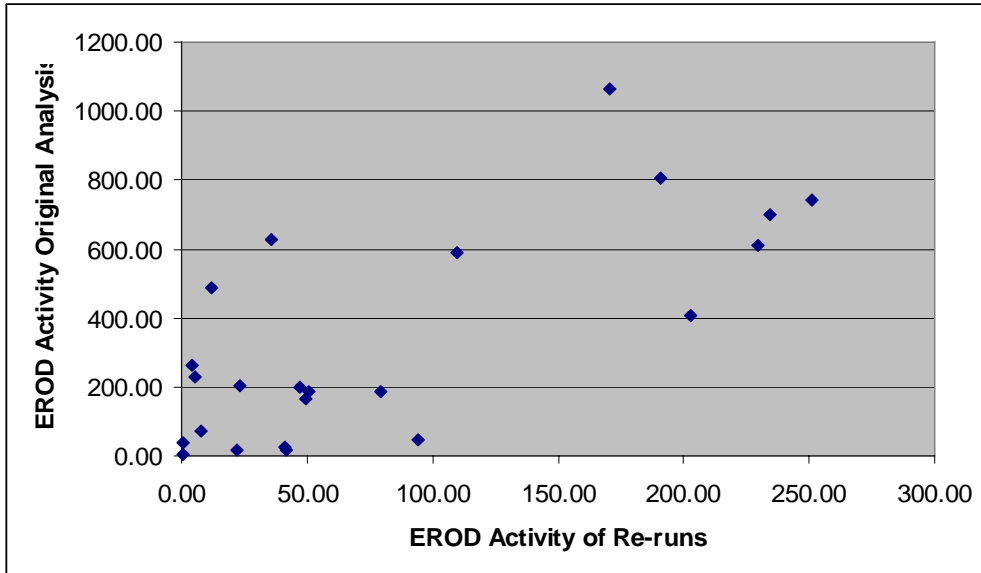
Figure 1. Scatterplot of EROD activity (pmol/min/mg) of female harlequin ducks captured during November 2001 in Prince William Sound, Alaska. On the Y-axis are values from original analyses (with low protein data points removed) and on the X-axis are values from analyses of archived microsomes of the same samples run in 2005. Statistical description of this relationship is provided in the text.
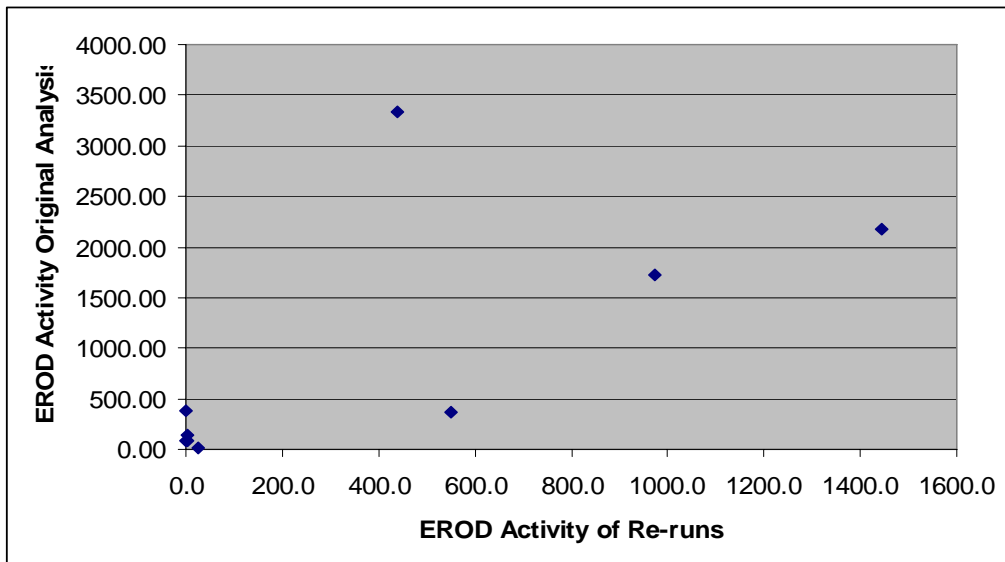

Figure 2. Scatterplot of EROD activity (pmol/min/mg) of captive female harlequin ducks held at the Alaska SeaLife Center during winter 2001-02. On the Y-axis are values from original analyses (with low protein data points removed) and on the X-axis are values from analyses of archived microsomes of the same samples run in 2005. Statistical description of this relationship is provided in the text.

The lack of 1 to 1 correspondence between original and re-run analyses could have been due to either degradation of archived samples or from differences in lab procedures between the 2 runs (see below). In either case, re-run data could not be directly compared to original data. Because not all samples had adequate quantity of archived material, sample size would be reduced for interannual comparisons if re-runs were used as a method of standardization. Use of relationships between original data and reruns as a method for applying a correction factor to original data would be inappropriate, given the considerable variation around the relationship. Also, if sample degradation were affecting results, there would presumably be differential degradation, depending on the time since samples were originally collected, which again would lead to difficulties with standardization based on re-runs. Storage, even at -80, has been shown to reduce catalytic activity (Goksøyr and Larsen 1991). Therefore, although reanalysis of archived amples provided important insights, this could not be used as a method for retrospectively correcting for interannual differences in originally reported data.

Inter-Lab Comparison

We evaluated the relationship between data reported from two different laboratories (Wood's Hole and University of California Davis) for hepatic EROD activity of harlequin ducks captured in March 2005. Results from the 2 labs were correlated (R^2 = 0.70; Figure 3), suggesting that patterns in the data were consistent and confirming that EROD activity is a repeatable method. Averages differed somewhat by laboratory (oiled = 194.9 at Wood's Hole and 161.3 at UCDavis, and unoiled = 96.6 at Wood's Hole and 55.3 at UCDavis). This finding further illustrates that, even after removing potential confounding effects of sample degradation, different lab procedures can give different results, although the relative relationship between treatments is consistently maintained.
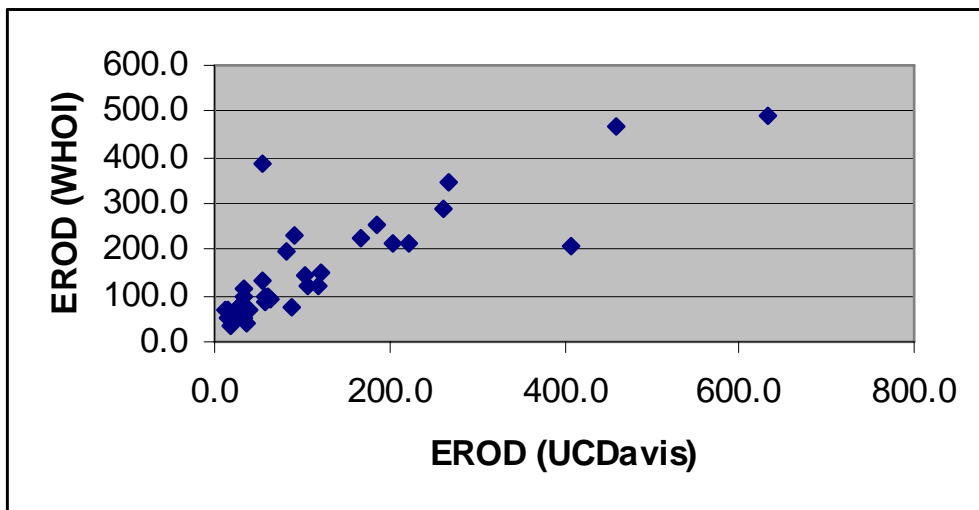

Figure 3. Scatterplot of harlequin duck EROD activity contrasting 2 labs that measured subsamples of the same livers collected from harlequin ducks in Prince William Sound in March 2005.

Interannual Variation in Lab Methods

All of the data reported above provided insight into potential sources of interannual variation. Although some sources were correctable (e.g., reporting the results in appropriate units), there remained unexplained variability, constraining the ability to compare results across years. Continued discussion with the Wood's Hole laboratory led to speculation that interannual variation may have been due to variation in lab procedures. Dr. Bruce Woodin, who was responsible for conducting the analyses at Wood's Hole, indicated that changes in procedures and machinery, a normal part of evolving and improving analysis methods, could be related to observed interannual variation. Dr. Woodin provided the following information:

> "At the time [that 2000 and 2001 analyses] were performed, we were diluting the samples into TEDG for catalytic measurement, and using the same dilutions for protein measurement (this was to conserve small samples). The problem is that TEDG alone in the protein assay gives a relatively strong background signal, which is subtracted before calculating the protein values. The problem with very small samples (like these biopsies) is that the TEDG signal can be a larger part of the signal than the contribution from the sample. If the signal measured for the TEDG blank in the assay is higher for any reason than the actual contribution from TEDG in the sample analysis, this would have the effect of causing underestimation of the protein (and consequently amplifying protein normalized catalytic function). We also have recently found that the machine we used for the 2000 and later samples has had a problem which gives higher signals in the first rows of the plate than in the lower rows of a plate (measured for the same contents in all wells), due to an incorrect setting in the instrument (the proper setting for which was not included in the manual!!). I routinely read the TEDG blank in the second row of the plate, and ahead of the samples. This problem effects the protein measurements, which are absolute measurements, but not the EROD measurements, which are changes with time."

This potential procedural effect is a parsimonious explanation for observed data differences across years. It also is consistent with analyses of original data by re-runs, which showed significant correlations but slopes different from 1. Also, the magnitude of re-run data is similar to that of 2005 data, when procedural issues were resolved. In further discussions with Dr. Woodin, he indicated that this methodological issue should not affect the relative differences among treatments, i.e., that results within a year provide a reliable measure of CYP1A variation within that year. Given this, a method of data standardization was derived to allow comparisons across years.

Data Standardization

To standardize EROD activity data across years, an index was created for each year, scaling the results from unoiled areas to 1 and the corresponding oiled area data by the same factor. When used to contrast data across years, this approach assumes that EROD

activity would be similar in unoiled areas in each year, under the assumption that residual Exxon Valdez oil is the primary inducer of CYP1A. This assumption is supported by the literature describing distribution and concentrations of CYP1A inducing compounds in PWS (Short et al. 2004, Trust et al. 2000). Exposure to these CYP1A inducing compounds in unoiled areas would be expected to be relatively low and stable under the conditions in PWS. Further, the captive bird work at the ASLC imposed conditions of consistent exposure to CYP1A inducing compounds for both controls and experimental birds, so the assumption is explicitly supported by data in this case.

Based on this index (Figure 4), the difference in EROD activity diminished through 2002 and areas were statistically similar (P > 0.10) in 2001 and 2002. This pattern is concordant with those described for some other nearshore species (Ballachey et al. 2006). However, EROD activity in March 2005 was higher (P = 0.005) in oiled areas than unoiled.
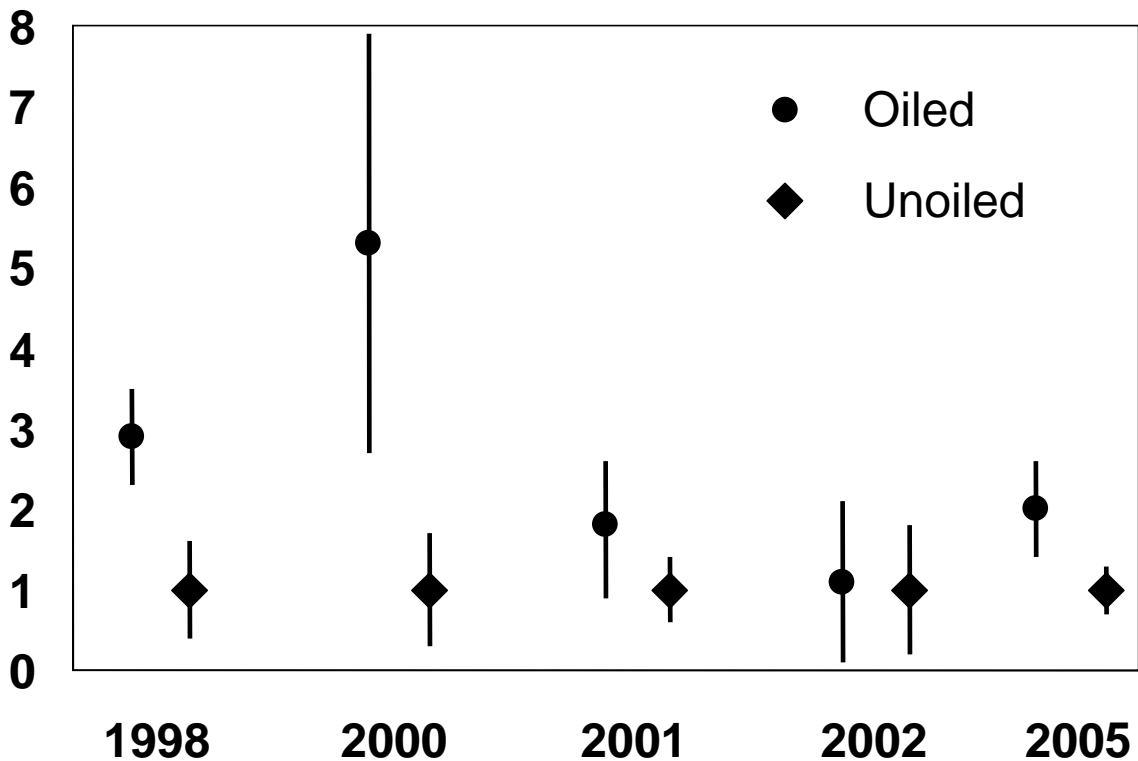

Figure 4. Variation in P450 Index (y-axis; the ratio of EROD in oiled areas: EROD in unoiled areas ± 95% CI) across years.

DISCUSSION

The originally reported data for EROD activity of harlequin ducks captured in PWS during winter, and those held at the ASLC, showed interannual variation that was much

larger than could be explained by any reasonable biological process. Because an important goal for the body of harlequin duck research was to track changes in exposure to *Exxon Valdez* oil (as well as other metrics of recovery) over time, this project was initiated to determine causes of observed variation and to derive methods to normalize the data to allow interannual comparison.

Some factors were evaluated and confirmed to be unrelated to observed interannual variation. In particular, individual attributes of age, sex, and body mass were not related to variation in EROD activity for birds captured during March 2005. In addition, recent data from November 2006 and March 2007 (D. Esler, unpubl. data) confirmed this finding, and further eliminated season as a factor related to CYP1A variation. Also, movements of birds could have confounding effects, if large and variable numbers of individuals moved between oiled and unoiled areas of PWS. However, the degree of movements has been carefully quantified (Iverson and Esler 2006) and harlequin ducks have been well-documented to show high winter site fidelity at very small geographic scales. Therefore, individual attributes and movements have been eliminated as factors explaining observed interannual variation in EROD activity.

Given these analyses, and the observation of interannual variation in reported EROD activity in captive harlequin ducks held under controlled conditions, the most parsimonious conclusion is that reported variation was due to variation in laboratory analyses or data processing. Careful attention to the data (i.e., using correct units and removing unreliable points based on protein yield) removed some of the interannual variation, enough variation remained to question the direct comparability of the raw data. In addition, reanalysis of samples did not produce results that could be directly compared with confidence, due to high variability in comparisons of re-runs with original data, concerns about sample degradation, and a reduction in available sample size.

After evaluating a number of ways of treating EROD activity data for harlequin ducks captured between 1998 and 2005, it was concluded that interannual comparisons were most appropriate when data were transformed to an index that standardized the average EROD activity in unoiled areas to 1 for each year. This method of data standardization requires the assumption that the average EROD activity in unoiled areas was similar in each year. This seems to be a reasonable assumption, given the literature on distribution and concentrations of EROD inducing compounds in PWS, and allows the distinct advantage of direct year to year comparison of averages, as well as a standard that can be applied to individuals to evaluate variation in survival probability in relation to individual-level variation in oil exposure across years.

It is important to recognize that, irrespective of data review and standardization, the conclusions drawn about temporal patterns of the relationship between oiled and unoiled areas remained unchanged. Using any iteration of the data set, the data suggest that CYP1A induction was higher on oiled areas than unoiled areas in 1998 and 2000, with values converging in 2001 and 2002. These data fit the expected pattern observed for other vertebrates, in which CYP1A declined over time as bioavailable lingering oil presumably diminished (Ballachey et al. 2006).

However, the 2005 results indicated a different pattern; these demonstrated significantly higher CYP1A induction in birds captured on oiled areas than unoiled areas. This is a robust result, as not only did harlequin ducks show this pattern but Barrow's goldeneye (*Bucephala islandica*) captured in the same sites during the same period also showed significantly higher CYP1A on oiled areas (Ballachey et al. 2006). In addition, two labs independently showed the same pattern for harlequin ducks, and strongly support the conclusion that higher availability of a CYP1A-inducing compound existed in areas oiled during the *Exxon Valdez* oil spill in relation to unoiled areas in March 2005.

Because oil exposure has been considered a primary constraint to recovery of harlequin duck populations (Esler et al. 2002) and definition of recovery status of harlequin ducks is based, in part, on the degree of exposure to lingering oil, continued CYP1A monitoring until the signal is gone is recommended.

Despite difficulties encountered in analyzing and interpreting the CYP1A data for harlequin ducks, the use of CYP1A for evaluating the degree and duration of exposure to *Exxon Valdez* oil is well-supported. The data from captive birds at the Alaska SeaLife Center clearly showed induction of CYP1A by birds exposed to oil (Table 3). Similarly, recent studies of harlequin duck CYP1A induction following the *Selendang Ayu* spill in the Aleutian Islands showed patterns of elevated CYP1A in bays that were oiled relative to bays that were not oiled (Miles et al. 2007). We conclude that CYP1A induction offers important insights about the degree, duration, and effects of exposure of harlequin ducks to residual *Exxon Valdez* oil, and that scaling of the data described here allows standardization and comparison of results across years.

ACKNOWLEDGMENTS

LITERATURE CITED

Ballachey, B.E., J.L. Bodkin, and D. Irons. 2006. Oil exposure biomarkers and population trends of Prince William Sound marine vertebrates (Restoration Project //0774), US Geological Survey, Alaska Science Center, Anchorage, Alaska.

Ben-David, M., T. Kondratyuk, B.R. Woodin, P.W. Snyder, and J.J. Stegeman. 2001. Induction of cytochrome P450 1A1 expression in captive river otters fed Prudhoe Bay crude oil: evaluation by immunohistochemistry and quantitative RT-PCR. Biomarkers 6:218-235.

Bodkin, J.L., B. Ballachey, T.A. Dean, F.K. Fukuyama, S.C. Jewett, L.L. McDonald, D.H. Monson, C.E. O'Clair, and G.R. Van Blaricom. 2002. Sea otter population status and the process of recovery following the 1989 *Exxon Valdez* oil spill. Marine Ecology Progress Series 241:237-253.

Bodkin, J. L., B. E. Ballachey, D. Esler, and T. Dean. 2003. Patterns and processes of population change in selected nearshore vertebrate predators. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project //423).

Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: a practical information theoretic approach. 2nd Edition. Springer-Verlag, New York.

Esler, D., J.A. Schmutz, R.L. Jarvis, and D.M. Mulcahy. 2000. Winter survival of adult female harlequin ducks in relation to history of contamination by the Exxon Valdez oil spill. Journal of Wildlife Management 64:839-847.

Esler, D., T.D. Bowman, K.A. Trust, B.E. Ballachey, T.A. Dean, S.C. Jewett, C.E. O'Clair. 2002. Harlequin duck population recovery following the *Exxon Valdez* oil spill: Progress, process, and constraints. Marine Ecology Progress Series 241: 271-286.

Galt, J.A., W.J. Lehr, and D.L. Payton. 1991. Fate and transport of the *Exxon Valdez* oil spill. Environmental Science and Technology 25:202–209.

Goksøyr, A. 1995. Use of cytochrome P450 1A (CYP1A) in fish as a biomarker of aquatic pollution. Archives of Toxicology (Supplement 17): 80-95.

Goksøyr, A., and H. E. Larsen. 1991. The cytochrome P450 system of the Atlantic salmon (*Salmo salar*): I. Basal properties and induction of P450 1A1 in liver of immature and mature fish. Fish Physiology and Biochemistry 9:339-349.

Harwell, M. A., and J. H. Gentile. 2006. Ecological significance of residual exposures and effects from the *Exxon Valdez* oil spill. Integrated Environmental Assessment and Management 2:204-246.

Iverson, S. A., and D. Esler. 2006. Site fidelity and the demographic implications of winter movements by a migratory bird, the harlequin duck. Journal of Avian Biology 37:219-228.

Jewett S.C., T.A. Dean, B. R. Woodin, M. K. Hoberg, and J. J. Stegeman. 2002. Exposure to hydrocarbons ten years after the Exxon Valdez oil spill: evidence from cytochrome P4501A expression and biliary FACs in nearshore demersal fishes. Marine Environmental Research 54(1):21-48.

Kaiser, G. W., A. E. Derocher, S. C. Crawford, M. J. Gill, and I. A. Manley. 1995. A capture technique for marbled murrelets in coastal inlets. Journal of Field Ornithology 66:321-333.

Kammann, U., T. Lang, M. Vobach, and W. Wosniok. 2005. Ethoxyresorufin-O-deethylase (EROD) Activity in Dab (Limanda limanda) as Biomarker for Marine Monitoring. Environmental Science and Pollution Research 12:140-145.

Lee, R. F., and J. W. Anderson. 2005. Significance of cytochrome P450 system responses and levels of bile fluorescent aromatic compounds in marine wildlife following oil spills. Marine Pollution Bulletin 50:705-723.

Lee, Y.-Z., F. A. Leighton, D. B. Peakall, R. J. Norstrom, P. J. O'Brien, J. F. Payne, and A. D. Rahimtula. 1985. Effects of ingestion of Hibernia and Prudhoe Bay crude oils on hepatic and renal mixed-function oxidase in nestling herring gulls (*Larus argentatus*). Environmental Research 36:248–255.

Mather, D. D., and D. Esler. 1999. Evaluation of bursal depth as an indicator of age class of harlequin ducks. Journal of Field Ornithology 70:200-205.

Miles, A.K., P.L. Flint, K.A. Trust, M.A. Ricca, S.E. Spring, D.E. Arietta, T. Hollmén, and B.W. Wilson. 2007. Polycyclic aromatic hydrocarbon exposure in Steller's eiders (*Polysticta stelleri*) and harlequin ducks (*Histrionicus histrionicus*) in the eastern Aleutian Islands, Alaska. Environmental Toxicology and Chemistry 26:2694-2703.

Monson, D.H., D.F. Doak, B.E. Ballachey, A. Johnson, and J.L. Bodkin. 2000. Long-term impacts of the *Exxon Valdez* oil spill on sea otters, assessed through age-dependent mortality patterns. Proc. Nat'l. Acad. Sciences, USA 97(12):6562-6567.

Peakall, D. B., R. J. Norstrom, D. A. Jeffrey, and F. A. Leighton. 1989. Induction of hepatic mixed function oxidases in the herring gull (*Larus argentatus*) by Prudhoe

Bay crude oil and its fractions. Comparative Biochemistry and Physiology 94C:461–463.

Peterson, C.H. 2001. The "Exxon Valdez" oil spill in Alaska: acute, indirect and chronic effects on the ecosystem. Advances in Marine Biology 39:1-103.

Peterson, C.H, S.D. Rice, J.W. Short, D. Esler, J. L. Bodkin, B.E. Ballachey, D.B. Irons. 2003. Long-term ecosystem responses to the Exxon Valdez oil spill. Science 302:2082-2086.

Rattner, B. A., M. J. Melancon, T. W. Custer, R. L. Hothem, K. A. King, L. J. LeCaptain, J. W. Spann, B. R. Woodin, and J. J. Stegeman. 1993. Biomonitoring environmental contamination with pipping black-crowned night heron embryos: Induction of cytochrome P450. Environmental Toxicology and Chemistry 12:1719-1732.

Short, J.W., M.R. Lindeberg, P.M. Harris, J.M. Maselko, J.J. Pella, and S.D. Rice. 2004. Estimate of oil persisting on the beaches of Prince William sound 12 years after the *Exxon Valdez* oil spill. Environmental Science & Technology 38:19-25.

Short, J.W., J.M. Maselko, M.R. Lindeberg, P.M. Harris, and S.D. Rice. 2006. Vertical distribution and probability of encountering intertidal *Exxon Valdez* oil on shorelines of three embayments within Prince William Sound. Environmental Science & Technology 40:3723-3729.

Sleiderink, H. M., I. Oostingh, A. Goksøyr, and J. P. Boon. 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. Archives of Environmental Contamination and Toxicology 28:423-430.

Trust, K. A., A. Fairbrother, and M. J. Hooper. 1994. Effects of 7,12-dimethylbenz[a]anthracene on immune function and mixed-function oxygenase activity in the European starling. Environmental Toxicology and Chemistry 13:821-830.

Trust, K. A., D. Esler, B. R. Woodin, and J. J. Stegeman. 2000. Cytochrome P450 1A induction in sea ducks inhabiting nearshore areas of Prince William Sound, Alaska. Marine Pollution Bulletin 40:397-403.

Whyte, J. J., R. E. Jung, C. J. Schmitt, and D. E. Tillitt. Ethoxyresorufin-*O*-deethylase (EROD) activity in fish as a biomarker of chemical exposure. Critical Reviews in Toxicology 30:347-570.

Wolfe, D. A., M. J. Hameedi, J. A. Galt, G. Watabayashi, J. Short, C. O'Clair, S. Rice, J. Michel, J. R. Payne, J. Braddock, S. Hanna, and D. Sale. 1994. The fate of the oil

spilled from the *Exxon Valdez*. Environmental Science and Technology 28:561A–568A.