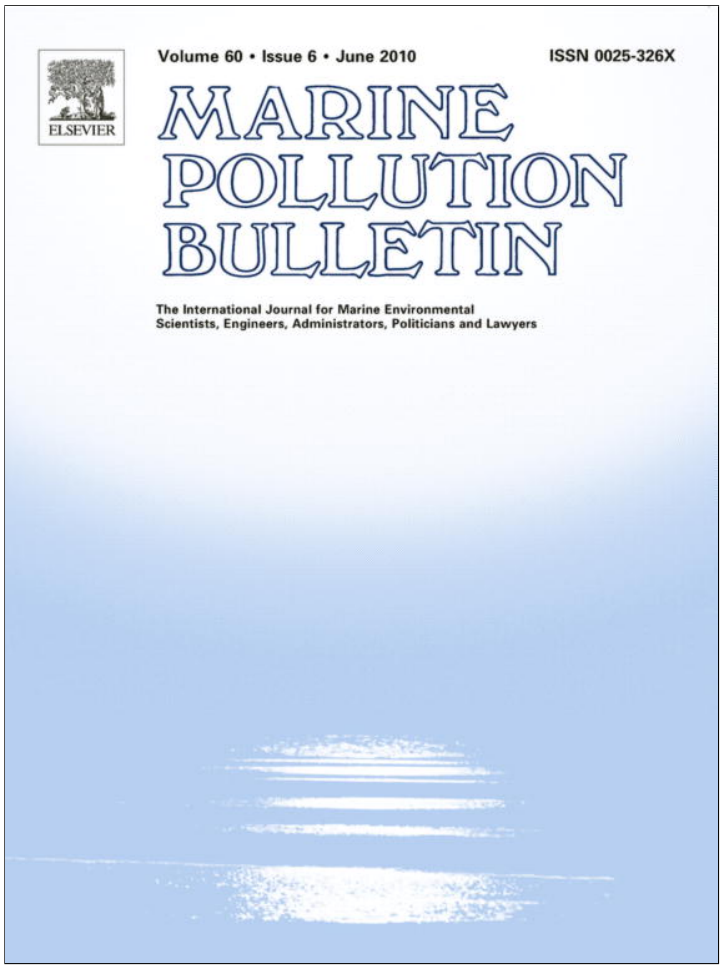

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PCB exposure in sea otters and harlequin ducks in relation to history of contamination by the *Exxon Valdez* oil spill

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ABSTRACT

Exposure to contaminants other than petroleum hydrocarbons could confound interpretation of *Exxon Valdez* oil spill effects on biota at Prince William Sound, Alaska. Hence, we investigated polychlorinated biphenyls (PCBs) in blood of sea otters and harlequin ducks sampled during 1998. PCB concentrations characterized by lower chlorinated congeners were highest in sea otters from the unoiled area, whereas concentrations were similar among harlequin ducks from the oiled and unoiled area. Blood enzymes often elevated by xenobiotics were not related to PCB concentrations in sea otters. Only sea otters from the unoiled area had estimated risk from PCBs, and PCB composition or concentrations did not correspond to reported lower measures of population performance in sea otters or harlequin ducks from the oiled area. PCBs probably did not influence limited sea otter or harlequin duck recovery in the oiled area a decade after the spill.

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1. Introduction

The grounding of the T/V *Exxon Valdez* in March 1989 released approximately 42 million liters of crude oil into Prince William Sound (PWS) and the Gulf Alaska. The direct effects of acute mortality within the nearshore biological community immediately after the *Exxon Valdez* oil spill (EVOS) were well documented (Spies et al., 1996). Perhaps more importantly, chronic direct and indirect population-level effects were attributed to bioavailable oil persisting longer than expected throughout the subsequent 10 – plus years post-spill (summarized by Peterson et al., 2003).

Induction of the cytochrome p450 (CYP) mixed function oxygenase system is a particularly germane biomarker for exposure to polycyclic aromatic hydrocarbons (PAHs) found in crude oil, and has been used increasingly as an indicator of oil exposure in fish and wildlife populations (e.g., Trust et al., 2000; Jewett et al., 2002; Sarkar et al., 2006; Miles et al., 2007). However, exposure to other co-occurring persistent organic pollutants such as polychlorinated biphenyls (PCBs) that are capable of inducing CYP may confound PAH exposure inferred from CYP induction and subsequent conclusions regarding chronic effects of oil. PCBs are ubiquitous in higher latitude marine food webs because of long-range transport from industrialized regions, as well as local point sources (Arctic Monitoring and Assessment Programme, 2004). The positioning of chlorine substituents around the biphenyl structure largely influences the binding affinity of a particular PCB congener to arylhydrocarbon (*Ah*) receptor sites that induce CYP, which in conjunction with the degree of chlorination and biotransformation rates primarily governs the environmental transport and persistence of PCBs (De Voogt et al., 1993). Congeners known to induce CYP include the highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-like non-*ortho* chlorine substituted congeners followed by the mono-*ortho* and di-*ortho* substituted derivatives, whereby the introduction of each *ortho* chlorine substituent decreases *Ah* agonist activity (De Voogt et al., 1993). However, PCBs not necessarily mediated by *Ah* affinity (i.e., non-coplanar PCBs) can also have negative impacts in marine mammals by suppressing immune function response (Levin et al., 2007) or triggering changes in blood serum chemistry indicative of hepatic damage (Mazet et al., 2000; Hanni et al., 2003). Furthermore, a prevalence of heavier, highly chlorinated PCB congeners or recalcitrant congeners with chlorine substitutions in the 4,4′ or 3,4′,5 positions in organisms generally indicates either recent exposure from a nearby source or long-term bioaccumulation. In contrast, a prevalence of more volatile lower chlorinated PCB congeners typically indicates exposure from distant sources or biotransformation and degradation of parent compound (Zell and Ballschmiter, 1980).

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While considerable debate exists as to whether populations and communities affected by the EVOS have (Harwell and Gentile, 2006) or have not (Peterson et al., 2003) fully recovered, not all factors that could contribute to depressed recovery during the decade post-spill have been examined thoroughly. For example, a multitude of factors potentially influencing recovery of two nearshore sentinel species, sea otters (*Enhydra lutris*) and harlequin ducks (*Histrionicus histrionicus*), affected by the EVOS have received considerable study (e.g., Trust et al., 2000; Bodkin et al., 2002; Ballachey et al., 2003; Dean et al., 2002; Esler et al., 2002). From a xenobiotic perspective, most of these studies compared demographic parameters or biomarker responses in sea otters and harlequin ducks from oiled and unoiled areas relative to chronic exposure to PAHs originating from the EVOS. In contrast, possible confounding effects due to PCB exposure have received sparse consideration despite their known global distribution and environmental persistence. The one previous study of PCB exposure in harlequin ducks from areas affected by the EVOS reported no differences in PCB concentrations between ducks from oiled and unoiled areas and poor correlations between PCBs and CYP induction, which suggested that PCBs did not constrain harlequin duck recovery (Trust et al., 2000). However, concentrations and composition of PCBs in sea otters affected by the EVOS have not been reported nor compared relative to patterns observed in harlequin ducks. It is plausible that even low levels of PCBs may have been an additive limiting factor for sea otter recovery post-spill given high reproductive sensitivities to PCB exposure reported in con-familial mink (*Mustela vision*) and Eurasian otter (*Lutra lutra*) (Kannan et al., 2000).

Sea otters and harlequin ducks also share common foraging strategies by diving for benthic invertebrates in nearshore habitats. This behavior results in a generalized shared pathway for exposure to contaminants sequestered in benthic sediments that are bioaccumulated by their invertebrate prey (Kuzyk et al., 2005), or directly ingested when sediments are disturbed during foraging excavations (Short et al., 2006). Both species forage occasionally in overlapping habitats but absolute exposure to PCBs from foraging activity likely differs between the two species. Sea otters commonly forage to depths of 50 m (Bodkin et al., 2004) whereas harlequin ducks typically constrain diving depths to <20 m in the intertidal and shallow subtidal zones (Robertson and Goudie, 1999). Sea otters generally select different prey (e.g., urchins) or larger size classes of prey (e.g., mollusks and crustaceans) (Dean et al., 2002) than harlequin ducks (Robertson and Goudie, 1999; Esler et al., 2002). Hence, a more detailed analysis of PCB patterns in sea otters and harlequin ducks may reveal confirmatory or contradictory evidence for overall PCB effects on the recovery of these species during the first post-EVOS decade, and is an important aspect of EVOS related injury and recovery assessment.

The primary goal of this study was to assess PCB exposure in sea otters relative to recovery from the EVOS, and to provide a more detailed analysis of PCBs in harlequin ducks beyond the reported assessment by Trust et al. (2000). We used archived sea otter and previously published harlequin duck (Trust et al., 2000) data collected 9 years post-EVOS to determine: (1) concentrations and composition of PCBs in blood samples from sea otters and harlequin ducks inhabiting an oiled and unoiled area in PWS, (2) PCB composition relative to recent or point source exposure, (3) the influence of reproductive status and age on PCB concentrations in sea otters, and (4) the relations between PCB concentrations and serum enzyme concentrations in sea otters. We did not measure CYP induction in sea otters in this study; rather we examined concentrations of PCBs relative to their potential *Ah*-receptor agonist activity.

2. Methods

2.1. Sample collection

Sea otters were captured at Knight (oiled area) and Montague (unoiled area) Islands in western PWS, Alaska during July–August 1998 using tangle nets or diver-operated Wilson traps. Captured otters ($n_{oiled} = 18$ and $n_{unoiled} = 10$) were assessed for gender and reproductive dependency status (i.e., females with or without a dependent pup). One premolar tooth was extracted for aging via cementum annuli, and up to 35 ml of blood was collected and serum extracted via centrifugation for PCB and biochemical analyses. Captured sea otters averaged approximately 6 years of age at both Knight ($\bar{x} = 6.5$, SE = 1.0, range = 1–18) and Montague Islands ($\bar{x} = 6.6$, SE = 1.1, range = 1–20). Additional details for sea otter sample collection and preservation were described in Ballachey et al. (2003). Harlequin duck blood plasma samples were collected from live individuals captured at Montague Island ($n = 10$), and Crafton Island and Main Bay (oiled) ($n = 10$) located 8–15 km to the northwest of Knight Island during March–April 1998. Trust et al. (2000) described specific details for harlequin duck sample collection and preservation. The oiled and unoiled area for both species was separated by at least 24 km of open water.

2.2. Analytical chemistry

Blood samples from sea otters (serum) and harlequin ducks (plasma) were analyzed for PCB congeners (sea otters = 96 congeners, harlequin ducks = 93 congeners) and Σ PCBs based on aroclor standards (i.e., specific mixtures of congeners for commercial application) by the Geochemical and Environmental Research Group (Texas A&M, College Station, Texas, USA). Specific details for analytical methods using capillary gas chromatography equipped with an electron capture device for both species were described by Trust et al. (2000). The US Fish and Wildlife Service's Analytical Control Facility (USFWS-ACF, Shepherdstown, WV) approved quality control and assurance procedures and analytical results for both PCB data sets. We report PCB congeners according to their first International Union of Pure and Applied Chemistry (IUPAC) number. Coeluting congeners in sea otters and/or harlequin ducks included IUPACs 7/9, 8/5, 16/32, 18/17, 22/51, 24/27, 33/20, 47/75, 41/64, 60/56, 74/61, 87/115, 95/80, 101/90, 107/108/144, 118/108/149, 138/160, 141/179, 149/123, 153/132, 156/171/202, 170/190, 171/202, 187/182/159, and 195/208. We excluded three congeners (IUPACs 30, 42, and 176) quantified in sea otters but not in harlequin ducks for comparison of patterns in both species.

Quest Laboratories (Portland, Oregon, USA) analyzed all sea otter samples for serum chemistry. We report the following six serum analytes, whose levels can be indicative of liver damage from low dose xenobiotic exposure (Mazet et al., 2000), for comparison with sea otter PCBs: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transpeptidase (GGT), uric acid, total cholesterol, and total triglycerides. Ballachey et al. (2003) provided specific details for serum chemistry analytical methods.

2.3. Data treatment – statistical analyses

We analyzed detection frequencies and concentrations in blood samples using four functional groups of PCB congeners by species and area (oiled and unoiled): (1) total PCBs comprised all 93 congeners analyzed, (2) CYP congeners comprised the sum of non-*ortho* IUPACs 77, 81, 126, and 169, mono-*ortho* IUPACs 105, 114, 118, 123, 156, 167, and 189, and di-*ortho* IUPACs 128, 138, 158, 166,

and 170 that can elicit a biomarker response by binding to *Ah*-receptor sites (De Voogt et al., 1993), (3) ICES congeners comprised the sum of IUPACs 28, 52, 101, 118, 138, 153, 180 recommended for monitoring in marine environments as indicators of bioaccumulative PCB pollution by the International Council for the Exploration of the Sea (Anonymous, 1986), and (4) IUPAC 138 because of its recalcitrant nature and it was the only congener detected in all samples (Trust et al., 2000; see results). Only concentrations of detected congeners were statistically analyzed and concentrations reported as ng/g on a wet weight basis unless stated otherwise. We $\log_e$ transformed all concentrations prior to parametric statistical analyses to meet assumptions of homoscedacity.

We conducted Fisher exact tests to determine whether the odds of detecting PCBs comprising each congener group in sea otters or harlequin ducks differed between the oiled and unoiled area, followed by the Mantel Haenszel Heterogeneity Test to determine whether the odds of detecting congener groups by species strata were equal. Body size typically governs the volume of blood safely drawn from wildlife. A lower volume of blood available from harlequin ducks resulted in higher limits of detection of PCBs relative to sea otters. Thus, we increased the minimum limit of detection for sea otters (0.048 ng/g) to the lowest limit of detection achieved for harlequin ducks (0.137 ng/g) to evaluate patterns of PCB concentrations by species on a similar scale. We then conducted a second analysis for each congener group using the original detection limits to determine if overall conclusions were dependent on our adjustment of the detection limit. Tests were conducted with NCSS 2000 (Hintze, 2006).

Table 1
Geometric mean concentrations (ng/g, wet weight)[a,b] (and ranges) of congeners comprising non-, mono-, di-*ortho* PCBs, ICES PCBs, and Σ aroclors in blood from harlequin ducks (plasma) and sea otters (serum) sampled from an oiled and unoiled area of Prince William Sound, Alaska, 1998.

Ortho substitution or PCB group	IUPAC#	Harlequin duck		Sea otter	
		Oiled (n = 10)	Unoiled (n = 10)	Oiled (n = 18)	Unoiled (n = 10)
Non-*ortho*	77	ND –	ND –	ND –	ND –
	126	ND –	ND –	ND –	ND
	169	ND –	ND –	ND –	ND –
	81	0.422 (ND_4–2.78)	0.178 (0.539–4.59)	ND –	ND –
Mono-*ortho*	105	ND –	ND –	0.055 (ND_{13}–0.598)	0.092 (ND_5–2.92)
	114	ND –	ND –	ND –	ND –
	118[c]	ND –	ND –	0.072 (ND_{10}–0.905)	0.481 (ND_1–1.86)
	123	ND –	ND –	0.192 (ND_2–2.24)	0.217 (ND_1–0.800)
	156	ND –	0.122 (ND_9–0.641)	ND –	ND –
	167	ND –	ND –	0.053 (ND_{14}–1.36)	0.359 (ND_2–1.44)
	189	ND –	0.125 (ND_9–0.748)	0.030 (ND_{17}–0.811)	0.050 (ND_6–0.313)
	$TEQ_{mono\text{-}ortho}$[d]			0.233 (ND_{92}–1.20)	0.608 (ND_{45}–1.70)
Di-*ortho*	128	ND –	ND –	0.043	0.151
	138[c]	1.00 (0.299–8.58)	0.921 (0.418–11.4)	0.809 (0.200–17.6)	2.17 (1.19–4.63)
	158	ND –	ND –	0.037 (ND_3–2.81)	0.035 (ND_9–1.54)
	166	0.167 (ND_9–1.15)	ND –	0.030 (ND_3–0.130)	ND –
	170	ND –	ND –	0.191 (0.054–1.530)	0.428 (0.180–1.40)
ICES	28	0.253 (ND_5–2.03)	0.150 (ND_8–1.46)	0.157 (ND_4–3.41)	0.070 (ND_6–0.692)
	52	ND –	0.175 (ND_8–1.61)	0.052 (ND_{13}–2.05)	0.077 (ND_7–3.33)
	101	ND –	0.254 (ND_5–1.75)	0.038 (ND_{16}–6.31)	0.033 (ND_9–0.980)
	153	0.308 (ND_6–3.88)	0.129 (ND_9–0.384)	0.364 (ND_1–5.85)	0.253 (ND_1–1.02)
	180	0.396 (ND_2–1.54)	0.316 (ND_1–0.707)	0.258 (ND_1–4.69)	0.369 (ND_1–1.10)
Σ Aroclors[e]	–	ND –	ND –	16.4 (ND_{14}–114)	22.2 (ND_5–125)

[a] Geometric means were estimated by substituting a value of ½ the limit of detection (LOD) for samples with a non-detected congener.
[b] ND = concentration was below the LOD. For geometric means, ND = all samples were below the LOD. For ranges, subscripted values following ND indicate the number of samples below the LOD.
[c] Also an ICES congener.
[d] Arithmetic mean reported for comparison to published TEQ average values. Harlequin duck TEQs were not calculated due to low mono-*ortho* congener detection frequencies.
[e] Total PCBs based on aroclor standards.

We constructed PCB profiles to examine average proportions of detected individual congeners by species and area, and report geometric mean concentrations of CYP congeners, ICES congeners, and aroclor based Σ PCBs. We calculated toxic equivalents (TEQs) for detected mono-*ortho* congeners in sea otters using updated World Health Organization toxic equivalency factors to aid interpretation of toxicological significance (Van den Berg et al., 2006). We did not calculate mono-*ortho* TEQs for harlequin ducks or non-*ortho* TEQs for either taxon due to rare detection of these congeners (see results, Table 1). For descriptive purposes, we substituted a value of ½ the limit of detection for all non-detected congeners when calculating geometric means, and report the number of samples with concentrations below the limit of detection for each congener (Table 1).

We used principal components analysis (PCA) to characterize covarying combinations of PCB homolog groups by area and species. Use of individual PCB congeners would have resulted in a horizontally elongated ordination matrix where the number of samples is less than the number of variables (McCune and Grace, 2002). We substituted a value of ½ the limit of detection for a single congener within a homolog group when no congeners comprising a homolog group were detected for a particular sample, which reduced overrepresentation of rare homolog groups. Principal components were derived from the covariance matrix of the centered log-ratios of the proportional total of homolog groups for each individual sample (Howel, 2007). We then used multiple response permutation procedure (MRPP) to non-parametrically test if coordinate scores from the PCA differed by area for each species. We report the *A* test statistic as a measure of effect size along with corresponding *P* values using a Bonferroni adjusted significance level ($\alpha = 0.5/2$ comparisons = 0.025). We used PC-ORD 5.10 to conduct PCA and MRPP analyses (McCune and Mefford, 2006).

We conducted two-factor ANOVAs to determine differences in detected concentrations of the four groups of congeners by area and species. To examine additional factors influencing PCBs in sea otters, we used ANCOVA to determine the effect of animal age, dependency status, and area on detected concentrations of the four groups of congeners. Gender could not be used as an explanatory variable because males comprised only three samples from Knight Island and one sample from Montague Island, which negated the necessary degrees of freedom for inclusion in the ANCOVA models. We used Welch's corrected *t*-tests to compare concentrations of the PCB congener groups between males and independent females to assess whether pooling males with non-dependent females confounded interpretation of dependency effects. We also used ANCOVA to test relations between detected

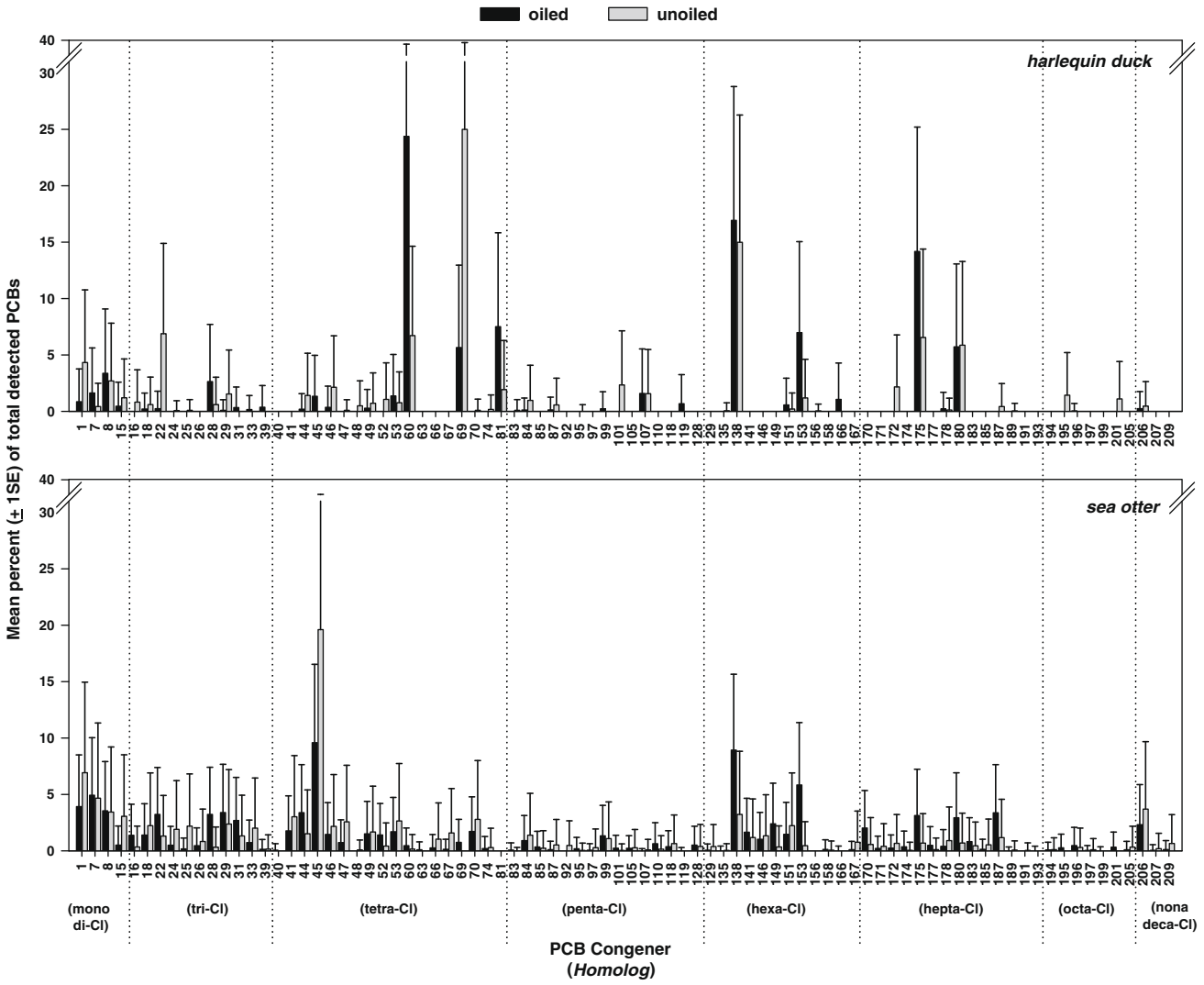

Fig. 1. Profiles for detected PCB congeners in blood samples from harlequin ducks (plasma) and sea otters (serum) inhabiting an oiled and unoiled area of Prince William Sound, Alaska, 1998. Data are grouped by successive IUPAC congener number indicating increasing chlorination and corresponding homolog groups.

concentrations of the four groups of congeners and the six blood biochemical analytes while controlling for area, age, and dependency status effects. We do not report area, age, or dependency status effects because our objective was to determine if PCBs influenced biochemical indices. For all ANOVAs, we initially fit all two-way interactions for each model and retained only significant interactions. We then refitted main effects, and interpreted the resulting model. To verify ANCOVA assumptions, homogeneity of slopes were assessed by fitting interactions between covariates and treatments. Main effects were refitted if interactions were insignificant. Tests were conducted with SAS 9.2 (SAS Institute, 2006).

3. Results

3.1. PCB detection

Using adjusted detection limits for sea otters, individual PCB congeners were more likely to be detected in sea otters (odds ratio = 2.6, 95% CI = 2.2–3.2, $P < 0.0001$) and harlequin ducks (odds ratio = 1.4, 95% CI = 1.0–1.9 $P = 0.11$) from the unoiled than the oiled area. The CYP congeners also were more likely to be detected in sea otters from the unoiled area (odds ratio = 1.6, 95% CI = 1.1–2.5, $P = 0.02$), but detection odds did not differ for harlequin ducks (odds ratio = 1.2, 95% CI = 0.5–3.2, $P = 0.83$). The odds of detecting ICES congeners were equivalent in sea otters (odds ratio = 1.4, 95% CI = 0.7–2.5, $P = 0.37$) and harlequin ducks (odds ratio = 1.1, 95% CI = 0.5–2.3, $P = 0.86$) from both areas. The odds of detecting individual PCB congeners were significantly greater for sea otters than harlequin ducks ($\chi^2_{[1]} = 13.5$, $P = 0.0002$), but did not differ by species for CYP and ICES congeners ($\chi^2_{[1]} \leqslant 2.7$, $P \geqslant 0.10$).

Results were similar when we used the unadjusted detection limits for sea otters. The odds of detecting individual (odds ratio = 2.1, 95% CI = 1.7–2.4) and CYP (odds ratio = 1.8, 95% CI = 1.1–2.7) congeners remained higher for otters from the unoiled area ($P < 0.001$) and again did not differ by area for ICES congeners (odds ratio = 1.0, 95% CI = 0.5–1.9). The odds of detecting individual PCB congeners remained greater for sea otters than harlequin ducks ($\chi^2_{[1]} = 4.9$, $P = 0.02$), and did not differ by species for ICES congeners ($\chi^2_{[1]} = 0.1$, $P = 0.80$). Only the odds of detecting CYP congeners changed by species, which were greater for sea otters than for harlequin ducks ($\chi^2_{[1]} = 5.9$, $P = 0.01$). IUPAC 138 was detected in all sea otter ($n = 28$) and harlequin duck ($n = 20$) samples regardless of the limit of detection used. We retained the original detection limits for all subsequent analyses because the odds of congener detection were invariant to lower detection limits for all but one evaluation by species.

3.2. PCB composition and concentrations

Profiles of PCB congeners spanned a wider range congeners in sea otters compared to harlequin ducks (Fig. 1). The ICES congeners

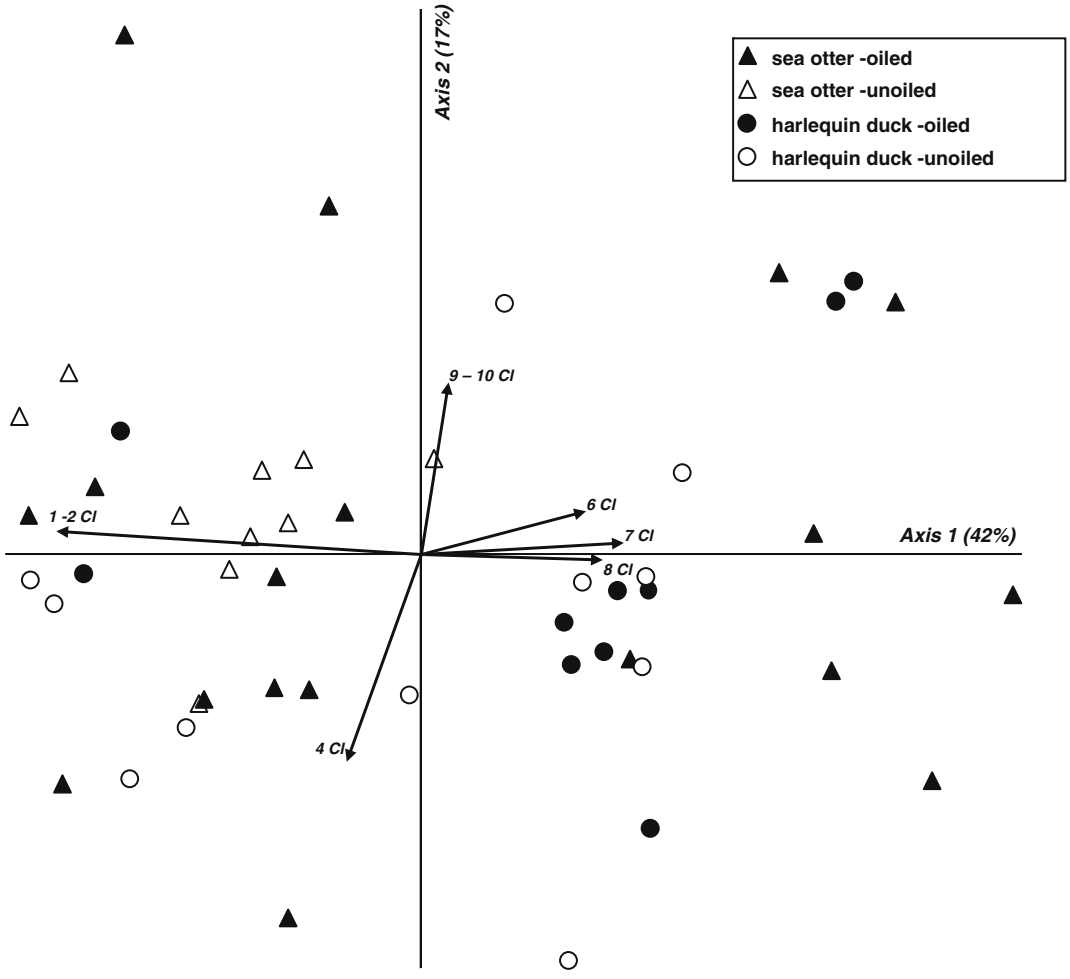

Fig. 2. Principal components analysis for proportional PCB homolog groups in blood samples from harlequin ducks (plasma) and sea otters (serum) inhabiting an oiled and unoiled area of Prince William Sound, Alaska, 1998. Joint plots (arrows) indicate strength and direction of the relationships between homolog groups and the ordination scores.

comprised much larger percentages of Σ PCB concentrations in sea otters from the oiled area ($\bar{x}$ = 23%, range = 2–51%) than the unoiled area ($\bar{x}$ = 2%, range = 0–11%), whereas percentages were more similar in harlequin ducks from both areas (oiled: $\bar{x}$ = 32%, range = 6–80%; unoiled: $\bar{x}$ = 26%, range = 9–53%). The CYP congeners accounted for similar mean percentages of Σ PCBs in sea otters (oiled:

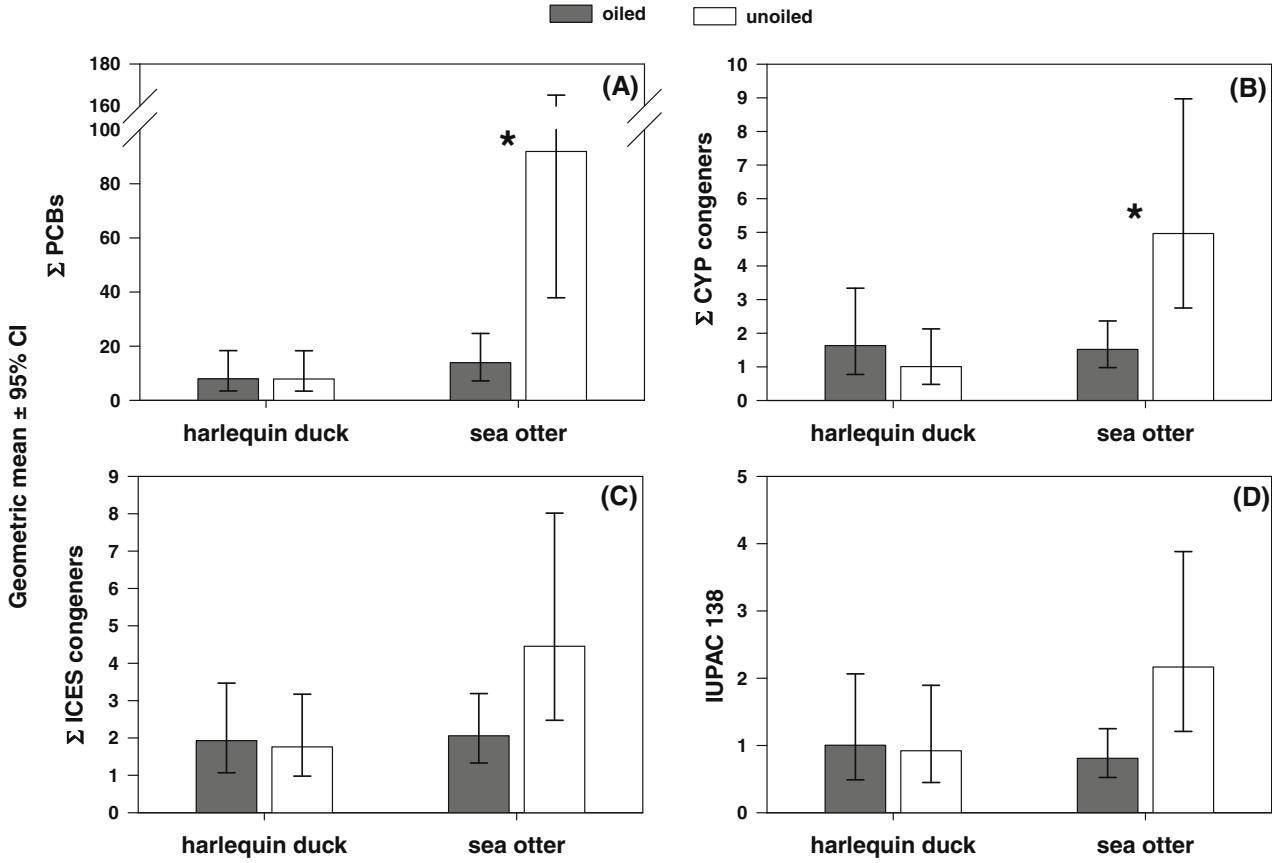

Fig. 3. Back-transformed geometric mean concentrations (ng/g, wet weight) and 95% confidence intervals for detected PCBs in blood samples from harlequin ducks (plasma) and sea otters (serum) inhabiting an oiled and unoiled area of Prince William Sound, Alaska, 1998. PCBs are grouped according to the sum of (A) 93 congeners, (B) non-, mono-, and di-*ortho* congeners capable of inducing the cytochrome p450 (CYP) mixed function oxygenase system, (C) IUPACs 28, 52, 101, 118, 138, 153, 180 recommended for monitoring in marine environments by the International Council for the Exploration of the Sea, and (D) IUPAC 138. Asterisks above groups denote significant ($P < 0.05$) differences determined by ANOVA. Note variable *y*-axis scales.

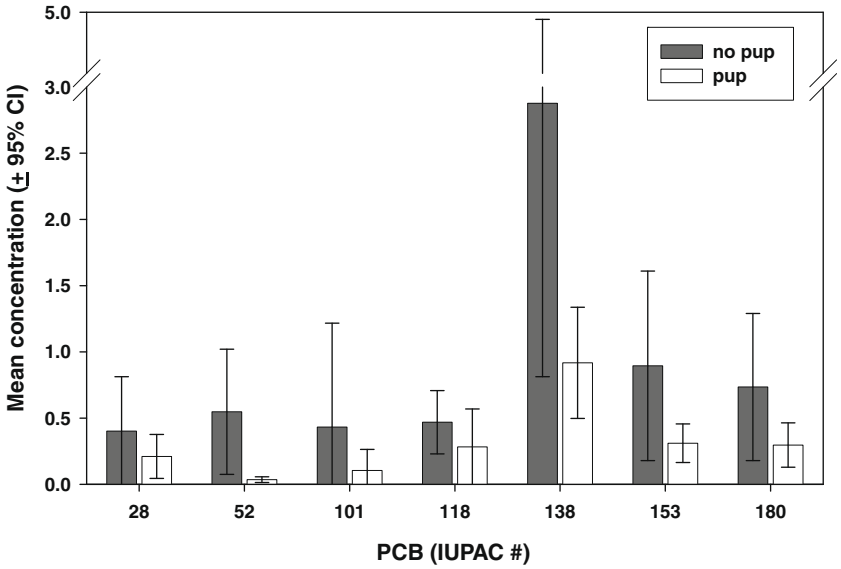

Fig. 4. Arithmetic mean concentrations (ng/g, wet weight) and 95% confidence intervals for ICES congeners in blood serum samples from sea otters with (grey bars) and without (white bars) a dependent pup, Knight (oiled) and Montague (unoiled) Islands, Prince William Sound, Alaska, 1998.

$\bar{x}$ = 12%, range = 2–30%; unoiled: $\bar{x}$ = 7%, range = 2–14%) and harlequin ducks (oiled: $\bar{x}$ = 17%, range = 4–43%; unoiled: $\bar{x}$ = 15%, range = 4–37%). IUPAC 138 accounted for a large percentage of the detected CYP congeners in sea otters (oiled: $\bar{x}$ = 70%, range = 26–100%; unoiled: $\bar{x}$ = 46%, range = 15–70%) and nearly all in harlequin ducks (oiled: $\bar{x}$ = 100%, unoiled: $\bar{x}$ = 99%, range = 89–100%). The lighter mono- and di-chlorobiphenyls comprised notable but variable percentages of Σ PCBs in sea otters (oiled: $\bar{x}$ = 13%, range = 0–54%; unoiled: $\bar{x}$ = 18%, range = 7–18%) and harlequin ducks (oiled: $\bar{x}$ = 6%, range = 0–35%; unoiled: $\bar{x}$ = 9%, range = 0–31%).

Within the CYP congener group, the most dioxin-like non-*ortho* IUPACs 77, 126, and 169 were not detected in any sea otter or harlequin duck samples (Table 1). The non-*ortho* but less toxic IUPAC 81 was detected only in harlequin ducks, where geometric mean concentrations were 2.3 times greater in harlequin ducks from the oiled area. All but two mono-*ortho* congeners (IUPACs 114 and 156) were detected in at least one sea otter sample from both areas. Geometric mean concentrations were typically higher in sea otters from the unoiled area; maximum concentrations were <3 ng/g. Similarly, TEQs were nearly three times higher in sea otters from the unoiled area. All di-*ortho* congeners were detected in at least one sea otter sample from the oiled area, and IUPAC 166 was the only congener not detected in any sea otter sample from the unoiled area. Geometric mean concentrations varied inconsistently among areas, with higher concentrations of IUPACs 128 and 170 ($\leqslant$0.43 ng/g) in sea otters from the unoiled area, and equivalent concentrations of IUPAC 158 (ca. 0.4 ng/g) in otters from both areas. In contrast, no mono-*ortho* congeners were detected in any harlequin duck sample from the oiled area while IUPAC 156 and 189 were the only mono-*ortho* congeners detected in ducks from the unoiled area; maximum concentrations were <1 ng/g. With the exception of IUPAC 166, which was only detected in harlequin ducks from the oiled area, di-*ortho* congeners other than IUPAC 138 were not detected in any harlequin duck sample.

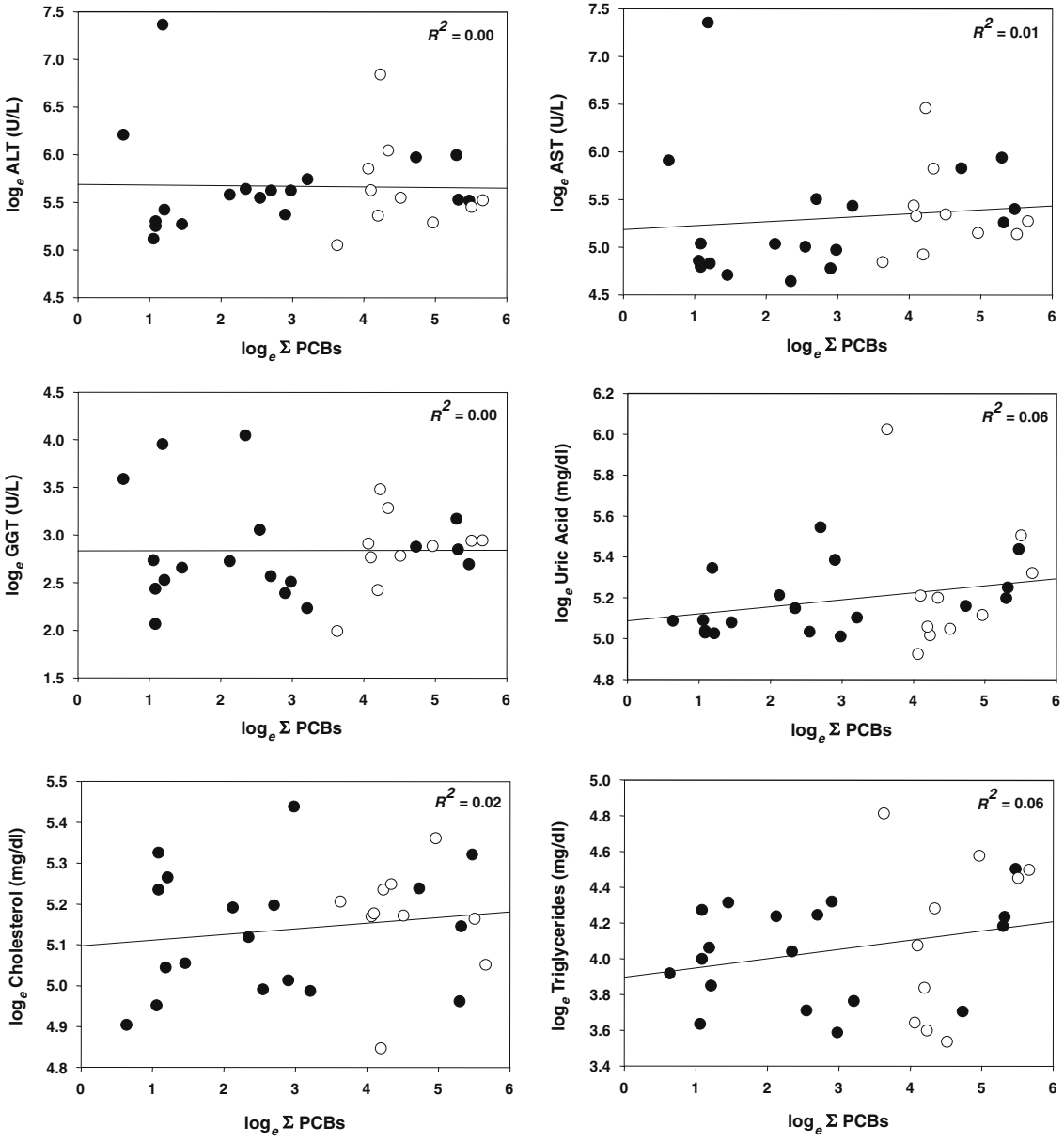

Fig. 5. Partial residual plots illustrating weak relationships between the sum of 93 congeners (Σ PCBs, ng/g wet weight) detected in blood serum and six blood serum analytes after accounting for area, age, and dependency effects for sea otters inhabiting an oiled (closed circles) and unoiled (open circles) area of Prince William Sound, Alaska, 1998. Data on the *x* and *y* axes are $\log_e$ transformed, note variable *y*-axis scales.

Within the ICES congener group, sea otters from the unoiled area had the highest geometric mean concentration of IUPAC 180 (0.37 ng/g), and the maximum concentration for any congener was 6.3 ng/g for IUPAC 101 at the oiled area (Table 1). Harlequin ducks had the highest geometric mean concentration of IUPAC 180 (0.4 ng/g) at the oiled area, and the maximum concentration for any congener was 3.9 ng/g for IUPAC 153 at the oiled area. Total PCBs based on aroclor standards were detected in 32% of sea otter samples but not in any harlequin duck sample (Trust et al., 2000). Geometric mean concentrations were slightly higher in sea otters from the unoiled area but ranges from both areas overlapped each other (Table 1).

Two principal components accounted for 59% of the cumulative variation in proportional concentrations among PCB homolog groups (Fig. 2). Most variation (42%) was accounted for by axis 1, which correlated negatively with mono- and di-chlorobiphenyls ($r < -0.42$) and positively with the higher chlorinated hexa-, hepta-, and octa- chlorobiphenyls ($r > 0.63$). In contrast, axis 2 (17%) correlated positively with nona- and deca-chlorobiphenyls ($r = 0.64$) and negatively with tetra-chlorobiphenyls ($r = -0.70$). Sea otters from the unoiled area generally had higher proportional concentrations of mono- and di-chlorobiphenyls, as indicated by most samples grouping along the left side of axis 1. In contrast, sea otters from the oiled area aligned along the entire axis length and harlequin ducks from both areas were scattered throughout the ordination space. Coordinate scores from the PCA were marginally different for sea otters ($A = 0.08$, $P = 0.01$) but did not differ for harlequin ducks ($A = -0.01$, $P = 0.48$) by area.

Significant area * species interactions ($F_{[1,44]} \geqslant 4.7$, $P \leqslant 0.04$) confounded interpretation of main effects on Σ PCBs and CYP congeners, so differences between areas were tested separately for each species. Concentrations of these two congener groups were significantly higher in sea otters from the unoiled area ($F_{[1,26]} \geqslant 10.9$, $P \leqslant 0.003$) but did not differ in harlequin ducks by area ($F_{[1,18]} \leqslant 0.84$, $P \geqslant 0.37$) (Fig. 3). Geometric mean concentrations of Σ PCBs and CYP congeners in sea otters from the unoiled area were 91.9 and 5.0 ng/g compared to 13.9 and 1.5 ng/g in sea otters from the oiled area, respectively. Although the significant area * species interactions precluded statistical comparisons in the same model, PCB concentrations in sea otters from the oiled area were notably comparable to harlequin ducks from both areas as evidenced by overlapping 95% confidence intervals. In contrast, concentrations of Σ ICES congeners and IUPAC 138 did not differ by species ($F_{[1,45]} \leqslant 2.6$, $P \geqslant 0.11$) or area ($F_{[1,45]} \leqslant 3.0$, $P \geqslant 0.09$) (Fig. 3).

3.3. Additional sources of PCB variation in sea otters

After accounting for area effects among sea otters, animal age and dependency status did not explain significant variation in concentrations of Σ PCBs (age: $F_{[1,24]} = 0.03$, $P = 0.60$, status: $F_{[1,24]} = 0.6$, $P = 0.45$), CYP congeners (age: $F_{[1,24]} = 0.02$, $P = 0.90$, status: $F_{[1,24]} = 2.2$, $P = 0.16$), or IUPAC 138 (age: $F_{[1,24]} = 0.06$, $P = 0.81$, status: $F_{[1,24]} = 3.2$, $P = 0.09$). While age had no effect on concentrations of Σ ICES congeners (age: $F_{[1,24]} = 0.08$, $P = 0.78$), concentrations were significantly lower ($F_{[1,24]} = 6.8$, $P = 0.02$) in otters with dependent pups (lsmean = 1.88 ng/g, 95% CI: 1.14–3.08) compared to otters without pups (lsmean = 4.17 ng/g, 95% CI: 2.78–6.26). Mean concentrations of all congeners comprising the ICES group were consistently lower in sea otters with dependent pups (Fig. 4). The homogeneity of slopes assumption was met for all models since interactions for age * dependency status and age * area were insignificant ($F_{[1,27]} \leqslant 3.4$, $P \geqslant 0.08$) among all four groups of congeners. Interpretation of dependency status was not likely confounded by gender because concentrations of the four groups of congeners did not differ between males ($n = 4$) and females without dependent pups ($n = 12$) ($t_{[14]} \leqslant 1.4$, $P \geqslant 0.18$).

After accounting for area, age, and dependency effects, concentrations of all four-congener groups were unrelated to blood serum levels of ALT ($F_{[1,23]} \leqslant 0.3$, $P \geqslant 0.6$), AST ($F_{[1,23]} \leqslant 0.9$, $P \geqslant 0.4$), GGT ($F_{[1,23]} \leqslant 0.4$, $P \geqslant 0.6$), uric acid ($F_{[1,23]} \leqslant 2.8$, $P \geqslant 0.1$), and triglycerides ($F_{[1,23]} \leqslant 1.7$, $P \geqslant 0.2$). Only relations between PCBs and cholesterol were confounded by significant dependency status * PCB group interactions ($F_{[1,20]} \geqslant 5.3$, $P \leqslant 0.03$). Thus, relations were examined by dependency status separately, and cholesterol remained unrelated to concentrations of all PCB groups in sea otters with ($F_{[1,8]} \leqslant 3.5$, $P \geqslant 0.1$) and without ($F_{[1,12]} \leqslant 0.8$, $P \geqslant 0.4$) pups. Partial residual plots for Σ PCBs against the six blood serum biochemical analytes illustrate no associations (Fig. 5).

4. Discussion

4.1. Relevance of PCBs to sea otter and harlequin duck recovery

PCBs have been implicated as contributing factors in the declines of several populations of aquatic mustelids such as Eurasian otters (Kannan et al., 2000), North American river otters (*Lontra canadensis*), and mink (Elliott et al., 1999). Birds can be susceptible to reproductive impairment from exposure to PCBs (particularly dioxin-like congeners), yet acute and chronic PCB effects are variable among taxa (Hoffman et al., 1996; Rice et al., 2003). While our results could not be directly related to studies that used different tissue matrices (typically liver), concentration bases (wet vs. lipid), or PCB quantification techniques, ample evidence indicated that PCBs did not constrain sea otter population recovery post-EVOS and substantiated the conclusions of Trust et al. (2000) that discounted PCB effects on harlequin duck recovery.

First, we failed to detect highly toxic non-*ortho* congeners (IUPACs 77, 126, and 169) with a high affinity to *Ah*-receptor sites for CYP induction. The absolute toxicological interpretability of this result is limited because advanced analytical chemistry techniques (e.g., EPA method 1668a) capable of detecting these planar congeners at very low limits of detection (ca. 0.1 pg/g) were not readily available from USFWS-ACF laboratories at the time of our study. However, toxicologically relevant mono-*ortho* congeners that have a greater *Ah* agonist ability than di-*ortho* congeners were detected at low concentrations (maximum concentration < 3 ng/g) in sea otters and rarely detected in harlequin ducks. Mono-*ortho* TEQs in sea otters in our study were similar to those determined for sea otters in southeast Alaska and remained markedly lower than those determined for sea otters inhabiting point source contaminated habitats in the Aleutian archipelago (Bacon et al., 1999). Although the non-*ortho* IUPAC 81 comprised a rather high percentage of Σ PCBs in harlequin ducks, De Voogt et al. (1993) suggested that CYP induction by this congener was considerably less than the other planar congeners. Furthermore, a recent study that found no relationship between PCBs and CYP1A induction in harlequin ducks and Steller's eiders (*Polysticta stelleri*) from southwest Alaska (Miles et al., 2007) corroborated the lack of CYP induction by PCBs in harlequin ducks reported by Trust et al. (2000). The Miles et al. (2007) study documented concentrations of Σ PCBs in harlequin ducks similar to those in our study, which included detection of non-*ortho* congeners 77 and 169 at relatively low concentrations (0.11–2.9 ng/g).

Second, interpretation of PCB effects on PWS sea otters depends on the assumption that PCBs in blood serum or plasma are representative of other tissue burdens, and is hampered by the lack of established blood based PCB toxicity thresholds for sea otters. Plasma PCB concentrations in hooded seals (*Cistophora cristata*) represented recent dietary or lipid mobilized exposure, and correlated poorly with PCBs sequestrated over time in liver and blubber (Wolkers et al., 2006). However, Murk et al. (1998) reported a strong

correlation between liver and blood plasma PCB TEQs in Eurasian otters that lack a blubber layer. With these caveats in mind, sea otters from the oiled area probably had consistently low PCB risk while risk for sea otters from the unoiled area seemed mixed. For example, Kannan et al. (2000) suggested a conservative lowest observerable adverse effects level (LOAEL) for Σ PCBs in marine mammal liver or blood of 8.7 μg/g lipid weight. In contrast, Murk et al. (1998) suggested a no observed effect concentration (NOEC) of 4.0 μg/g lipid weight for Σ ICES congeners in liver or blood plasma based on the lack of associated suppression of hepatic vitamin A levels in Eurasian otters. If we conservatively assume an average serum lipid concentration of 0.5%, approximate lipid weight concentrations of Σ PCBs and ICES congeners in sea otters were 2.8 and 0.4 μg/g from the oiled area, and 18.4 and 0.9 μg/g from the unoiled area, respectively. The Kannan et al. (2000) threshold indicated potential negative effects from PCBs in sea otters from the unoiled area and low risk in those from the oiled area. Kannan et al. (2008) also reported that livers from a small sample of sea otters ($n = 3$) from PWS during a similar time period had Σ PCBs exceeding the 8.7 μg/g lipid weight threshold. However, comparison to the Murk et al. (1998) threshold indicated low risk to both oiled and unoiled populations; in fact the upper 95% confidence intervals for Σ ICES congeners in sea otters from the oiled (0.6 μg/g lipid weight) and the unoiled area (1.6 μg/g lipid weight) were 6.6 and 2.5 times lower than the NOEC for hepatic vitamin A suppression, respectively. Comparisons to toxicity thresholds developed for mink, a species highly sensitive to PCBs, also suggest low PCB risk to sea otters. Heaton et al. (1995) reported a reproductive NOAEL and LOAEL for Σ PCBs based on aroclor standards of 0.06 and 2.19 μg/g wet weight, respectively, while Leonards et al. (1995) reported a median reproductive effects level (EC_{50}) for congener specific Σ PCBs of 1.2 μg/g wet weight. The maximum aroclor based Σ PCB concentration in our study (0.04 μg/g) was less than the mink NOAEL and over 50 times lower than the mink LOAEL. Mean concentrations and upper 95% confidence intervals for congener based Σ PCBs were below the mink EC_{50} for both otter populations.

Third, PCB immunotoxicity in sea otters may be independent of the non-*ortho* congener pattern. An *in vitro* study by Levin et al. (2007) using blood from southern sea otters reported significant reductions in monocyte phagocytosis using wet weight 10 μg/g doses of IUPACs 138 + 153 and IUPACs 153 + 180, and 20 μg/g doses of IUPACs 138 + 153 + 169 + 180. They deduced that this reduction could increase susceptibility to infectious pathogens. The summed maximum concentrations of these congeners in our study (0.022 μg/g) were nearly three orders of magnitude lower than the 20 μg/g dose. In related studies, liver Σ PCB concentrations of 2.4 μg/g (wet weight; Nakata et al., 1998) and 17.0 μg/g (lipid weight; Kannan et al., 2007) were associated with death from infectious disease in southern sea otters. The lipid weight average (18.4 μg/g) and associated upper 95% confidence interval (33.0 μg/g) for Σ PCB concentrations in sea otters from the unoiled area in our study exceeded the detrimental level suggested by Kannan et al. (2007), but the wet weight upper 95% confidence interval (1.7 μg/g) fell below the Nakata et al. (1998) concentration. In addition, maximum Σ PCB concentrations in sea otters from the oiled area (upper 95% confidence interval 0.025 μg/g wet weight, 4.9 μg/g lipid weight) were well below suggested detrimental levels. Sea otters suffering from parasitic infection or depressed nutritional condition can also have elevated levels of blood serum biochemical indices compounded by exposure to xenobiotic contaminants (Hanni et al., 2003). Comparing these indices among individuals without baseline controls (Mazet et al., 2000) requires the assumption that any unexplained variation in blood serum chemistry induced by capture stress or nutritional status is similar for all individuals. Thus, we did not directly compare levels of blood serum analytes among different groups of sea otters. However, blood serum analytes were unrelated to concentrations of all four-congener groups measured in our study when controlling for variation due to area, age, and dependency status. Little evidence existed to suggest that PCBs exerted immunotoxic or physiological effects on sea otters in our study.

Finally, PCB patterns do not match measures of population performance during the period of study. Demographic attributes of harlequin ducks (Esler et al., 2002) and sea otters (Bodkin et al., 2002) remained depressed at oiled areas when compared to unoiled areas. Our reevaluation of PCBs in harlequin ducks failed to detect significant differences in concentrations or composition between the oiled and unoiled area. Even if we use conservative toxicity thresholds for sea otters that assume PCBs blood based concentrations are somewhat isometric with PCB burdens in other tissue matrices, higher concentrations of Σ PCBs and CYP congeners at Montague Island and statistically equivalent concentrations of Σ ICES and IUPAC 138 between the oiled and unoiled area simply did not correspond to estimated higher sea otter abundances and survival at Montague Island compared to Knight Island.

4.2. Local or distant sources of PCB exposure

Patterns of PCB composition in sea otters and harlequin ducks did not suggest recent exposure to persistent PCBs but rather exposure to distantly derived weathered PCBs or biotransformation into metabolic products. Congener patterns in consumers typically differ from those in their prey and the original parent formulation, whereby metabolism or bioaccumulation of PCBs largely depends on the molecular structure of a particular congener and species-specific pharmacokinetic abilities (e.g., Boon et al., 1997). As a generalization, lower chlorinated PCBs are less recalcitrant whereas more highly chlorinated congeners IUPACs 118, 138, 153, and 180 (which partially comprise the ICES group) bioaccumulate strongly in marine food webs (De Voogt et al., 1993). High and invariant proportions of recalcitrant congeners did not characterize PCB composition in sea otters in our study. In fact, IUPAC 153 (considered one of the most recalcitrant PCBs) was not detected in all sea otter samples from either area. Furthermore, fewer congeners were detected in harlequin ducks relative to sea otters, and the lack of separation for harlequin ducks along homolog gradients indicated distant source exposure. In contrast, sea otters (Bacon et al., 1999), bald eagles (*Haliaeetus leucocephalus*) (Anthony et al., 2007), glaucous-winged gulls (*Larus glaucescens*) (Ricca et al., 2008), and coastal fish (Miles et al., 2009) near former military installations in the Aleutian archipelago of Alaska with known PCB contamination harbored higher proportions of more highly chlorinated or recalcitrant PCB congeners.

Studies of contamination in abiotic and lower trophic level media provided additional evidence for low availability of point source PCBs in PWS food webs. For example, concentrations of Σ PCBs in PWS ambient seawater during 2004 were too low to induce CYP through aquatic bioconcentration, although this inference did not extend to bioaccumlative exposure from ingesting contaminated prey (Short et al., 2008). Lower chlorinated congeners indicative of atmospheric deposition dominated PCB composition in seawater (Short et al., 2008), which was similar to the pattern of PCB composition in sea otters and harlequin ducks from our study. Concentrations of Σ PCBs in sediments and benthic fish from PWS are also likely low. Sediments and flathead sole (*Hippoglossoides elassodon*) sampled at the Port of Valdez during the mid to late 1980s had PCB concentrations in the 10th and 20th percentiles, respectively, relative to samples from Dutch Harbor, Alaska and the coasts of Washington, Oregon, and California (Brown et al., 1998).

4.3. Inter- and intra-species variation in PCB exposure

Inter-specific differences in PCB exposure may have been expected owing to different foraging strategies, yet why did sea otters from the oiled area have concentrations of PCBs similar to those found in harlequin ducks from both areas? Moreover, why were sea otters from the unoiled area exposed to different PCB mixtures characterized by a higher relative percentage of lower chlorinated congeners compared to harlequin ducks from both areas and sea otters from the oiled area? The reasons for these patterns are unclear but variation in foraging strategies and prey availability provides an interesting and plausible explanation. Sea otters can display a high degree of individual dietary specialization in response to limited food resources, particularly at high population densities (Tinker et al., 2008), which in turn could yield variable exposure to PCB mixtures among individuals and local populations. For example, southern sea otters (*Enhydra lutris neries*) incur an increased risk of exposure to disease inducing pathogens when specializing on non-preferred prey (Johnson et al., 2009). Estimates of otter abundance from Montague Island were approximately 2–5 times greater during the 1990s compared to Knight Island (Bodkin et al., 2002). Corresponding density dependent responses acted ostensibly through higher sea otter prey availability and energy consumption rates at Knight Island compared to Montague Island where sea otters foraged on more limited prey that included smaller and more diverse species of bivalves (Dean et al., 2002). These demographic and dietary patterns may help explain the different patterns of PCB composition and concentrations in sea otters between areas observed in our study. Conversely, the generalist foraging strategy employed by harlequin ducks to meet metabolic demands during winter (Goudie and Ankey, 1986) and optimize mass before spring migration (Bond and Esler, 2006) coupled with similar prey availabilities at Montague and Knight Islands during our study (Esler et al., 2002) may explain overlapping patterns of PCB exposure in harlequin ducks.

Other explanations for areal differences in PCBs among sea otters include variable PCB deposition, and differences in migration mediated contaminant transport possibly coupled with associated anthropogenic activities. First, Knight Island is more protected from the Gulf of Alaska within PWS. In contrast, Montague Island is directly exposed to the Gulf of Alaska, and waters from the Gulf enter and then exit PWS through the pass between Montague and Hitchenbrook Islands (Weingartner, 2007). Thus, the physical juxtaposition of Montague Island may confer a higher probability of oceanic deposition of lower chlorinated PCBs and help explain higher PCB concentrations in sea otters captured near the northern exposed end of the Island. Second, commercial fishing operations target spawning migratory Pacific herring (*Clupea pallasi*) that are substantially more abundant at Montague than at Knight Island (Mundy, 2005). In addition, chum salmon (*Oncorhynchus keta*) aquaculture occurs at Montague Island, whereby smolts are reared originally in pens from spring to early summer and returning adults are targeted for harvest by a commercial fleet (J.L. Bodkin, *personal observation*). Elevated levels of PCBs can occur in commercial salmon feed (Carlson and Hites, 2005), thus commercial feed used for chum salmon aquaculture could contaminate the Montague Island food web with PCBs to a greater extent than at Knight Island. Perhaps more importantly, however, is that bulk transport of distantly derived PCBs to local food webs is facilitated by the high lipid content and migratory nature of salmon (Krümmel et al., 2003) and herring (West et al., 2008). The prevalence of lower chlorinated PCBs in sea otters from Montague Island where biomasses of returning salmon and herring are much higher than at Knight Island provides more support for animal migration mediated PCB exposure than direct point source input associated with commercial fishing and aquaculture.

Some evidence indicates that mammals are less effective at metabolizing and excreting PCBs than ducks (Hoffman et al., 1996; Kamrin and Ringer, 1996; Rice et al., 2003), which may account for the differences observed between areas for sea otters but not harlequin ducks. In addition, sea otters are year-round residents at both Islands, whereas harlequin ducks only reside in nearshore habitats from late fall to early spring before leaving for terrestrial biomes to breed (Robertson and Goudie, 1999). Sea otters could then accumulate contaminants derived from PWS food webs over a longer time-interval than harlequin ducks. However, we measured PCBs in blood for both species and although concentrations in blood plasma samples can reflect mobilized PCB previously sequestered in lipids, they also represent short-term exposure (Wolkers et al., 2006).

4.4. Sea otter age and pup dependency effects on PCBs

Lactation is a primary mode of PCB depuration in marine mammals (Addison and Brodie, 1987; Wolkers et al., 2006; Neale et al., 2009), yet pup dependency status could not explain higher concentrations of Σ PCBs, CYP congeners, and IUPAC 138 in sea otters from Montague Island compared to Knight Island. However, sea otters without dependent pups had concentrations of Σ ICES congeners more than twice as high as those with dependent pups. Selective maternal transfer of penta-chlorobiphenyls with lower lipophilicity relative to more lipophilic hepta-chlorobiphenyls has been demonstrated in pinnipeds (Wolkers et al., 2006). In contrast, maternal transfer of all ICES congeners regardless of their molecular weight was inferred in sea otters from our study. A key reason for this discrepancy may again relate to the absence of blubber in sea otters. Pinnipeds largely rely upon a blubber layer whereas sea otters rely upon pelage for endothermy. Thus, the lack of a blubber layer could result in the mobilization of a different suite of bioaccumilative congeners during lactation in sea otters compared to pinnipeds. Age-related differences in PCB accumulation may also occur in marine mammals. Older male marine mammals can accumulate higher PCB concentrations, whereas PCBs in female marine mammals can decrease with age largely due to lactational depuration (Neale et al., 2009). The lack of an age effect among any of the four PCB groups further suggests low long-term exposure to PCBs in PWS, although this result should be viewed cautiously since our dataset was skewed towards younger animals with few males.

4.5. Conclusion

PCBs probably did not contribute to depressed recovery of sea otters and harlequin ducks populations inhabiting oiled areas of PWS 10 years post-EVOS based on the evidence of our study. In particular, while comparisons with some conservative toxicological thresholds indicated potential risk for sea otters occupying unoiled areas, patterns of PCB exposure in this study simply did not match reported measures of site-specific population performance in sea otters and harlequin ducks. Sea otters and harlequin ducks from both areas were probably exposed to weathered PCBs or PCBs of distant origin, with sparse evidence for elevated levels from point sources.

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