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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,

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