

POLYCYCLIC AROMATIC HYDROCARBON EXPOSURE IN STELLER'S EIDERS (*POLYSTICTA STELLERI*) AND HARLEQUIN DUCKS (*HISTRONICUS HISTRONICUS*) IN THE EASTERN ALEUTIAN ISLANDS, ALASKA, USA

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**Abstract**—Seaducks may be affected by harmful levels of polycyclic aromatic hydrocarbons (PAHs) at seaports near the Arctic. As an indicator of exposure to PAHs, we measured hepatic enzyme 7-ethoxyresorufin-*O*-deethylase activity (EROD) to determine cytochrome P4501A induction in Steller's eiders (*Polysticta stelleri*) and Harlequin ducks (*Histrionicus histrionicus*) from Unalaska, Popof, and Unga Islands (AK, USA) in 2002 and 2003. We measured PAHs and organic contaminants in seaduck prey samples and polychlorinated biphenyl congeners in seaduck blood plasma to determine any relationship to EROD. Using Akaike's information criterion, species and site differences best explained EROD patterns: Activity was higher in Harlequin ducks than in Steller's eiders and higher at industrial than at nonindustrial sites. Site-specific concentrations of PAHs in blue mussels (*Mytilus trossilus*) seaduck prey; PAH concentrations higher at Dutch Harbor, Unalaska, than at other sites) also was important in defining EROD patterns. Organochlorine compounds rarely were detected in prey samples. No relationship was found between polychlorinated biphenyl congeners in avian blood and EROD, which further supported inferences derived from Akaike's information criterion. Congeners were highest in seaducks from a nonindustrial or reference site, contrary to PAH patterns. To assist in interpreting the field study, 15 captive Steller's eiders were dosed with a PAH known to induce cytochrome P4501A. Dosed, captive Steller's eiders had definitive induction, but results indicated that wild Steller's eiders were exposed to PAHs or other inducing compounds at levels greater than those used in laboratory studies. Concentrations of PAHs in blue mussels at or near Dutch Harbor (~1,180–5,980 ng/g) approached those found at highly contaminated sites (~4,100–7,500 ng/g).

**Keywords**—Hydrocarbons 7-Ethoxyresorufin-*O*-deethylase Seaducks Alaska

## INTRODUCTION

Steller's eiders (STEI; *Polysticta stelleri*) are protected by law [1] as a result of global population declines [2,3]. Several life-history studies have provided possible insights regarding the decline [3–6], but few have addressed the role of environmental contaminants [4,7]. Contaminant exposure often is suggested to contribute to wildlife declines, but in situ studies that associate population declines to contaminants are rare, particularly those studies that are inclusive of highly mobile, endangered species.

In Alaska (USA), some populations of STEI winter in protected bays that support heavy maritime traffic and seafood processing [8]. Maritime activities in these areas likely increase the probability of exposure to contaminants, particularly petroleum-based products. Polycyclic aromatic hydrocarbons (PAHs) are produced from the combustion or physical degradation of petroleum compounds. Lipophilic PAHs are persistent in the environment, and effects on mammals and fish are well known. Effects on wild avian populations, however, have been less definitive [9]. In vertebrates, the cellular bio-

chemical metabolism of PAHs is catalyzed by induction of the hepatic cytochrome P4501A (P4501A) system. In two studies, Trust et al. [10] and Custer et al. [11] documented induction of P4501A in wild seaducks in response to PAH exposure that was associated with reduced population numbers or somatic chromosomal damage, respectively. Esler et al. [12] also documented reduced survival of female Harlequin ducks (HADU; *Histrionicus histrionicus*) in relation to contamination by the *Exxon Valdez* oil spill.

The attraction of wintering STEI to ice-free bays provided an opportunity to examine P4501A induction and PAH exposure in seaducks. Fox and Mitchell [4] suspected that hydrocarbon exposure at boat harbors in Norway impacted STEI populations. Alaskan harbors and bays from the eastern Aleutian Islands to the Cook Inlet shelter large numbers of wintering STEI [8]. Moreover, we observed STEI roosting or feeding near discharge plumes from fish-processing plants and wastewater treatment facilities. The offal discharged in seafood-processing wastewater largely is composed of lipid-based matter that may contain organic contaminants that have been bioaccumulated in harvested fish. Furthermore, this waste contains nitrogenous compounds that increase biochemical oxygen demand in the system [13,14] at the point of discharge. Interactions between nitrogenous or lipid compounds and cycling of organic contaminants are important because of the affinity

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of contaminants, such as PAHs, to organic carbon [15–17]. Therefore, PAHs may be concentrated in this waste, compounding STEI exposure.

Our goal was to ascertain the efficacy of using biochemical responses in wintering STEI as a means to identify problems associated with elevated contaminants at industrial but remote, near-Arctic bays. Because of the protected status of STEI, we also evaluated HADU as a possible surrogate. Harlequin ducks use habitat proximate to that of STEI but usually forage closer to shore.

In the present study, we examined 7-ethoxyresorufin-*O*-deethylase (EROD) activity in hepatic tissue from seaducks as a measure of the catalytic function of hydrocarbon-inducible P4501A response. Because survival and release of these seaducks were essential, the liver tissue extracted as a biopsy specimen was too small to measure PAHs as well. Furthermore, the relationship of PAHs to P4501A induction is difficult to establish in ducks, because they rapidly metabolize PAHs [18,19]. To establish possible sources of PAH exposure as well as confounding contaminants that might induce P4501A, PAHs, aliphatic hydrocarbons (AHs), and organochlorines (OCs) were measured in prey of these seaducks. In particular, 30 polychlorinated biphenyl (PCB) congeners were measured in blood of STEI and HADU as a possible confounding factor affecting P4501A induction [20]. We included coplanar PCB congeners reported as being highly toxic P4501A inducers as well as other congeners (e.g., PCBs 118, 138, 153, and 180) that are common in the marine environment and have been suggested to induce P4501A [10,21]. Measures of these contaminants were compared between industrial and nonindustrial areas and related to EROD activity in STEI and HADU. Last, to facilitate interpretation of EROD activity in wild STEI, we exposed captive STEI to a laboratory standard PAH compound and examined P4501A induction.

## MATERIALS AND METHODS

### Study area

The present study was conducted at Unalaska Island in the eastern Aleutian chain and at the closely adjacent Popof and Unga Islands in the Shumigan Island group near the southeastern tip of the Alaska Peninsula during the winters of 2002 and 2003 (Fig. 1). Sites were selected based on observations of their use by STEI [8]. At Unalaska Island, these sites were identified as Dutch Harbor, which included an offshore seafood-processing plant outfall, a municipal wastewater discharge area, an existing small-boat harbor, and a proposed new small-boat harbor location; Nateekin Bay, located across Unalaska Bay from the industrialized Dutch Harbor; and Captains Bay, an a priori reference (i.e., clean site) that had no processor outfalls and little shipping traffic. Sites at the Shumagin Islands included Sand Point at Popof Island, in the vicinity of a seafood-processing plant outfall and boat harbor, and Coal Harbor, an uninhabited, a priori reference site at Unga Island. Home range (~5 km) estimated during the present study indicated no movement between industrialized and reference sites by STEI or HADU [22].

### STEI and HADU captures

Seaducks were captured using boats to herd flocks toward floating mist nets and decoys. Approximately 3 to 4 d of effort were expended at each site for captures in January and February of 2002 and 2003. Thirty-eight STEI and 65 HADU

captured at Unalaska Island and 32 STEI and 15 HADU captured at Popof and Unga Islands were used for the present study. Seaducks were captured over a two-year period because of the time and effort required for captures under severe weather conditions at each site.

Captured seaducks were transported a short distance to a mobile field laboratory, where they were banded, examined for general condition, and had weight, sex, and age (adult or immature) recorded. A biopsy specimen of the liver (~0.05 g) was then extracted only from healthy birds according to established procedure (D. Mulcahy, Alaska Science Center, Anchorage, AK, USA, unpublished guidelines). Liver samples were immediately frozen and stored in liquid nitrogen, then retained at -80°C until processing. If birds exhibited good postoperative condition, a 4- to 5-ml sample of blood was drawn via jugular venipuncture, transferred into Vacutainer® heparinized tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for plasma and nonheparinized tubes for serum; plasma samples were centrifuged within 15 min at 2,500 rpm for 10 min. The resulting plasma was frozen for PCB analyses at -80°C until processing. Birds were released at capture locations following a postoperative recovery period of approximately 3 h and examination by a licensed veterinarian. All procedures on any birds used in the present study were reviewed and approved by the U.S. Government and the University of California–Davis (Davis, CA, USA) Animal Use and Care Committee, and all surgeries or invasive procedures were performed by veterinarians.

### EROD analyses

Approximately 5 mg (wet wt) of liver per bird was analyzed for EROD activity at the Department of Animal Sciences, University of California–Davis. For quality assurance and quality control, embryonated mallard (*Anas platyrhynchos*) eggs were injected with 2 mg/egg of  $\beta$ -naphthoflavone (BNF; a known P4501A inducer) and their livers assayed at 24 h. Liver samples were prepared by separating the hepatic microsomes using differential centrifugation. Microsomes were extracted from livers homogenized in 0.1 M NaPO<sub>4</sub> buffer at pH 7.4, centrifuged at 100,000 g for 1 h, and then resuspended (~2 ml/g tissue) in 50 mM Tris solution that contained 1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, and 20% (v/v) glycerol at pH 7.4. We measured EROD activity as described by Trust et al. [10] according to the method of Burke and Mayer [23], adapted to a fluorescence microwell plate scanner [24]. Microsomal preparations were run in triplicate in a 96-well plate at 25°C using a Packard FluoroCount microplate fluorometer (Packard Instrument, Meriden, CT, USA). Each well contained 1  $\mu$ l of microsomes and 159  $\mu$ l of 2.5  $\mu$ M (final concentration) 7-ethoxyresorufin in 50 mM Tris-buffer at pH 8.0. The addition of 40  $\mu$ l of 1.34 mM (final concentration) of nicotinamide adenine dinucleotide phosphate initiated activity. Fluorescence was measured at an excitation wavelength of 530 nm and an emission wavelength of 590 nm at 1-min intervals for 6 min. 7-Ethoxyresorufin-*O*-deethylase activity was expressed as pmol/min/mg protein. Protein was determined using the Bradford reagent (Sigma, St. Louis, MO, USA) [25].

### Prey sampling

Three composite samples each of the most common potential prey species as well as flocculi (organic material likely associated with fish-processing discharge) were collected sub-

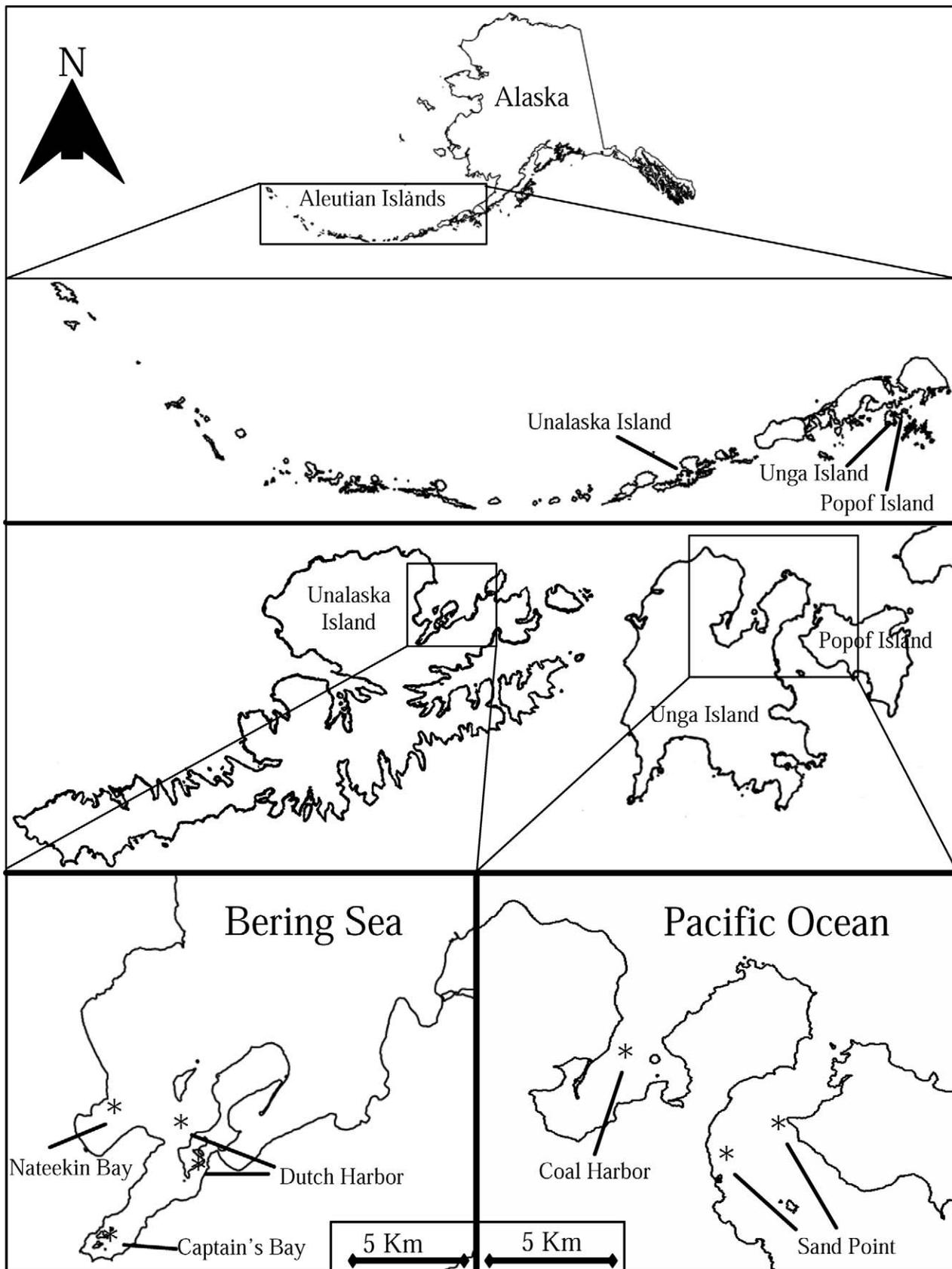


Fig. 1. Location of capture or collection sites for Steller's eiders (*Polysticta stelleri*) and Harlequin ducks (*Histrionicus histrionicus*) during the winters of 2002 and 2003 and seaduck prey samples during the winter of 2002 in Unalaska and Popof/Unga Islands (AK, USA).

tidally using a self-contained breathing apparatus or intertidally at each of four sites (Nateekin Bay was not sampled because of weather) during seaduck captures in 2002. Blue mussels (*Mytilus trossilus*) are common in adult STEI diets [26] and were collected in sterile, polypropylene bags at all four sites; small gastropods (e.g., *Tegula* spp.) or crustaceans (Amphipoda, Isopoda, *Pandulus* sp.) also were collected at sites but were more difficult to obtain. Each composite sample consisted of approximately 15 g (blotted wet wt) of soft tissue or flocculi; each invertebrate composite comprised approximately 20 blue mussels (length, 2–3 cm) or crustaceans and 30 to 50 gastropods. Specimens were kept cool until soft tissues could be extracted (within 1–2 h); these tissues were then stored in acid-rinsed jars and frozen until analysis.

All samples were collected with small nets or by hand using polypropylene gloves. Stainless-steel processing tools used on invertebrates or containers were either hexane-rinsed or replaced between each sample.

#### Analytical chemistry

Avian blood plasma was analyzed for 30 PCB congeners (PCBs 4, 11, 15, 40, 47, 52, 54, 77, 80, 105, 118, 126, 128, 133, 136, 138, 153, 155, 156, 158, 167, 169, 170, 171, 180, 189, 194, 197, 202, and 209) at Mississippi State University Chemical Laboratory (Mississippi State, MS, USA). Sample-specific detection limits for PCB congeners averaged 0.36 ng/g (standard error [SE], 0.009 ng/g) across all samples analyzed. Invertebrate and flocculi samples were analyzed for 44 PAHs, 27 AHs, and 22 OCs ( $\alpha$ -,  $\beta$ -,  $\delta$ -, and  $\gamma$ -benzene hexachloride;  $\alpha$ - and  $\gamma$ -chlordane; oxychlordane; *cis*- and *trans*-nonachlor; dieldrin; endrin; hexachlorobenzene; heptachlor epoxide; mirex; *o,p'*- and *p,p'*-dichlorodiphenyltrichloroethane; *o,p'*- and *p,p'*-dichlorodiphenyldichloroethylene; *o,p'*- and *p,p'*-dichlorodiphenyldichloroethane; toxaphene; and total [ $\Sigma$ ] PCBs; see Table 1 for PAHs and AHs). Sample-specific detection limits averaged 3.7 ng/g (SE, 0.008 ng/g) for PAHs, 10.0 ng/g (SE, 0.0 ng/g) for AHs, and 2.0 ng/g (SE, 0.0 ng/g) for OCs. Analytical accuracy and precision were assessed using spiked-sample recovery and duplicate analyses on 5% of all samples and certified to be within acceptable standards by the U.S. Fish and Wildlife Service Analytical Control Facility (Shepherdstown, WV, USA). Spiked recoveries averaged 94% (SE, 5%) for PCB congeners, 92% (SE, 8%) for PAHs, 86% (SE, 11%) for OCs, and 82% (SE, 16%) for AHs. Relative percent difference averaged 2.8% (SE, 1.0%) for PAHs, 0% (SE, 0%) for OCs, 27% (SE, 8.2%) for PCB congeners, and 34.6% (SE, 6.7%) for AHs; PCB congeners and AHs had lower precision largely because duplicate samples for a number of these compounds were less than the limit of detection.

#### Dosing study

An additional 11 STEI captured at Dutch Harbor in March 2003 and four captured at the Alaska Peninsula in September 2003 were used to facilitate interpretation of EROD activity in wild ducks. These birds were transported to the Alaska Sealife Center in Seward (AK, USA), held in quarantine for five to nine months, and determined by veterinarians to be in good health prior to experimentation.

In January 2004, these 15 STEI were separated by gender and then randomly assigned to one of three groups ( $n = 5$ ). Birds were orally administered gelatin capsules containing BNF at 20 mg/kg body weight in one group and at 100 mg/kg body weight in another. Dose levels were established con-

servatively according to the method described by Renaud et al. [27] and M. Melancon (Patuxent Wildlife Research Center, Laurel, MD, USA, personal communication) at a level known to induce P4501A activity but not acutely harm mallard ducklings. The third group consisted of control birds that received an empty gelatin capsule to experience similar handling stress. Birds were fasted for approximately 12 h before the initial dosing, after which they were provided food and water ad libitum. A single oral dose was administered every 24 h for two consecutive days after the initial dosing. Birds were weighed each day within 2 h of dosing to calculate dosage and assure that birds retained a safe weight. After 3 d of dosing (fourth day of study), Alaska Sealife Center and U.S. Geological Survey veterinarians used laparoscopy to extract a liver sample (~0.10 g) from each bird, which was then stored at  $-80^{\circ}\text{C}$  until analysis. All birds recovered from the procedure.

#### Statistical analyses

Both EROD activity and PCB congeners were quantified in individual seaducks in 2002 (30 STEI and 25 HADU) and in 2003 (40 STEI and 55 HADU). Also, PAHs and OCs were measured in avian prey collected during 2002 ( $n = 3$  composite samples/site of blue mussels, crustaceans, gastropods, or organic flocculi); however, only blue mussels were found consistently or in sufficient quantity at all sites. Blue mussels also were analyzed for AHs. All EROD and contaminants data were log-transformed for analyses.

Using Akaike's information criterion (AIC), we asked a priori if EROD activity was best explained by sites of suspected PAH contamination (Dutch Harbor, Nateekin Bay, and Sand Point) versus reference sites (Coal Harbor and Captains Bay), species (i.e., HADU vs STEI),  $\Sigma$ PCB concentrations of the 30 congeners analyzed in avian blood, year of capture, or gender. We first constructed sets of candidate analysis of variance or analysis of covariance models comprised of combinations of these main effect factors with EROD as the response variable (JMP, Ver 5.0.1.2; SAS Institute, Cary, NC, USA). Residual sum of squares necessary for AIC were then calculated for each analysis of variance or analysis of covariance model [28]. The second-order AIC ( $\text{AIC}_c$ ) corrected for sample size bias—that is,  $\text{AIC}_c = -2(\log\text{-likelihood}) + 2K/(n - K - 1)$ , where  $K$  is the number of estimable parameters and  $n$  is the sample size—was used to determine which candidate model or models contained the suite of factors that most parsimoniously explained variation in EROD activity when contrasted with all candidate models [28]. The AIC method is considered to be better suited for analysis and inference of observational data compared with null hypothesis testing for statistical significance [28]. Models with delta ( $\Delta$ )  $\text{AIC}_c$  of two or less were primarily evaluated by comparing negative log-likelihood values ( $-\log L$ ),  $\text{AIC}_c$  values,  $\text{AIC}_c$  weights, and evidence ratios of  $\text{AIC}_c$  weights ( $w_i/w_j$ , where  $w_i$  is the weight of the best  $\Delta\text{AIC}_c$  model and  $w_j$  is the weight of each of the next-best models in the set) relative to the number of parameters [28]. To determine the relative importance of each factor, we calculated factor weights by summing  $\text{AIC}_c$  weights across all models [28]. We then ran a second AIC that compared the best main effects model to a full model containing interactions of the main effects.

Because site was deemed to be important (see *Results*), we evaluated site differences in PAHs in blue mussel samples relative to EROD activity in a subsequent AIC procedure that only included factors from the best model determined from

Table 1. Geometric mean concentrations (ng/g) of polycyclic aromatic hydrocarbons (PAHs; arranged from lowest to highest molecular weight)<sup>a</sup> and aliphatic hydrocarbons (AHs) analyzed in blue mussels (*Mytilus trossulus*) collected during the winter of 2002<sup>b</sup>

PAHs	DH	CB	SP	CH	AHs	DH	CB	SP	CH
C2-Naphthalene	52.9	— <sup>c</sup>	—	—	<i>n</i> -Decane	86.0	44.9	47.6	33.6
Fluorene	14.7	—	— <sup>d</sup>	—	<i>n</i> -Docosane	102	16.1	15.5	—
1,6,7-Trimethyl-naphthalene	40.9	—	—	—	<i>n</i> -Dodecane	125	24.4	25.3	21.0
C3-Naphthalene	175	—	—	—	<i>n</i> -Dotriacontane	13.5	*	—	—
Anthracene	7.5	—	*	—	<i>n</i> -Eicosane	136	—	—	—
Phenanthrene	89.3	11.2	21.1	—	<i>n</i> -Heneicosane	109	15.7	*	—
C1-Fluorene	12.7	—	—	—	<i>n</i> -Hentriacontane	20.5	34.1	25.7	11.1
C4-Naphthalene	581	—	—	—	<i>n</i> -Heptacosane	22.5	30.2	12.5	23.0
1-Methylphenanthrene	50.7	—	—	—	<i>n</i> -Heptadecane	109	16.6	33.8	24.3
Fluoranthene	86.4	11.1	24.9	—	<i>n</i> -Hexacosane	*	20.0	*	12.8
Pyrene	46.4	*	16.0	—	<i>n</i> -Hexadecane	157	19.3	25.3	21.6
C1-Fluoranthene and pyrene	14.8	—	—	—	<i>n</i> -Nonacosane	22.5	53.4	*	12.7
C1-Phenanthrene	235	—	*	—	<i>n</i> -Nonadecane	108	—	—	*
C2-Phenanthrene	293	—	13.7	—	<i>n</i> -Octacosane	25.6	11.5	*	*
Benzofl[ <i>a</i> ]anthracene	27.7	—	17.2	—	<i>n</i> -Octadecane	60.2	—	—	—
C3-Phenanthrene	43.8	—	*	—	<i>n</i> -Pentacosane	61.2	30.3	—	14.3
Dibenzothiophene	10.5	—	—	—	<i>n</i> -Pentadecane	190	40.7	35.6	40.8
Chrysene	34.4	—	12.4	—	<i>n</i> -Tetradecane	81.1	22.8	—	9.2
Benzofl[ <i>b</i> ]fluoranthene	15.8	—	12.0	—	<i>n</i> -Tetradecane	156	25.2	27.8	21.6
Benzofl[ <i>e</i> ]pyrene	75.0	—	61.0	*	<i>n</i> -Tetriacontane	*	*	*	—
Benzofl[ <i>k</i> ]fluoranthene	243	7.5	313	13.7	<i>n</i> -Tricosane	*	13.2	—	—
Perylene	13.1	—	—	—	<i>n</i> -Tricosane	93.5	23.4	23.4	8.5
2,6-Dimethylnaphthalene	37.3	*	32.6	*	<i>n</i> -Tridecane	130	*	9.2	—
	10.2	—	—	—	<i>n</i> -Tritriacontane	20.2	*	—	—
					<i>n</i> -Undecane	84.0	10.6	9.4	8.7
					Phytane	667	68.7	22.6	—
					Pristane	1,310	156	59.7	—
Total mean PAHs (± 95% confidence interval)	2,908 (3,310)	42.8 (19.9)	571 (221)	30.2 (11.7)	Total mean AHs (± 95% confidence interval)	4,710 (4,560)	749 (286)	428 (72.7)	271 (20.0)

<sup>a</sup> PAHs not listed: The C2- and C3-fluorenes; C1-, C2-, and C3-dibenzothiophenes; and C3- and C4-chrysenes were not detected at any site. Benzofl[*h*]perylene and indeno[1,2,3-*cd*]pyrene were trace (detected in <50% of the samples) at DH, CB, and CH but were not detected at SP. Acenaphthene was trace at DH and CH but was not detected at CB and SP. Naphthalene, 1- and 2-methylnaphthalene, C1-naphthalene, acenaphthalene, biphenyl, C1- and C2-chrysenes, and C4-phenanthrene were trace at DH only; and dibenz[*a,h*]anthracene was trace at CB only.

<sup>b</sup> DH = Dutch Harbor, Unalaska Island; CB = Captains Bay, Unalaska Island; SP = Sand Point, Popof Island; CH = Coal Harbor, Unga Island. All locations are in Alaska (USA).

<sup>c</sup> — = PAH < 1 ng/g (limit of detection).

<sup>d</sup> \* = PAH was trace; mean not calculated.

Table 2. Akaike's information criteria (AIC) of 21 candidate models used to determine best approximating model that explain patterns of 7-ethoxyresorufin-*O*-deethylase (EROD) activity in Steller's eiders (*Polysticta stelleri*) and Harlequin ducks (*Histrionicus histrionicus*) collected at Unalaska and Popof/Unga Islands (AK, USA) during the winters of 2002 and 2003<sup>a</sup>

Model	RSS	<i>K</i>	−Log <i>L</i>	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	Akaike weight	Model <i>r</i> <sup>2</sup>
EROD	38.38	2	−102.24	−200.40	136.43	0.00	
Site	32.21	6	−115.38	−218.17	118.66	0.00	0.16
Species	19.42	3	−153.33	−300.50	36.32	0.00	0.49
PCB	38.23	3	−102.53	−198.89	137.94	0.00	0.004
Year	36.66	3	−105.67	−205.18	131.65	0.00	0.04
Gender	36.19	3	−106.64	−207.11	129.71	0.00	0.06
Site + species	14.39	7	−175.81	−336.83	0.00	0.39	0.63
Site + species + PCB	14.22	8	−176.70	−336.38	0.45	0.31	0.63
Site + species + year	14.23	8	−176.65	−336.27	0.56	0.30	0.63
Site + species + gender	14.39	8	−176.81	−334.59	2.23	0.00	0.63
All factors (site + . . . + gender)	14.07	10	−177.49	−333.41	3.42	0.00	0.63
Site + species	14.39	7	−175.81	−336.83	0.00	0.89	0.63
Site + species + interaction	14.39	7	−177.12	−332.66	4.17	0.11	0.63

<sup>a</sup> 7-Ethoxyresorufin-*O*-deethylase activity is the random effects model and the dependent variable in all models. Provided are the main factor and relevant multifactor models only, and then the best model evaluated with the factors of the best model plus interaction. For all models, *n* = 150. RSS = residual sum of squares; *K* = parameters; (*L*) = likelihood; AIC<sub>c</sub> = second-order AIC corrected for sample-size bias; ΔAIC<sub>c</sub> = difference between the best model and each candidate model; PCB = total polychlorinated biphenyl congeners.

the first AIC procedure. Because PAHs could not be measured precisely in the small biopsy specimens of liver tissue that were available, site-specific PAH concentrations in blue mussels were used to represent exposure of seaducks to PAHs. Thus, the site factor was replaced with PAHs in blue mussels (sampled in 2002) and evaluated with PCBs and species relative to EROD (2002 data only) in a third AIC procedure.

We used one-way analysis of variance and Tukey-Kramer mean comparison tests to evaluate differences in EROD activity among treatments and control birds in the dosing experiment. The AIC procedure was not used in this case, because we conducted a true controlled experiment in which statistical inference derived from null hypothesis testing is valid [28]. All contaminant values or corresponding geometric means ( $\bar{x}$ ) were calculated from untransformed data, and they are reported in terms of wet weight.

## RESULTS

### STEI, HADU, and EROD

All seaducks that were examined had measurable EROD activity. The AIC model selection procedure indicated that patterns of EROD activity were best defined by the species and site model (ΔAIC<sub>c</sub> = 0.00, Akaike weight = 0.39) (Table 2). The next best models contained species, site, and ΣPCB congeners (ΔAIC<sub>c</sub> = 0.45, Akaike weight = 0.31) as well as species, site, and year (ΔAIC<sub>c</sub> = 0.56, Akaike weight = 0.30). Based on −log *L* or AIC<sub>c</sub> values, however, these models did not improve the fit of the best model. Moreover, the factor weight for species and site was 1.0 each, whereas that for ΣPCB congeners and year was less than 0.31. The model with site, species, and gender also was considered (ΔAIC<sub>c</sub> = 2.23), but the Akaike weight (0.11), −log *L*, factor weight (0.11), and evidence ratio ( $w_i/w_j$  = 3.54) indicated that gender did not improve the fit of the best model. The inclusion of an interaction term into a second AIC procedure with species and site did not improve the best model and indicated that EROD differences between species were not confounded by site effects (Table 2). We concluded that ΣPCB congeners, year, or gender did not improve the fit of the best approximating model. We retained ΣPCB congeners in subsequent AIC procedures,

however, both because of the potential for these to induce EROD activity when compared with other stressors and because the evidence ratio indicated that the species/site model was only 1.26-fold better than the model that included ΣPCB congeners.

Species and site differences best defined the pattern of EROD activity in seaducks. Overall EROD activity was lower in STEI ( $\bar{x}$  = 35.0 pmol/min/mg protein, *n* = 70) than in HADU ( $\bar{x}$  = 151.1 pmol/min/mg protein, *n* = 80). Among sites, mean EROD for STEI ranged from higher EROD activity in birds captured at Sand Point, Dutch Harbor, and Nateekin Bay to lower activity in STEI captured at the two a priori reference sites at Captains Bay and Coal Harbor (Fig. 2). In HADU, the means ranged from 46.1 to 280.4 pmol/min/mg protein, with the highest levels in birds captured at Nateekin Bay and Sand Point and the lowest activity measured in HADU captured at Captains Bay (no HADU were captured at Coal Harbor).

Among individual seaducks, levels of EROD activity ranged from 4.6 to 412 pmol/min/mg protein in STEI and from

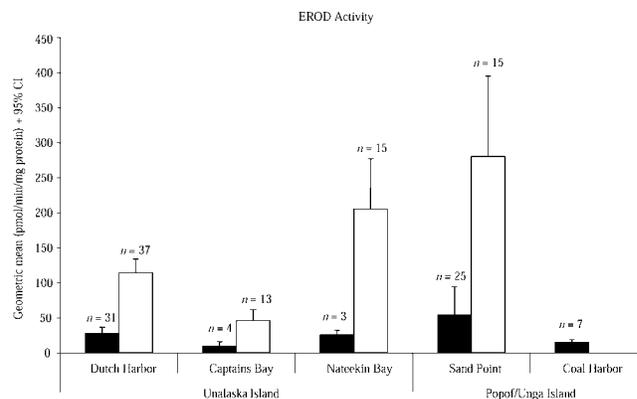


Fig. 2. Geometric mean concentrations (with 95% confidence interval [CI]) of hepatic P4501A 7-ethoxyresorufin-*O*-deethylase (EROD) activity in Steller's eiders (STEI; *Polysticta stelleri*; ■) and Harlequin ducks (HADU; *Histrionicus histrionicus*; □) from sites at Unalaska and Popof/Unga Islands (AK, USA) during the winters of 2002 and 2003. *n* = number of ducks collected at each site.

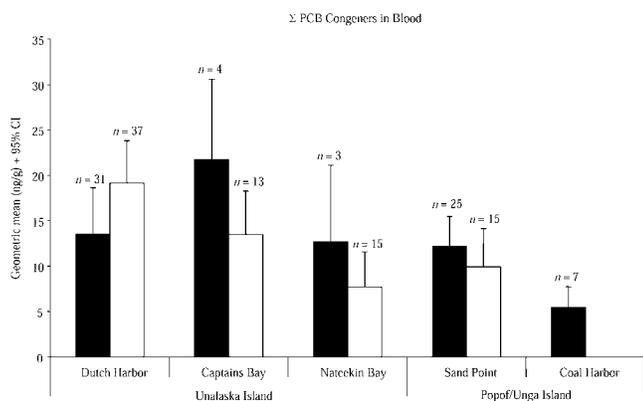


Fig. 3. Geometric mean concentrations (with 95% confidence interval [CI]) of total polychlorinated biphenyl ( $\Sigma$ PCB) congeners in blood plasma from Stellar's eiders (STEI; *Polysticta stelleri*; ■) and Harlequin ducks (HADU; *Histrionicus histrionicus*; □) from sites at Unalaska and Popof/Unga Islands (AK, USA) during the winters of 2002 and 2003.  $n$  = number of ducks collected at each site.

20 to 688 pmol/min/mg protein in HADU. Notably, only 3 of 70 STEI had EROD activity of greater than 100 pmol/min/mg protein, whereas 35 of 80 HADU measured greater than 100 pmol/min/mg protein.

#### PCB congeners in plasma

Total PCB congeners as measured in seaduck blood samples probably were not an important influence on EROD activity. The pattern of mean EROD activity in STEI and HADU at sites differed substantially from that of mean  $\Sigma$ PCB congener concentrations (Figs. 2 and 3). Total PCB congeners in blood and EROD activity in individual seaducks were not correlated ( $r^2 = 0.004$ ,  $p = 0.45$ ), which further supported inferences from the AIC procedure. Because  $\Sigma$ PCB congeners had no discernable effect on EROD activity, descriptive statistics for PCBs in seaduck blood are provided as follows.

Concentrations of  $\Sigma$ PCB congeners were highest in seaducks collected from Unalaska Island's Dutch Harbor and Captains Bay and lowest at Nateekin Bay (Fig. 3). Low  $\Sigma$ PCB congener concentrations also were detected at Popof/Unga Island's Sand Point and Coal Harbor (STEI only).

Concentrations of  $\Sigma$ PCB congeners were similar between

species ( $\bar{x}_{\text{STEI}} = 11.5$  ng/g;  $\bar{x}_{\text{HADU}} = 12.9$  ng/g) and slightly higher in males ( $\bar{x} = 13.8$  ng/g) than in females ( $\bar{x} = 9.8$  ng/g). Approximately 50% or more of the 30 PCB congeners were detected in blood of STEI and HADU captured at Dutch Harbor (18 and 19 congeners, respectively), Captains Bay (18 and 17 congeners, respectively), Nateekin Bay (11 and 18 congeners, respectively), Sand Point (15 and 13 congeners, respectively), and Coal Harbor (15 congeners, STEI only). The majority of congeners detected comprised 5% or less of the total value. Polychlorinated biphenyl congeners 138 ( $\bar{x} = 1.96$  ng/g), 153 ( $\bar{x} = 1.61$  ng/g), and 118 ( $\bar{x} = 1.20$  ng/g) were prevalent in both species at all sites; PCB congener 155 also was prevalent at Nateekin Bay ( $\bar{x} = 1.66$  ng/g). The lower-chlorinated PCB congeners 11 ( $\bar{x} = 1.91$  ng/g) and 15 ( $\bar{x} = 2.28$  ng/g) were prevalent and comprised the greater proportion of  $\Sigma$ PCB congeners at Sand Point. Congeners 52 and 202 ( $\bar{x} = 0.76$  ng/g each) were prevalent in STEI at Coal Harbor, and congeners 11 ( $\bar{x} = 2.18$  ng/g) and 136 ( $\bar{x} = 1.28$  ng/g) were prevalent in HADU at Captains Bay.

#### Contaminants in avian prey samples

Concentrations of PAHs in blue mussels best explained patterns of EROD in seaducks when substituted for the sites factor in the AIC model selection process. The model that contained species and PAHs in blue mussels had a  $\Delta\text{AIC}_c$  of 0.00 and an Akaike weight of 0.73 (Table 3); factor weights were 1.00 for species and 0.98 for PAHs. No support was found for the idea that PCB congeners (factor weight, 0.25) explained variation in EROD activity when accounting for the effects of species and mussel PAHs. The inclusion of an interaction term with species and site did not improve the best model (Table 3).

Concentrations of  $\Sigma$ PAHs in blue mussels were highest at the Dutch Harbor site ( $\bar{x} = 2,908$  ng/g) and virtually undetected in blue mussels from Captains Bay (Unalaska Island) and Coal Harbor (Unga Island) (Table 1); blue mussels were not collected at Nateekin Bay. Thirty-six of the 44 targeted PAHs were detected in blue mussels at Dutch Harbor, but few constituted more than 5% of the  $\Sigma$ PAHs. Most notable were the substituted or alkylated PAHs C3- and C4-naphthalenes, C1- and C2-phenanthrenes, and nonalkylated benzo[*e*]pyrene. Fewer (and mostly nonalkylated) PAHs were detectable in blue

Table 3. Akaike's information criteria (AIC) procedure of eight candidate models to determine the best model that explained patterns of 7-ethoxyresorufin-*O*-deethylase (EROD) activity: Substitution of polycyclic aromatic hydrocarbons (PAHs) in blue mussels (*Mytilus trossilus*) for the sites factor, modeled with species (Stellar's eiders [*Polysticta stelleri*] and Harlequin ducks [*Histrionicus histrionicus*]) and total polychlorinated congeners (PCB) in birds from the same sites at Unalaska and Popof/Unga Islands (AK, USA)<sup>a</sup>

Model	RSS	$K$	$-\text{Log}(L)$	$\text{AIC}_c$	$\Delta\text{AIC}_c$	Akaike weight	Model $r^2$
EROD	8.43	2	-39.03	-73.79	35.33	0.00	
PCB	8.13	3	-39.87	-73.16	35.96	0.00	0.04
Species	4.06	3	-55.84	-105.10	4.02	0.09	0.52
PAH mussels	6.12	3	-46.39	-86.21	26.43	0.00	0.27
PCB + species	4.05	3	-55.86	-102.74	9.90	0.01	0.52
Species + PAH mussels	3.27	4	-60.81	-112.64	0.00	0.73	0.59
PCB + PAH mussels	6.11	4	-46.42	-83.86	28.78	0.00	0.27
PCB + species + PAH mussels	3.24	5	-61.00	-110.49	2.15	0.25	0.59
Species + PAH mussels	3.27	4	-60.81	-112.64	0.00	0.71	0.59
Species + PAH mussels + Int	3.22	5	-61.18	-110.86	1.82	0.29	0.62

<sup>a</sup> Winter 2002 data only. Also provided is the best model evaluated with the factors of the best model plus interaction (Int). For all models,  $n = 46$ . RSS = residual sum of squares;  $K$  = parameters;  $(L)$  = likelihood;  $\text{AIC}_c$  = second-order AIC corrected for sample-size bias;  $\Delta\text{AIC}_c$  = difference between the best model and each candidate model.

mussels at the remaining sites: Eight at Captains Bay, comprised mainly of fluoranthene, phenanthrene, and benzo[*e*]pyrene; 15 at Sand Point, dominated by benzo[*e*]pyrene, benzo[*b*]fluoranthene, perylene, and fluoranthene; and six at Coal Harbor, mostly benzo[*e*]pyrene (Table 1).

Total PAHs and AHs in blue mussels were highly correlated ( $r^2 = 0.73$ ,  $p < 0.0001$ ). Mean  $\Sigma$ AHs in blue mussels were highest at Dutch Harbor, similar to  $\Sigma$ PAHs, with correspondingly low concentrations at Sand Point, Captains Bay, and Coal Harbor (Table 1). All or most targeted AHs were detected at Dutch Harbor and Captains Bay, with the highest proportions being pristane and phytane. Fewer but more evenly distributed (proportionately) AHs were found at Sand Point and Coal Harbor; notably, pristane and phytane were found at Sand Point but were not detectable at Coal Harbor.

Potential seaduck prey other than blue mussels were not available in sufficient quantity at all sites, which precluded associating their contaminant concentrations with seaduck EROD activity. More importantly,  $\Sigma$ PAHs in these prey were magnitudes lower than those detected in blue mussels and less likely to induce EROD activity. The gastropod *Tegula* spp. was common in sufficient abundance subtidally only at Unga and Popof Islands, with higher concentrations of  $\Sigma$ PAHs found at Sand Point ( $\bar{x} = 146$  ng/g) in the vicinity of a fish-processing outfall and boat harbor than those from uninhabited Coal Harbor ( $\bar{x} = 10.5$  ng/g). For crustaceans, the shrimp *Pandulus* sp. was collected subtidally at Dutch Harbor, and amphipods and isopods in the intertidal at Dutch Harbor and Captains Bay;  $\Sigma$ PAHs were low and similar at these sites (crustaceans combined: Dutch Harbor,  $\bar{x} = 48.3$  ng/g; Captains Bay,  $\bar{x} = 44.9$  ng/g). Flocculi were available in sufficient quantity near outfalls at Sand Point and at Dutch Harbor. Concentrations of  $\Sigma$ PAHs in flocculi were higher at Sand Point ( $\bar{x} = 92.8$  ng/g) than at Dutch Harbor ( $\bar{x} = 48.3$  ng/g).

Fluoranthene, perylene, and phenanthrene comprised 5 to 30% of the individual constituents of  $\Sigma$ PAHs in crustaceans from Dutch Harbor. Pyrene comprised 45% of  $\Sigma$ PAHs in *Tegula* spp. from Sand Point, whereas dibenz[*ah*]anthracene comprised 43% of  $\Sigma$ PAHs in *Tegula* spp. from Coal Harbor. Perylene comprised all the  $\Sigma$ PAHs in flocculi from Dutch Harbor, whereas 16 PAH compounds comprised 1 to 12% of the  $\Sigma$ PAHs in flocculi at Sand Point.

Except for dichlorodiphenyldichloroethylene and  $\Sigma$ PCBs, OCs were not detected in any prey samples. Only *p,p'*-dichlorodiphenyldichloroethylene was detected in one blue mussel composite sample from Dutch Harbor (3.0 ng/g), and no other chlorinated pesticides were detected. Also,  $\Sigma$ PCBs were detected in only two composite blue mussel samples from Dutch Harbor (15.0 and 120.0 ng/g). Subsequently, it was unnecessary to relate OCs and  $\Sigma$ PCBs in seaduck prey to EROD activity.

#### *EROD and dosed, captive STEI*

Cytochrome P4501A activity in captive STEI exposed to 20 mg/kg body weight of BNF had fourfold EROD activity compared to control birds (3.9 pmol/min/mg protein), indicating a definitive biochemical response by STEI from exposure to PAHs. Average EROD activity at the 20 and 100 mg/kg body weight treatments were significantly higher than in the control group ( $f = 4.15$ ;  $p = 0.04$ ; degrees of freedom = 2, 12). Average EROD activity in STEI exposed to 100 mg/kg body weight of BNF (13.6 pmol/min/mg protein) was similar to that in the 20 mg treatment (16.2 pmol/min/mg protein).

## DISCUSSION

The present study indicated that incidental PAH contamination at remote, near-Arctic industrial areas induced P4501A response in wintering seaducks. Mean EROD activity in HADU from Nateekin Bay and Sand Point were similar to that reported for HADU from areas with known hydrocarbon contamination (e.g., Prince William Sound [AK, USA] impacted by the *Exxon Valdez* oil spill) [10,12]. Although Nateekin Bay is not within the industrialism of Dutch Harbor, elevated EROD activity in HADU from that location is not surprising. Estimated home-range size and observed local movements indicated that HADU and STEI using Nateekin Bay routinely used contaminated areas around Dutch Harbor [22]. Nateekin Bay also may have historical point sources of contamination resulting from activity associated with World War II.

Induction of hepatic P4501A as measured by EROD activity was evident in both species, and activity was more pronounced in HADU among sites than in STEI. Although HADU can use deeper waters, they more commonly use shallower (<0.8 m) shoreline waters for foraging or roosting, whereas STEI use deeper (>5 m) offshore waters, with occasional foraging visits to intertidal areas during high tide [29,30]. Hydrocarbons may be more elevated in habitats closer to shore because of accumulation at the water-land barrier, whereas greater dispersion and, thus, dilution occurs in more open water [10]. Low PAH concentrations in crustaceans collected intertidally at Captains Bay, however, indicated interspecific differences in hydrocarbon bioaccumulation, which has been substantiated in other studies (i.e., crustaceans bioaccumulated lower concentrations and fewer PAHs than blue mussels did [31]).

The possibility that HADU may manifest higher biochemical responses to similar levels of organic contaminants compared to STEI is supported by the lower level of EROD activity demonstrated by captive STEI exposed to the recommended high dose of BNF [27]. The P4501A system in STEI may not have been induced to reach maximum activity, but experimentation with legally protected STEI was restrictive. Free-ranging STEI at industrial sites had (mean) EROD activity two- to threefold greater than dosed STEI, and activity in STEI from the two a priori reference sites also were higher than in control and comparable to that in dosed STEI, indicating potentially elevated levels of causative agents at all study sites. Those few individual STEI with activity higher than 100 pmol/min/mg protein may have been exposed to multiple stressors or consistently used highly contaminated habitat. We suggest that the low-dose/high-dose response similarity demonstrated that STEI are sensitive to PAHs at low doses and that sensitivity did not correlate linearly. The STEI biochemical detoxification system may not be capable of responding more effectively at high versus low doses; at higher doses, STEI may be more susceptible to harm from PAHs or other stressors.

Higher levels of EROD in HADU probably were indicative of intersite differences in hydrocarbon contaminant levels in one type of generalized, nearshore habitat but were not reflective of all possible habitats at the study sites. Because of interspecific differences in EROD activity, HADU did not suffice as direct surrogates for STEI. Because biochemical response was more pronounced in HADU than in STEI, however, and because nearshore habitat may harbor higher levels of contamination than offshore habitat, HADU may be better indicators of potential habitat injury from organic contaminants than STEI.

Induction of P4501A from hydrocarbon exposure was demonstrated in captive STEI. We caution that the captive birds were exposed to only one PAH, whereas free-ranging birds may be exposed to many xenobiotics or combinations of compounds that induce P4501A. Assuming PAH concentrations in prey were positively correlated with seaduck EROD activity, PAHs likely were a causative factor of elevated P4501A in free-ranging birds in the present study. The evidence was less supportive for PCBs, because no relationship was evident between EROD activity and  $\Sigma$ PCB congeners measured in the same duck. Although our analyses included only 30 of a possible 209 PCB congeners, our suite of congeners included highly toxic, dioxin-like, or coplanar congeners that are known P4501A inducers (e.g., PCBs 77, 126, and 169) as well as suspected inducers known to be commonplace in the marine environment [10,21]. Again, we caution that xenobiotics not investigated may have induced P4501A.

Interestingly, EROD activity was correlated with blue mussels that probably depurated PAHs rapidly following a decrease in hydrocarbon exposure [31]. High concentrations of PAHs in blue mussels may indicate continual exposure at Dutch Harbor, at least during the critical STEI wintering period. Concentrations of  $\Sigma$ PAHs in blue mussels from Dutch Harbor were comparable to those reported in blue mussels from nationally recognized, highly polluted sites, such as Lauritzen Canal (4,100 ng/g; San Francisco Bay, CA, USA) and Eagle Harbor (5,224–7,498 ng/g; Puget Sound, WA, USA) [31,32]. Profiles of PAHs in blue mussels at Dutch Harbor sites had a high proportion of substituted or alkylated PAHs that indicated more exposure to petroleum spillage than combustion of petroleum products [33]. Benzo[*e*]pyrene (known for high toxicity and usually formed from the combustion of petroleum-based products) comprised less than 18% of the  $\Sigma$ PAHs in blue mussels from Dutch Harbor or Captains Bay sites. Although benzo[*e*]pyrene comprised most of the  $\Sigma$ PAHs in blue mussels from Popof/Unga Island sites, concentrations of  $\Sigma$ PAHs were low at these sites. The virtual nondetection of PAHs at Captains Bay and Coal Harbor confirmed that these sites were appropriate as reference sites for lower PAH exposure.

Geographic patterns of AH composition in blue mussels were similar to PAH profiles, but certain higher-proportioned AHs indicated sources from organic origins. Pristane and phytane comprised the majority of AHs at Dutch Harbor sites and were common at Sand Point but not at Coal Harbor. These nonpetroleum-based oils occur naturally and could be elevated as a result of fish-processing effluent. Proportional compositions of AHs were more evenly distributed at both Sand Point and Coal Harbor, where similar AH compounds comprised 5% or more of the AHs. Although highly correlated with PAHs, AHs apparently do not induce P4501A [34,35].

Blue mussels were a useful media to determine potential PAH exposure when tissue volumes from seaduck specimens limited direct correlations with EROD activity. In seaduck prey other than blue mussels, concentrations of PAHs were low, comprised fewer constituents, and generally were associated with either complete or partially combusted fossil fuels or coal tar (e.g., fluoranthene, phenanthrene, dibenz[*ah*]anthracene, chrysene, pyrene, or perylene).

The lack of detectable OCs in seaduck prey indicated that either environmental levels were low, prey were very efficient at metabolizing these xenobiotics, or PCB congener accumulation in blood of STEI and HADU were acquired from

sources in addition to prey. For example, contaminants that accumulate in the oily surface microlayer of oceanic waters [36] could be transferred to seaducks via preening, but the low concentration of  $\Sigma$ PCB congeners in seaduck blood indicated that levels in the environment probably were not highly elevated.

The relationship between P4501A induction and PAHs rather than PCB congeners also was reported in HADU from Prince William Sound and lesser scaup (*Aythya affinis*) from Indiana (USA) near the Great Lakes [10,11]. Concentrations of PCB congeners detected in seaducks in the present study were higher than those reported in the Prince William Sound study [10], but the one-to-one correlation of concentrations of blood plasma PCB congeners and P4501A induction in each of 165 seaducks demonstrated no relationship. Similarly, a study of Arctic seabirds with high levels of PCB congeners demonstrated no relationship to P4501A activity [37]. Studies that compared liver and blood concentrations of PCB congeners indicated concentrations that were 0.9- to 2.0-fold greater in liver than in blood [38]. Based on this information, if we conservatively assume that  $\Sigma$ PCB congeners were twofold greater in liver than in blood, then tissue concentrations of PCB congeners were substantially higher in aquatic birds at Chesapeake Bay and near the Great Lakes (USA) and in seabirds from Norway than we detected at the eastern Aleutian Islands [11,20,39].

Profiles of PCB congeners showed little variability among species, but site-specific patterns were evident, suggesting potentially different sources. Non-*ortho*-planar PCB congeners (PCBs 77 and 169) that are among the most likely P4501A inducers [21] were detected in STEI or HADU from some sites. A pattern of prevailing nonplanar PCB congeners (PCBs 118, 128, 138, 153, 171, 180, 189, 194, 197, 202, and 209) also was evident. Many of these congeners commonly are reported in seabirds, but detectable accumulation of these congeners in birds apparently results from lower elimination capability of the higher-chlorinated compounds rather than exposure or uptake capacity [37,39]. We detected retention, however, of purportedly easier-to-eliminate, lower-chlorinated PCB congeners (PCBs 11, 15, and 52) at Captains Bay and Sand Point, which may indicate either higher environmental levels of these congeners at those sites, relatively recent exposure, or interspecific differences in elimination capability.

Comprehensive contaminant studies of listed or protected species *in situ* are rare. Advances in interpretation of molecular biomarkers and nonlethal microsurgical techniques proved to be useful for understanding potential impacts of contaminants without apparent harm to STEI. Other than the use of feathers, few studies have employed these techniques in investigations of wild avian species and contaminants, probably because of the high monetary, personnel, and temporal investments of capture, handling, and surgeries.

In summary, STEI and HADU were exposed to an array of organic contaminants, but with the exception of PAHs, ubiquitous PCBs and other organic compounds appeared to be at low environmental levels, which correspond to generalized deposition models of OC outfall in the Arctic environment [40]. Our evidence implicates elevated PAHs associated with commercial maritime activities at near Arctic bays for P4501A response in wintering seaducks.

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