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# Persistent organic pollutants in forage fish prey of rhinoceros auklets breeding in Puget Sound and the northern California Current

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## ABSTRACT

Organochlorine contaminants in upper trophic-level consumers inhabiting Puget Sound are consistently higher than in those species inhabiting other west coast locations. We analyzed persistent organic pollutants (POPs) in the six most common fish prey of rhinoceros auklets breeding on Protection Island (Puget Sound), Tatoosh Island (WA coast), and Destruction Island (WA coast). Wet-weight concentrations of POPs ranged widely (PCBs: 1.6-25.0 ng/g; DDTs: 0.2-56.0 ng/g; PBDEs:<LOQ-49.0 ng/g), but overall patterns showed fish from Puget Sound were 2-4 times more contaminated and had similar contaminant profiles compared to fish from the outer coast. Unexpectedly elevated PCB and PBDE concentrations in Chinook salmon from the outer coast likely reflected Columbia River influences. Calculating contaminant loads for auklet nestlings magnified differences observed between inland and outer coast fish prey. Monitoring of breeding auklets, their prey and other resident marine birds is needed to assess biomagnification impacts in the Puget Sound marine ecosystem.

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## 1. Introduction

Persistent organic pollutants (POPs) have been documented in disparate aquatic ecosystems throughout the world as a consequence of their wide-spread usage, long-range transport, and recalcitrance to metabolism (Strandberg et al., 1998; Muir et al., 1999; Ross et al., 2009). This class of pollutants includes a variety of industrial compounds, organochlorine pesticides, and flame retardants, including polychlorinated biphenyls (PCBs), dichlorodiphenyl-trichloroethan (DDT), polybrominated diphenyl ethers (PBDEs), chlordanes, hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB). Fat soluble and not readily degradable in the environment, POPs can occur in high concentrations in individuals via bioaccumulation and in food webs via biomagnification.

(G.M. Ylitalo).

Persistent organic pollutants are a cause for concern for nearshore marine ecosystems already threatened by a variety of human activities and pressures. In Puget Sound, these include pollution, overharvest of fishery stocks, the introduction of non-native and invasive species, climate change, and habitat loss and degradation associated with development and regional population expansion (Fresh et al., 2011). These impacts have led to changes in food web dynamics, an increase in hypoxic "dead zones", declines in pelagic fish populations such as Pacific herring (Clupea harengus pallasi; Stick and Lindquist, 2009), and many threatened and endangered marine species (Pearson et al., 2010).

Signs of ecosystem deterioration in the form of increasing levels and types of persistent organic pollutants have already been detected in a variety of Puget Sound marine organisms. At key middle trophic levels, Pacific herring are at least three times more contaminated with PCBs in Puget Sound than in the Strait of Georgia (West et al. 2008). Studies of juvenile salmonids in West Coast estuaries found high levels of PCBs and DDTs in the more urban estuaries, including Puget Sound (Johnson et al., 2007a, 2007b). At higher trophic levels, studies of adult and subadult salmonids have shown high concentrations in Chinook salmon (Onchorynchus

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tschawytscha; O'Neill and West, 2009; Cullon et al., 2009). Residency in the contaminated Puget Sound environment was likely a major factor contributing to the higher and more variable PCB concentrations in these fish (O'Neill and West, 2009). Higher trophic level harbor seal (*Phoca vitulina*) pups from Puget Sound, WA had PCB levels seven times that of pups from the Strait of Georgia, BC (Ross et al., 2004), and recreating and analyzing their diets for both locations using documented dietary preferences (a food basket approach) corroborated the PCB differences as well as the dietary source of the contaminants (Cullon et al., 2005).

At the top of the food web, killer whales (Orcinus orca) that spend time in Puget Sound (fish-eating Southern Resident killer whales and marine mammal-consuming transient killer whales) are among the most contaminated marine mammals in the world, with relatively high levels of PCBs and PBDEs found in individuals throughout the Puget Sound and Georgia Basin (Ross et al., 2000: Ross, 2006; Krahn et al., 2007, 2009). Concentrations of some POPs in harbor seal pups from a southern Puget Sound site have declined since the mid-1980s (Ross et al. 2013), and PBDE concentrations in great blue heron (Ardea herodias) and double-crested cormorant (Phalacrocorax auritus) eggs have declined after peaking in the mid-1990s (Elliott et al., 2005). These hopeful signs of reduced inputs into nearshore systems notwithstanding, Puget Sound continues to represent a regional PCB "hotspot," and movement of persistent organic pollutants through the food web may be extensive (Ross, 2006).

The forage fish guild in the Puget Sound food web is dominated by Pacific herring, Pacific sandlance (*Ammodytes hexapterus*), surf smelt (*Hypomesus pretiosus*) and juvenile *Oncorhynchus* salmonids (Rice et al. 2012), and these species are likely important vectors for POPs to upper trophic level taxa such as piscivorous fish, marine mammals, and seabirds (West et al., 2008). Pacific herring have been analyzed from sites throughout Puget Sound and Georgia Strait (West et al., 2008), but there is a need for broader foodweb based toxics research. While a food basket approach (Cullon et al., 2005) has provided information on contaminant loads associated with composite samples representing the diets of harbor seals, no regional studies have captured and analyzed captured prey of upper trophic level predators.

Seabird diet, in addition to tracking foraging patterns of the seabird themselves, has been used to indicate fish population status and fishery recruitment (Bertram et al., 2005; Thayer and Sydeman, 2007). Seabirds foraging in nearshore waters also experience a suite of environmental stressors, and many responses to these stressors (e.g., mortality, body condition, disease/parasites, pollutants) may serve as indicators of overall ecosystem health (Mallory et al., 2010). Being central place foragers, breeding seabirds are generally constrained to areas close to their colonies, thus seabirds breeding in inland waters may be particularly vulnerable to local stressors including contaminants. Tracking seabird diet can also enhance our understanding of trophic position and contaminant monitoring, as well as potential bioaccumulation and biomagnification effects in the food web (Hebert and Weseloh, 2006; Jarman et al., 2007).

Rhinoceros auklets (*Cerorhinca monocerata*) are medium-sized members of the Family Alcidae. Most of the North American population breeds in underground burrows on a small number of islands in British Columbia, Washington State and southeast Alaska, although some colonies exist as far south as California (*Gaston and Dechesne*, 1996). In Washington, auklets breed on rocky islands on the outer Washington coast and on undeveloped islands in the otherwise developed Puget Sound's inland waters (*Speich and Wahl*, 1989; *Pearson et al.*, 2013). These birds capture prey through wing-propelled pursuit-diving, feeding mainly on schooling fishes in nearshore areas (*Thayer and Sydeman*, 2007). During breeding, the foraging distance of rhinoceros auklets at

colonies ranges from  $\sim$ 40 km in inland Washington waters up to 80–90 km on the outer Washington coast (Wahl and Speich 1994). After chicks hatch, each adult brings back one load (1–30 fish)/night crosswise in their bill for approximately 50 days until the chicks fledge (Wilson, 1977).

Research on the diet of rhinoceros auklets in Washington State has indicated that fish prey delivered to chicks varies considerably among three distinct study locations (Pearson et al. unpubl.). In Puget Sound, rhinoceros auklets breeding on Protection Island show a diet relying on few prey species, primarily Pacific sandlance (76% by weight) and Pacific herring (16% by weight). Auklets breeding on the outer Washington coast on Destruction Island show a reliance on northern anchovy (Engraulis mordax; 48% by weight), smelt (Osmerus spp. 30% by weight), and rockfish (Sebastes spp. (12% by weight), with much lower reliance on Pacific sandlance (2% by weight) and Pacific herring (2% by weight). Auklets breeding on Tatoosh Island on the Washington coast at the tip of the Olympic Peninsula showed the most variable diet delivered to chicks, including Pacific sandlance (45% by weight), Pacific herring (15% by weight), rockfish (10% by weight), Pacific saury (Cololabis saira; 7% by weight), smelt (7% by weight), and salmonids

Based on concentrations of contaminants detected in other species in the Puget Sound food web, we hypothesized that patterns of persistent organic pollutants in the prey of rhinoceros auklet chicks would differ among breeding colony locations. Specifically, we predicted that fish prey from the inland Washington water colony in Puget Sound would have greater mean concentrations of persistent organic pollutants than fish prey from study colonies on the outer Washington coast (Tatoosh Island, Destruction Island). Moreover, we predicted that the overall calculated contaminant intake of rhinoceros auklet chicks (via prey delivered by provisioning adults) would be greater in Puget Sound than on the outer Washington coast. Specifically, we examined the following questions: (1) Do contaminant levels (concentrations of POPs) of fish prey differ among rhinoceros auklets breeding colonies on Washington's outer coast and inland marine waters? (2) Do contaminant levels (concentrations of POPs) of fish prev of rhinoceros auklets breeding on Washington's outer coast and inland marine waters differ among prey species? (3) Do calculated contaminant burdens (based on observed diet differences and using a quasi-food basket approach) differ among observed chick diets on breeding colonies?

## 2. Materials and methods

## 2.1. Study sites

The study colonies were located in the inland waters of Puget Sound (Protection Island) and the outer coast of Washington state (Tatoosh Island; Destruction Island; Fig. 1). Protection Island (48°08′N, 122°55′W) is a 143-ha island located 3.2 km off the mouth of Discovery Bay at the eastern end of the Strait of Juan de Fuca. Approximately 36,000 rhinoceros auklet pairs breed on its grass-dominated habitats on slopes and cliff edges (Pearson et al., 2013). Tatoosh Island (48°24′N, 124°44′W), is a 6-ha complex of flat-topped rocky islets located 0.6 km off the northwest tip of the Olympic Peninsula. Recent monitoring suggests at least 200 rhinoceros auklet pairs have burrows on the island's cliff top grass and shrub habitats (Pearson et al., 2013). Destruction Island (47°40′N, 124°24′W) is a 15-ha island located 4.8 km west of the Olympic Peninsula and 29 km south-southeast of La Push. An estimated 6500 rhinoceros auklet pairs breed in grass, shrub and willow habitats on cliff tops and the island's steep slopes (Pearson et al., 2013).

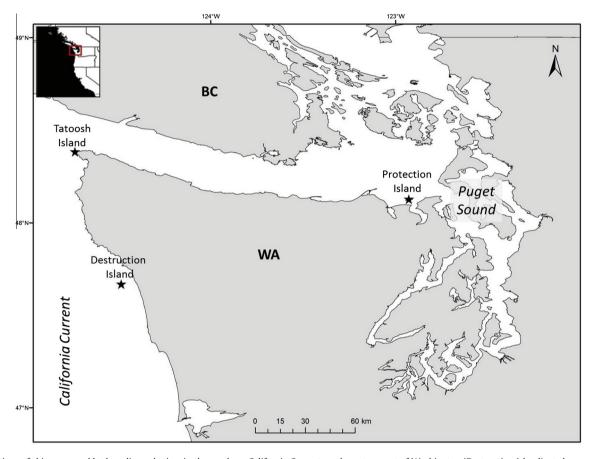


Fig. 1. Locations of rhinoceros auklet breeding colonies: in the northern California Current on the outer coast of Washington (Destruction Island), at the confluence of the northern California Current and the Strait of Juan de Fuca (Tatoosh Island), and in Washington's inland marine waters of Puget Sound (Protection Island).

#### 2.2. Sample collection

We obtained bill loads of fish prey from rhinoceros auklet adults as they returned to the colony to feed chicks during the chick provisioning period. On Protection and Destruction Islands, we collected fish prey samples by spot-lighting returning individuals; this method involves sitting in the middle of the nesting colony beginning shortly after dusk (usually from 2130 h to 2400 h) and shining a headlamp beam onto adult auklets that landed nearby. When spotlighted, birds usually drop their bill loads of prey after freezing in their tracks or running or flying away. On Tatoosh Island, we collected fish prey using a combination of spot-lighting and burrow screening. The latter involves placing a screen barrier at the burrow entrance around dusk; this screen generally resulted in adults dropping their bill loads, which we collected within a few hours. Collection trips (three/season and 1-2 nights/trip) were spaced throughout the chick rearing period for both Protection (2006-2010) and Destruction (2008-2010) islands, adjusting for differences in phenology between islands (Pearson et al. unpubl.). These trips were categorized as early (Destruction Island: 23 June-8 July; Protection Island: 29 June-16 July), middle (DI: 14-21 July; PI: 14-29 July), and late (DI: 26 July-11 August; PI: 30 July-13 August). We controlled for phenology to account for differences in prey size and composition during early and late provisioning (e.g., Bertram and Kaiser, 1993, Hedd et al., 2006). Tatoosh Island collection trips (2006-2010) generally comported with the same schedule, but related research on the island afforded us more opportunities for sampling and thus greater breadth in the overall sampling period (early: 23 June-11 July; mid: 14-24 July; late: 26 July-17 August). We varied collection locations at each colony within and between trips to minimize effects on individual breeding pairs.

## 2.3. Sample processing

We placed bill load samples in individual plastic Ziploc® bags, which were immediately labeled. Bagged samples were stored in the field in an ice-filled cooler, transferred to freezers at the University of Washington upon returning to Seattle, and thawed in the lab prior to identification and measurement. We identified individual fish, measured their mass (wet weight) to the nearest 0.1 gram on an Ohaus portable electronic balance, and measured standard, fork and total length to the nearest millimeter; we recorded data in the lab within a week of collection. Prey samples were identified to the lowest possible taxon; in most cases, this was to species, but juvenile rockfish, salmonids, and smelt were sometimes identified only to genus. A number of larval smelt samples, which could not be positively identified, were analyzed by the Northwest Fisheries Science Center's Molecular Genetics Team for species determinations. A number of salmonid fin clips were sent to the Washington Department of Fish and Wildlife Molecular Genetics Laboratory to verify species identification. For contaminant analysis, we selected the seven most common prey species (n = overall sample sizes): Pacific sandlance (n = 11), Pacific herring (n = 12), northern anchovy (n = 12), surf smelt (n = 10), Chinook salmon (n = 16), chum salmon (Oncorhynchus keta; n = 16), and rockfish spp. (n = 8). Rockfish were the only prey type not collected from all three colonies (did not appear in the observed diet on the Protection Island colony); the remaining six species were collected 4

from all three colonies during sampling trips from 2007 to 2009. Standard lengths for each species/site pair are presented in Table 1.

## 2.4. Analyses of rhinoceros auklet prey for persistent organic pollutants

Prey samples of rhinoceros auklets were homogenized, extracted and analyzed for POPs using the gas chromatography/ mass spectrometry method of Sloan et al. (2005). Fish whole body samples (1–2 g) were extracted with methylene chloride using an accelerated solvent extractor after the addition of a surrogate standard (PCB 103; 75 ng). This procedure was followed by a clean-up step of the extract on a single stacked, gravity flow silica gel/alumina column to remove any highly polar compounds present in the sample. Using high-performance size exclusion liquid chromatography, the POPs were separated from the bulk lipid and other biogenic material present in each sample, and the cleaned extract was analyzed for POPs using a low-resolution quadrupole GC/MS system equipped with a 60 m DB-5 GC capillary column and a electron impact mass spectrometer in selected ion monitoring mode. The instrument was calibrated using sets of up to ten multi-level calibration standards of known concentrations.

We measured PCBs (polychlorinated biphenyls), DDTs (dichloro-diphenyl-trichloroethane and its metabolites), PBDEs (polybrominated diphenyl ethers), chlordanes (chlordanes, oxychlordane, heptachlors, nonachlor), HCHs (hexachlorocyclohexanes), and HCB (hexachlorobenzene) in the prey samples. Summed PCBs were calculated by adding concentrations of 40 PCB congeners (IUPAC numbers 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 138, 149, 151, 153, 156, 158, 170, 171, 177, 180, 183, 187, 191, 194, 195, 199, 205, 206, 208, and 209). Summed DDTs were calculated by summing concentrations of o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDT, and p,p'-DDT. Summed PBDEs were calculated by adding the concentrations of 15 PBDE congeners, including PBDEs 28, 47, 49, 66, 85, 99, 100, 153, 154, 155 and 183. Lower limits of quantitation (LOOs) of prev samples for individual PCBs and chlorinated pesticides ranged from 0.027 to 0.46 ng/g, wet weight: LOO values for individual PBDE congeners ranged from 0.23 ng/g to 2.6 ng/g.

Total non-volatile extractables (percent lipid) values were determined gravimetrically in whole bodies of fish prey using the method described in Sloan et al. (2005). Information on proportions of five lipid classes (i.e., wax esters/sterol esters, triglycerides,

Table 1

Mean (range) standard length, mass, lipid concentration, and stable isotope concentrations in whole body samples of seven fish species collected from outer coast (Destruction Island) Strait of Juan de Fuca (Tatoosh Island), and inside waters (Protection Island) rhinoceros auklet colonies. [n] = sample sizes for stable isotope concentrations if different than N

		Destruction Island	Tatoosh Island	Protection Island
Pacific sandlance Ammodytes hexapterus	N SL (mm) Mass (g) % lipids δ <sup>15</sup> N (‰) [n] δ <sup>13</sup> C (‰) [n]	4 113.3 (106–116) 5.1 (3.5–6.5) 3.1 (0.7–5.3) [0] [0]	3 123.3 (114–131) 7.6 (6.1–8.6) 3.0 (1.7–4.2) [0] [0]	4 110.8 (108–113) 6.1 (5.7–6.3) 5.8 (4.7–7.2) [0] [0]
Pacific herring Clupea pallasi	N SL (mm) Mass (g) % lipids $\delta^{15}$ N (‰) [n] $\delta^{13}$ C (‰) [n]	4 122.8 (116–138) 20.8 (16.5–30.0) 4.8 (2.3–6.6) 12.4 (12.2–12.5) –17.9 (–17.7 to –18.2)	4 131 (130–134) 24.6 (21.8–27.2) 3.4 (1.4–5.8) [0] [0]	4 116.5 (107–121) 18.2 (14.4–21.2) 3.4 (1.4–5.8) 12.4 (–) [1] –16.2 [1]
Northern anchovy Engraulis mordax	N SL (mm) Mass (g) % lipids $\delta^{15}$ N ( $\infty$ ) [n] $\delta^{13}$ C ( $\infty$ ) [n]	4 129.3 (123–136) 24.3 (21.2–28.1) 5.3 (4.0–6.7) 12.8 (12.7–13.0) –18.2 (–17.9 to –18.4)	4 130 (127-133) 21.8 (20.0-22.9) 4.4 (3.3-5.9) 13.1 (13.0-13.1) -18.1 (-17.8 to -18.6)	4 123.5 (109–134) 17.7 (10.6–22.5) 4.2 (0.5–8.2) 12.8 (12.5–13.5) –17.7 (–16.7 to –18.3)
Surf smelt Osmerus	N SL (mm) Mass (g) % lipids δ <sup>15</sup> N (‰) [n] δ <sup>13</sup> C (‰) [n]	2 123 (108–138) 18.7 (13.0–24.3) 1.2 (0.6–1.9) [0]	4 114.5 (65–139) 18.8 (2.2–28.3) 1.7 (0.9–2.9) [0]	4 131.8 (108–148) 25.1 (10.7–36.4) 5.2 (2.8–8.5) [0]
Chum salmon Oncorhyncus keta	N SL (mm) Mass (g) % lipids δ <sup>15</sup> N (‰) [n] δ <sup>13</sup> C (‰) [n]	2 99.5 (86-113) 12.4 (7.1-17.7) 2.1 (1.8-2.4) 12.2 (12.19-12.2) -18.7 (-18.1 to -19.2)	8 89.8 (80–97) 7.5 (5.7–11.0) 0.7 (0.4–1.0) 12.1 (10.6–13.0) –17.8 (–16.5 to –22.3)	6 109.5 (101–119) 13.6 (10.3–17.3) 0.8 (0.6–0.9) 12.3 (11.3–13.3) –16.0 (–14.7 to –18.0)
Chinook salmon Oncorhyncus tschawysha	N SL (mm) Mass (g) % lipids $\delta^{15}$ N (‰) [n] $\delta^{13}$ C (‰) [n]	3 112.3 (106–117) 18.1 (17.5–18.9) 1.2 (0.7–2.0) 12.7 [1] –19.0 [1]	3 92 (87-99) 10.5 (9.4-12.6) 0.8 (0.5-1.2) 11.6 (11.3-11.9) -16.5 (-16.1 to -16.9)	10 95 (68–119) 11.4 (4.1–21.5) 0.9 (0.6–1.6) 11.7 (10.5–13.1) [7] –19.9 (–15.8 to –23.2) [7]
Rockfish Sebastes spp.	N SL (mm) Mass (g) % lipids δ <sup>15</sup> N (‰) [n] δ <sup>13</sup> C (‰) [n]	4 66.5 (64–68) 4.5 (4.1–5.2) 5.2 (4.5–6.1) [0]	4 68 (67-69) 4.7 (4.3-5.1) 5.1 (4.3-5.9) [0]	0 - - - - -

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free fatty acids, cholesterol, phospholipids/polar lipids) contributing to the total lipid was obtained using a thin-layer chromatography/flame ionization method (Ylitalo et al., 2005). Quality control samples [i.e., method blank, replicate and a National Institute of Standards and Technology (NIST) Standard Reference Material [SRM 1947 (lake trout muscle)] were analyzed with each set of field samples as part of a performance-based quality assurance program (Sloan et al., 2006). Results obtained for SRM 1947 were in excellent agreement with certified and reference values published for these materials by the NIST. In addition, the other quality control samples met established laboratory criteria. The percent recovery of the surrogate standard ranged from 100% to 122%. Detection rates (% of samples with detectable levels) of prey samples were high for  $\Sigma$ PCBs (100%),  $\Sigma$ DDTs (100%), and  $\Sigma$ PBDEs (80%) and lower for chlordanes (68%), HCB (64%) and HCHs (49%); we confined our parametric analyses to those POPs with detection rates over 75% (PCBs, DDTs, PBDEs), Results for each species/site pair are presented in Table 1.

## 2.5. Stable isotope analyses

A subset of fish samples (N = 43) were lipid-extracted and analyzed for carbon and nitrogen stable isotopes (Herman et al., 2005). The values were calibrated against internal laboratory standards (aspartic acid and 15N-enriched histidine) analyzed after every 10 samples; unenriched histidine was also analyzed after every 25 samples as control material to determine set-to-set reproducibility. For quality control, all standards and reference material required standard deviations < 0.3‰ for  $\delta$ 15 N and < 0.2‰ for  $\delta$ 13C. A National Institute of Standards and Technology (NIST) fish muscle standard reference material (SRM 1946) was processed with every 20 analyses to monitor analytical accuracy. Results for each species/site pair are presented in Table 1.

## 2.6. Data analyses

We tested for differences in concentration of POPs among rhinoceros auklet prev species and breeding colonies using general linear models (GLMs; SYSTAT, 2007), with auklet colony site and prey species as the main factors, and length or weight, lipid concentration, and  $\delta^{15}N$  (when available) as covariates. We used a stepwise GLM to derive a predictive regression model that reduced effects of covariates, maximized the amount of variation explained by the model  $(r^2)$ , and contained as few covariates as possible (West et al., 2008). Data that did not approximate normal distributions by Shapiro-Wilk's W and the Kolmogorov-Smirnov and Liliefors tests were log (concentration + 1) transformed. Rockfish were not documented in the rhinoceros auklet diet in inland waters (Protection Island; Pearson et al., unpubl.); we thus confined our parametric analyses to the six prey species sampled at all three sites. Post-hoc analyses examined interaction of and pairwise differences for significant effects.

We tested for similarities in patterns of contaminant levels detected among prey fish samples using multivariate techniques in PRIMER v.6 (Clarke and Gorley, 2006). An analysis of the best explanatory variables (BEST) resulted in the removal of redundant or low-information variables (chlordanes, HCHs, HCB) and retention of three contaminant variables (PCBs, DDTs, PBDEs). The resulting dataset was standardized by computing proportional contributions of PCB, DDT, and PBDE concentrations in each sample, square-root transformed, and used to create a triangular matrix of Bray-Curtis similarity coefficients. The non-metric MDS constructed a two-dimensional unitless configuration or "map" of points that described groupings of contaminants based on the similarity of the relative contribution of each contaminant class in each sample, placing similar samples together and dissimilar

samples apart in low-dimensional space with the least amount of stress (see Clark and Warwick 2001). A non-parametric (randomization-based) multivariate analysis (analysis of similarities, ANOSIM) was used to examine similarity in POPs profiles among colony locations and prey species. This analogue to an ANOVA tests the null hypothesis that overall contaminant profiles do not differ among the three locations and six prey species (Clarke and Warwick, 2001). That is, samples within a location or prey species are more similar than between locations or prey species. The R statistic ranges from zero (there are no differences (or exact similarity) between groups to 1 (dissimilarities between the categories of a chosen factor (location, prey species) are larger than any dissimilarity among samples within that factor. Where the null hypothesis was rejected, post hoc pair-wise comparisons among locations or prey species were conducted.

To assess potential exposure to persistent organic pollutants in rhinoceros auklet chicks during their nestling period, we calculated average contaminant burdens delivered to chicks at the three auklet colonies using an approach similar to the "food basket" approach (Cullon et al., 2005). However, rather than use relative proportions of prey species in the diet to create composite diet samples for our locations and then analyze these mixed-species samples for contaminant levels (Cullon et al., 2005), we analyzed concentrations of PCBs, DDTs, and PBDEs for the six primary prey species and then multiplied these concentrations by their relative proportions in the diets observed at each of the three study colonies (Pearson et al. unpubl.). To calculate contaminant burdens over the nestling period, we then multiplied the contaminant levels of the three study-colony diets by the average bill load mass brought to rhinoceros auklet chicks and then by 100, to account for chicks being fed one bill load/night by each parent over a 50 day nestling period (Wilson and Manuwal, 1986).

## 3. Results

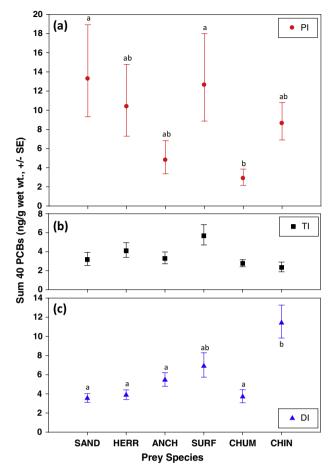
## 3.1. PCBs

Geometric mean PCB concentrations (ng PCBs/g fish wet wt.) in Pacific sandlance, and Pacific herring collected from Protection Island were 2.5 to 4.2 times greater than in the same species collected from Tatoosh and Destruction Islands; in surf smelt collected from Protection Island, mean PCB concentration was 1.8 to 2.2 times greater than in the same species collected from Tatoosh and Destruction Islands (Fig. 2a-c). While northern anchovy and chum salmon contained relatively low mean PCB concentrations that were similar among island locations, mean PCB concentrations in Chinook salmon collected from Protection and Destruction Islands were 3.7 to 4.9 times greater than for Chinook salmon collected from Tatoosh Island (Fig. 2a-c).

For all samples combined, fish length or weight, lipid concentration, and trophic status (as represented by  $\delta^{15}N$  concentration), were not significant covariates in the stepwise GLM for PCB concentration. The best model predicted PCB concentration in rhinoceros auklet prey using prey species and location (Table 2a). Including the interaction term (p = 0.012) with species (p = 0.005) and site (p < 0.001) further improved the model (GLM  $r^2 = 0.58$ ).

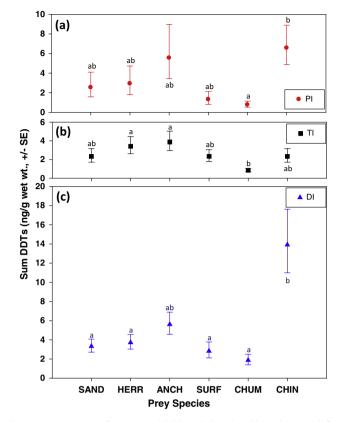
#### 3.2. DDTs

Geometric mean DDT concentrations (ng DDTs/g fish wet wt.) in prey collected from all three islands were generally low in Pacific sandlance, Pacific herring, northern anchovy, surf smelt, and chum salmon (Fig. 3a–c). Mean DDT concentration in Chinook salmon collected from Protection and Destruction Islands was 2.8 and 6.0 times greater, respectively, than for Chinook salmon collected from Tatoosh Island (Fig. 3a–c).



**Fig. 2.** Concentration of 40 PCBs (polychlorinated biphenyls) in fish collected from rhinoceros auklets on (a) Protection Island (PI), (b) Tatoosh Island (TI), and (c) Destruction Island (DI) breeding colonies on the outer coast and inland waters of Washington. Data are geometric means +/- SE for Pacific sandlance (SAND), Pacific herring (HERR), surf smelt (SURF), Northern anchovy (ANCH), chum salmon (CHUM), and Chinook salmon (CHIN). Letters denote significant post hoc differences among species using Bonferroni tests.

For all samples combined, fish length or weight, lipid concentration, and trophic status (as represented by  $\delta^{15}N$  concentration), were not significant covariates in the stepwise GLM for DDT concentration. The best model predicted DDT concentration in rhinoceros auklet prey using prey species and location (Table 2b). Including the interaction term (p = 0.54) with species (p < 0.001) and site (p = 0.033) further improved the model (GLM  $r^2 = 0.58$ ).



**Fig. 3.** Concentration of six DDTs (dichloro-diphenyl-trichloroethane and five others) in fish collected from rhinoceros auklets on (a) Protection Island (PI), (b) Tatoosh Island (TI), and (c) Destruction Island (DI) breeding colonies on the outer coast and inland waters of Washington. Data are geometric means +/- SE for Pacific sandlance (SAND), Pacific herring (HERR), surf smelt (SURF), Northern anchovy (ANCH), chum salmon (CHUM), and Chinook salmon (CHIN). Letters denote significant post hoc differences among species using Bonferroni tests.

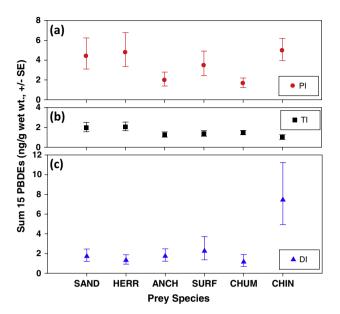
#### 3.3. PBDEs

Geometric mean PBDE concentrations (ng PBDEs/g fish wet wt.) in Pacific sandlance and Pacific herring collected from Protection Island were 2.2 to 3.5 times greater than in the same species collected from Tatoosh and Destruction Islands (Fig. 4a–c); in surf smelt collected from Protection Island, PBDE concentration was 1.5 to 2.1 times greater than in the same species collected from Tatoosh and Destruction Islands (Fig. 4a–c). While northern anchovy and chum salmon contained relatively low mean PBDE levels that were similar among island locations, mean PBDE concentrations in

**Table 2**Results of stepwise GLM testing primary effects (site; species) and covariates (fish length or mass, % lipid content, and trophic position ( $\delta^{15}N$  [‰]) on wet-weight concentrations of (a)  $\sum_{40}$  PCBs, (b)  $\sum_{6}$  DDTs, and (c)  $\sum_{15}$  PBDEs in samples of fish prey collected from three rhinoceros auklet breeding colonies.

Source	Type III SS	df	Mean squares	F-ratio	<i>p</i> -value	GLM r <sup>2</sup>
PCBs						
Species	7.177	5	1.435	4.121	0.002	0.402
Site	6.789	2	3.394	9.745	< 0.001	
Error	24.034	69	0.348			
DDTs						
Species	32.191	5	6.438	12.197	< 0.001	0.520
Site	3.059	2	1.530	2.898	0.062	
Error	36.421	69	0.528			
PBDEs						
Species	6.066	5	1.213	2.691	0.028	0.341
Site	6.783	2	3.391	7.522	0.001	
Error	31.107	69	0.451			

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**Fig. 4.** Concentration of 15 PBDEs (polybrominated diphenyl ethers) in prey fish collected from rhinoceros auklets on (a) Protection Island (PI), (b) Tatoosh Island (TI), and (c) Destruction Island (DI) breeding colonies on the outer coast and inland waters of Washington. Data are geometric means +/- SE for Pacific sandlance (SAND), Pacific herring (HERR), surf smelt (SURF), Northern anchovy (ANCH), chum salmon (CHUM), and Chinook salmon (CHIN).

Chinook salmon collected from Protection and Destruction Islands were 4.1 to 6.2 times greater than for Chinook salmon collected from Tatoosh Island (Fig. 4a-c).

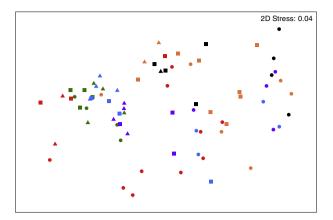
For all samples combined, fish length or weight, lipid concentration, and trophic status (as represented by  $\delta^{15}$ N concentration), were not significant covariates in the stepwise GLM for PBDE concentration. The best model predicted PBDE wet weight concentration in rhinoceros auklet prey using prey species (Table 2c). Including the interaction term (p = 0.03) with species (p = 0.022) and site (p < 0.001) further improved the model (GLM  $r^2 = 0.52$ ).

## 3.4. Multidimensional scaling

Collectively, the multidimensional scaling (MDS) pattern of the three main persistent organic pollutants (PCBs, DDTs, PBDEs) in our fish samples showed some segregation among the three study locations and the six species (Fig. 5). Pairwise tests revealed that rhinoceros auklet prey (all species combined) from Protection Island were differentiable from those collected from Tatoosh and Destruction Islands, but prey samples collected from the latter two islands were not differentiable from each other (Table 3). Pairwise tests also revealed that the contaminant profiles of many but not all rhinoceros auklet prey species (all sites combined) were differentiable from the other species, although notable exceptions were the Chinook salmon-Pacific herring pair-wise comparison and the surf smelt-chum salmon pair-wise comparison (Table 3). Some prey species (i.e., Pacific sandlance, Pacific herring, and surf smelt) showed strong segregation patterns among the three sampling colonies, particularly the Protection Island samples segregating from the Destruction and Tatoosh islands samples (Fig. 6a-f).

## 3.5. Rhinoceros auklet chick contaminant burdens

The estimated contaminant burdens of rhinoceros auklet chicks during their first weeks of life were a reflection of observed diet differences among breeding colonies and the measured differences in pollutants we found among colonies and prey species. Protection



**Fig. 5.** Multidimensional scaling map of persistent organic pollutants (PCBs, DDTs, PBDEs) in prey species (Pacific sandlance = purple, Pacific herring = blue, surf smelt = black, Northern anchovy = green, Chinook salmon = red, chum salmon = orange) for three locations (Protection Island = circles, Tatoosh Island = squares, Destruction Island = triangles) from which rhinoceros auklet prey were sampled. The stress value of <0.05 suggests the MDS gives an excellent representation of the data and indicates a very high probability that the groupings shown were not made by chance (Clarke and Warwick 2001). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

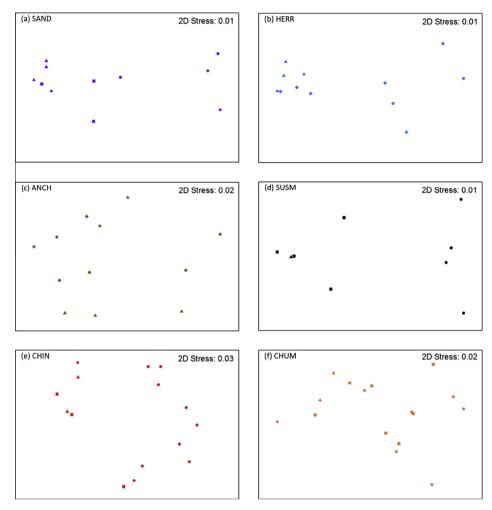
**Table 3**Results of 2-way ANOSIM of the effects of site and species on contaminant profiles of samples of fish collected from three rhinoceros auklet breeding colonies. Pairwise test compared sites (PI: Protection Island, DI: Destruction Island, TI: Tatoosh Island) and species (CHIN: Chinook salmon, CHUM: chum salmon, ANCH: Northern anchovy, HERR: Pacific herring. SAND: Pacific sandlance. SURF: surf smelt).

	R statistic	Significance level (p)
Global test (site)	0.334	<0.001
Pairwise tests		
PI, DI	0.484	<0.001
PI, TI	0.356	<0.001
DI, TI	0.132	<0.103
Global test (species)	0.385	<0.001
Pairwise tests		
CHIN, CHUM	0.372	<0.004
CHIN, ANCH	0.216	<0.044
CHIN, HERR	0.130	<0.169
CHIN, SAND	0.412	< 0.011
CHIN, SURF	0.672	<0.001
CHUM, ANCH	0.746	<0.001
CHUM, HERR	0.396	<0.004
CHUM, SAND	0.301	<0.016
CHUM, SURF	-0.024	<0.555
ANCH, HERR	0.361	<0.003
ANCH, SAND	0.626	<0.001
ANCH, SURF	0.996	<0.001
HERR, SAND	0.264	<0.044
HERR, SURF	0.597	<0.001
SAND, SURF	0.402	<0.007

Island chicks consume mostly Pacific sandlance and Pacific herring, Destruction Island chicks consume primarily northern anchovy and rockfish spp., and Tatoosh Island chicks consume Pacific sandlance, Pacific herring, rockfish, smelt, and salmonids (Table 4). The diet differences among the three colonies combined with our contaminant results for the main prey items and integrated over the nestling period, result in substantial differences in persistent organic pollutant burdens among the inland water and outer coast colonies, particularly for PCBs and PBDEs (Table 5).

## 4. Discussion

Our study found the best predictors of concentrations of PCBs, DDTs, or PBDEs in rhinoceros auklet prey are breeding colony



**Fig. 6.** Multidimensional scaling maps of persistent organic pollutants (PCBs, DDTs, PBDEs) for three locations (Protection Island = circles, Tatoosh Island = squares, Destruction Island = triangles) from which rhinoceros auklet prey were sampled. Prey species: (a) Pacific sandlance, (b) Pacific herring, (c) Northern anchovy, (d) surf smelt, (e) Chinook salmon, and (f) chum salmon.

**Table 4**Annual consumption estimates of prey species by rhinoceros auklet chicks during the nestling period at breeding colonies in Puget Sound (Protection Island, 2006–2010) and Washington's outer coast/northern California Current (Tatoosh Island, 2006–2009; Destruction Island, 2008–2010). Estimates are totals (in grams) for the nestling period calculated from proportional mass of all prey items observed in bill loads (Pearson et al. unpubl.) multiplied by two nightly feedings (one by each parent; Wilson, 1977) over a 50-day nestling period (Wilson and Manuwal, 1986).

Common name	Nestling period prey consumption estimates (g)							
	Protection Island	Tatoosh Island	Destruction Island					
Pacific sandlance	2394.5	1557.5	69.2					
Pacific herring	501.1	528.2	60.1					
Northern anchovy	44.9	294.6	1584.4					
Surf smelt	21.1	94.8	12.0					
Chum salmon	50.2	105.0	12.0					
Chinook salmon	20.2	30.5	12.0					
Rockfish spp.	0.00	372.4	399.8					
Other	125.9	501.1	1127.4					
Totals	3158.0	3484.0	3277.0					

location and prey species. Overall, fish collected in the urbanized Puget Sound region were much more likely to be contaminated than fish collected at the two locations that are less urbanized. Concentrations of POPs along gradients related to urbanization or contaminant exposure have similarly been found for Pacific herring in Puget Sound (West et al. 2008), largescale suckers (*Catostomus* 

macrocheilus) in the lower Columbia River (Nilsen et al. 2014), and topsmelt (Atherinops affinis) and Mississippi silverside (Menidia audens) in San Francisco Bay (Greenfield and Allen, 2013).

The multidimensional scaling (MDS) pattern of pollutants suggests differential contaminant profiles associated with the three study colony locations; that is, fish from the inland marine waters colony in Puget Sound had more similar contaminant signatures and clustered together in space as compared with the fish from the outer coast colonies. The patterns of similarity were more pronounced for the three prey species (Pacific sandlance, Pacific herring, surf smelt) that are resident species with spawning populations in Puget Sound (Penttila, 2007). The patterns of similarity were less pronounced for the three prey species (northern anchovy, Chinook salmon, and chum salmon) that generally range more widely and may be less environmentally isolated from other populations.

Of the resident species, only Pacific herring have been analyzed previously for persistent organic pollutants. The lower PCB and PBDE concentrations in herring from our outer coast locations as compared with more urbanized inland marine waters, while lower overall, mirror differences documented between Georgia Strait locations and more urbanized central Puget Sound (West et al., 2008). The differences in Pacific herring PCB and DDT levels observed in that study were driven by collection location and not trophic status (i.e.,  $\delta^{15}$ N), lipids, SL, age, weight, or year (West et al., 2008), just as we found in our study. Trophic position was

Table 5

Nestling-period contaminant burdens (PCBs, DDTs, PBDEs) for rhinoceros auklet chick diets from breeding colonies in Puget Sound (PI = Protection Island) and Washington's outer coast/northern California Current (TI: Tatoosh Island; DI: Destruction Island). Values are mean ng (±se) calculated from prey-specific concentrations (this study) accumulated for prey eaten over 50-day nestling period (Table 4).

	PCBs			DDTs			PBDEs	PBDEs		
	PI	TI	DI	PI	TI	DI	PI	TI	DI	
Pacific sandlance	34719.9	5035.3	248.9	6165.8	3633.6	231.6	9158.9	1577.7	50.1	
	(6946.4)	(764.7)	(26.9)	(572.3)	(104.4)	(15.4)	(2947.6)	(423.6)	(5.5)	
Pacific herring	5875.3	2218.4	240.5	1478.2	1822.2	233.0	2054.5	749.0	26.6	
	(1645.6)	(300.5)	(35.2)	(101.2)	(143.7)	(38.7)	(583.3)	(473.3)	(0.9)	
Northern anchovy	234.7	1038.3	8674.5	265.0	1244.5	8951.8	48.7	170.8	1216.8	
	(59.0)	(215.6)	(467.4)	(54.8)	(294.3)	(602.1)	(18.4)	(35.4)	(302.6)	
Surf smelt	303.8	587.8	93.8	30.8	248.9	36.1	654.0	237.0	21.6	
	(83.5)	(155.0)	(10.2)	(8.45)	(72.05)	(12.0)	(31.5)		-	
Chum salmon	163.1	311.00	45.1	45.0	105.3	23.5	56.5	90.7	4.0	
	(36.7)	(41.6)	(7.8)	(12.7)	(20.0)	(6.6)	(12.2)	(16.7)	-	
Chinook salmon	252.0	72.1	145.5	332.4	78.2	211.3	134.4	0.0	215.6	
	(63.8)	(8.9)	(33.7)	(125.5)	(22.9)	(105.1)	(45.3)	-	(187.2)	
Rockfish spp.	-	116.6	84.8	_	173.2	233.9		0.0	0.0	
		(37.2)	(12.4)		(53.6)	(51.6)		-	-	
Other	nd	nd	nd	nd	nd	nd	nd	nd	nd	
Total	41548.8	9379.5	9533.1	8317.1	7305.9	9921.1	11,518.0	2825.2	1534.8	
	(8835.0)	(1528.6)	(593.3)	(875.0)	(710.9)	(831.4)	(3638.3)	(948.9)	(496.2)	

also not correlated with organohalogen contaminants for a suite of 12 prey fish species in northern Hudson Bay, including sandlance (Braune et al., 2014). The lack of assessments of persistent organic pollutants in Pacific sandlance and surf smelt in Puget Sound and the northern California Current preclude comparisons, but the non-trivial concentrations in such critical mid-trophic level species in Puget Sound may be worrisome. The distinct contaminant signatures of resident species from inland marine waters may result from them being environmentally isolated from populations on the outer Washington coast (Penttila, 2007). Unfortunately, the herring populations on the outer Washington coast have not been extensively examined for contaminants.

Despite differences among our sampling locations, PCB and DDT concentrations in all of our Pacific herring samples were lower than those found by West et al. (2008). This could be due to where auklets obtained their herring. During breeding, auklets from Protection Island spend most of their time west of the island in the Strait of Juan de Fuca (Grover and Olla, 1983; Wahl and Speich, 1994) rather than down in the more urbanized sections of central and southern Puget Sound. We have also observed rafts of auklets north and east of the colony, which is also away from the urbanized core of Puget Sound. In addition, herring prey collected by auklets were generally smaller and likely younger than those analyzed by West et al. (2008). Herring we analyzed had location means ranging from 116-131 mm and 18-25 g, and most were likely ago-0 fish (Foy and Paul, 1999); herring analyzed by West et al. (2008) had location means ranging from 161-177 mm and 53-71 g and were likely age-1 fish.

The remaining three prey species (northern anchovy, Chinook and chum salmon) generally range more widely, which may produce less tight clustering of pollutant signatures. Northern anchovy range from California to Vancouver Island, and our three sampling locations appear to be part of the northern stock (Lecomte et al., 2004). The anchovy obtained in Puget Sound may thus not have been drawn from local sources. Chinook salmon juveniles range widely in nearshore areas along the Pacific Northwest coast, which may mix populations the birds may sample. Columbia River stocks dominate summer samples from southeast Alaska to the Washington outer coast (Tucker et al., 2012; Fisher et al., 2014). West of Vancouver Island, late fall sampling is dominated by Oregon watershed stocks, and by early spring some Puget Sound stocks and Fraser River stocks make up ca. 1/3 of the samples (Tucker et al., 2012). Chum salmon from multiple coastal

watersheds are distributed along the Washington outer coast in summer, moving northward by late summer (Weitkamp et al., 2012), which may result in the mixing of populations the birds can sample. Chum salmon can spend a fair amount of time in potentially impacted estuaries and accumulate PCBs and DDTs (Stehr et al., 2000), although our samples may not have differentiated significantly due to relatively low concentrations. Finally, the  $\delta^{13}$ C signal of northern anchovy, Chinook and chum salmon were similar (and, for the salmon, quite variable) among sampling locations. The fish may have similar marine distributions, or it may simply be due to the wide size range of sampled fish or their saltwater residency time (Johnson et al., 2007b).

Elevated POPs concentrations in Chinook salmon from the Protection Island colony supported our prediction of greater contaminant levels in an urbanized landscape and rivaled levels for Pacific salmon in other Pacific Northwest estuaries. While PCB concentrations were not as consistently high as found in other urbanized estuaries in the Pacific Northwest (Table 6), three of the 10 Chinook salmon from Protection Island exceeded the PCB threshold for adverse health effects (2400 ng/g lipid wt.; Meador et al., 2002). The same three Chinook salmon had DDT concentrations (>5200 ng/g lipid wt.) greater than Chinook salmon from southern Puget Sound and rivaling those from the Columbia River estuary (Table 6). In the Columbia River estuary, such DDT concentrations are indicative of the most urbanized sites (Johnson et al., 2007b); yearling migrants from interior Columbia and Snake river basins and fall subyearling migrants from the Snake River spend extended periods of time there feeding and rearing (Johnson et al., 2013). For PBDEs, concentrations for eight of the 10 Chinook salmon from Protection Island were considerably greater than for hatchery fish in the Columbia River basin (Table 6), with two of them exceeding whole-body level PBDE levels associated with increased disease susceptibility in subyearling Chinook salmon (1600 ng/g lipid wt.; Arkoosh et al., 2010).

Our prediction of lower contaminant levels in the less impacted outer coast ecosystem was supported by POPs concentrations in Chinook salmon from one colony (Tatoosh Island) but not the other (Destruction Island). The Chinook salmon from Tatoosh Island had concentrations of PCBs, DDTs, and PBDEs consistently lower than those from Protection Island (Table 6). For Destruction Island, two of the three Chinook salmon samples had concentrations of PCBs (1700–1800 ng/g lipid wt.) and DDTs (1100–3700 ng/g lipid wt.) in the range of levels for Puget Sound, Grays Harbor, Willapa

 Table 6

 Concentrations of persistent organic pollutants of juvenile Chinook salmon from the U.S. west coast. Data are site means (range); previously published data are site mean ranges.

Collection location		n	ng/g (lipid weight)		ng/g (wet weight)		
			PCBs	DDTs	PBDEs	Reference	
Puget Sound	Protection I.	10	1675 (328-4068)	2420 (172-9638)	6.6 (0.3-19.0)	This study	
Outer WA coast	Tatoosh I.	3	319 (164-441)	374 (158-747)	<loq< td=""><td>This study</td></loq<>	This study	
Outer WA coast	Destruction I.	3	1278 (364-1816)	1766 (498-3739)	17.9 (0.6-49.0)	This study	
Puget Sound	Multiple sites	3 <sup>a</sup>	980-3100	1150-2280	_	Johnson et al. 2007a	
Outer WA coast	Grays Harbor	1 <sup>a</sup>	1570	560	_	Johnson et al. 2007a	
Outer WA coast	Willapa Bay	1 <sup>a</sup>	1780	480	_	Johnson et al. 2007a	
Lower Columbia River	Multiple sites	31*	1310-14,200	1750-27,300	_	Johnson et al. 2007b	
Lower Columbia River	Multiple sites	31ª	1310-14,200	1750-27,300	_	Johnson et al. 2007b	
Lower Columbia River	Multiple hatcheries	1 <sup>b</sup>	_	_	<loq-0.78< td=""><td>Johnson et al. 2010</td></loq-0.78<>	Johnson et al. 2010	

<sup>&</sup>lt;LOQ = less than lower limit of quantitation.

Bay, and some sites in the Columbia River estuary (Table 6). For PBDEs, concentrations for two of the three Chinook salmon from Destruction Island were the highest we analyzed (4.2–49.0 ng/g wet wt.) and much greater than in hatchery fish in the Columbia River basin (Table 6). The highest concentration we recorded (6700 ng/g lipid wt.) far exceeded whole-body level PBDE levels associated with increased disease susceptibility in subyearling Chinook salmon (1600 ng/g lipid wt.; Arkoosh et al. 2010).

What could explain these unusual Chinook salmon POPs patterns for the outer coast colonies? The Chinook salmon brought to auklet chicks on Destruction Island, rather than originating from watersheds in the vicinity of the breeding colony, could have been Columbia River stocks that are commonly found on the outer Washington coast in summer (Fisher et al., 2014). Meanwhile, the Chinook salmon brought to Tatoosh Island auklet chicks could have originated from remote outer coast river systems, the Strait of Juan de Fuca, or even Puget Sound. The Strait of Juan de Fuca in summer has lower numbers than the outer coast of particularly contaminated Columbia River stocks (Fisher et al., 2014). Even if from Puget Sound, fish may originate from or have shorter residence times in contaminated portions of the Sound, which is thought to be responsible for elevated contaminant levels (O'Neill and West, 2009; Johnson et al., 2010).

Characterizing contaminant profiles on multiple species of forage fish communities provides information on a critical portion of the food web that influences upper trophic level consumers, including marine birds, Pacific salmon, and harbor seals. Pacific sandlance, herring, and surf smelt have been documented in the diets of many Pacific coast seabirds. In Alaska, they have been documented in the diets of rhinoceros auklets and other acids (tufted puffin Fratercula cirrhata, horned puffin Fratercula corniculata, common murre *Uria aalge*, thick-billed murre *U. lomvia*; Dragoo et al., 2003), pigeon guillemot (Cepphus columba) (Litzow et al., 2000), glaucous-winged gulls Larus glaucescens (Dragoo et al., 2003), black-legged kittiwake Rissa tridactyla (Dragoo et al., 2003). On Triangle Island, in British Columbia, rhinoceros auklets feed extensively on Pacific sandlance and Pacific herring (Vermeer, 1979). In Washington, these same forage fish species are important dietary components for rhinoceros auklets (Wilson, 1977; Lance and Thompson, 2005, Pearson et al. unpubl.) as well as common murre (Lance and Thompson, 2005; Schrimpf et al., 2012), and glaucouswinged/western gulls (Good, unpubl. data). The cumulative effects of contaminant-laced fish prey for marine bird and mammal consumers, particularly those breeding in or near urbanized estuaries, may be significant.

Not only do contaminants affect the health of forage fish and juvenile salmon, but PCBs, DDTs, and PBDEs may bioaccumulate and bioconcentrate, thus affecting their upper-level consumers. For DDTs, four Chinook salmon from Protection Island and one

from Destruction Island fell in the range for impacts related to DDT bioaccumulation and bioconcentration in estuarine systems (22–50 ng/g wet wt.; Nendza et al., 1997). In the lower Columbia River, DDTs and PCBs have been detected in eggs of bald eagles, cormorants and great blue herons (Thomas and Anthony, 1999; USFWS, 1999; Buck et al., 2005), where they may be contributing to reduced productivity of these upper trophic level piscivorous birds. No directed sampling of avian piscivores in Puget Sound has occurred since the 1980s (Henny et al., 1989; Speich et al., 1992; Blus et al., 1999), thus the potential impacts of POPs on resident and migratory marine birds remain largely unexplored.

Our modified food basket approach was comprised of diets observed over the course of the 50-day nestling period at the three rhinoceros auklet breeding colonies as well as the pollutant concentrations in the main prey species making up those diets. The estimates of potential contaminant burdens of rhinoceros auklet chicks during their first weeks of life reflected the significant differences among breeding locations for the most contaminated prey species. Estimated dietary PCB exposure for the Protection Island auklet chick diet/food basket was 4.5 times that of chicks on Destruction or Tatoosh islands (see Table 5), no doubt because of the substantial reliance on Pacific sandlance and Pacific herring at the inland waters colony. Estimated DDT exposure for auklet chicks was similar among the three island locations, but estimated dietary PBDE exposure for the Protection Island auklet chick diet/food basket was 4.5 and 7.5 times that of chicks on Tatoosh and Destruction Islands, respectively (see Table 5). Again, these differences likely stem from the substantial reliance on Pacific sandlance and Pacific herring by auklets breeding at the inland waters colony.

Similar geographical patterns in pollutant levels have been documented using analyses of harbor seal diets, which found contaminant concentrations greater in Puget Sound than in the Strait of Georgia, B.C. (Cullon et al. 2005). Concentrations of PCBs for the food basket constructed for Puget Sound were three (wet-weight) to seven (lipid-weight) times that found for the Strait of Georgia food basket. While DDT concentrations were very similar between the diets representing the two locations, concentrations of PBDEs  $((\mu g/kg \text{ wet wt and } \mu g/kg \text{ lipid wt}) \text{ for the food basket constructed})$ for Puget Sound were two (wet-weight) to five (lipid-weight) times that found for the Strait of Georgia food basket. These results corroborated direct measurements of contaminants of Puget Sound harbor seal, which were found to be seven times more contaminated than those inhabiting the Strait of Georgia (Ross et al., 2004). The ratio of PBDE:PCB concentrations we found in rhinoceros auklet diets (0.3 for Protection Island, 0.3 for Tatoosh Island, 0.2 for Destruction Island) were also similar to those found for harbor seal diets (Cullon et al., 2005). Chinook salmon PBDE:PCB ratios were greater than the other prey species, with values ranging up to

a =Composite samples from 10-15 fish.

b =Composite samples of 10 fish/composite.

0.70; one sample from Destruction Island had a PBDE:PCB ratio of 4.1., highlighting the continuing risk PBDEs pose to marine fauna.

The early-life contaminant burdens demonstrated in this study likely continue as rhinoceros auklets biomagnify and bioaccumulate POPs from their fish prey throughout their lifetime, especially upper trophic level predators that likely consume fish prey that are demonstrably more contaminated in Puget Sound (Cullon et al., 2005; O'Neill and West, 2009). While breeding birds were not sampled simultaneous with prey collection, POPs analyses of marine birds salvaged during field operations are indicative of forage fish predators and support the overall patterns seen in the fish prey. Concentrations of PCBs in tissues from three rhinoceros auklets collected from inland marine waters (3441-9183 ng/g lipid weight) were considerably greater than in tissues from an auklet collected from Destruction Island (1539–2562 ng/g lipid weight; unpubl. data). The same general pattern (Protection Island > Destruction Island) was seen in rhinoceros auklet tissues collected in 1981 (Blus et al., 1999). Our liver samples had lower DDE concentrations, which make sense given the time elapsed between the studies and the fact that DDT levels have generally declined since their ban in the U.S. in 1972 (Calambokidis et al., 1999; Lieberg-Clark et al., 1995). The highest contaminant levels detected in our salvaged birds was in a closely-related species, the tufted puffin (Fratercula cirrhata), collected in Puget Sound, which had lipid-corrected liver POPs concentrations nearly ten times the levels of rhinoceros auklets collected in Puget Sound. Exceeding other upper trophic-level consumers in the system such as Puget Sound resident Chinook salmon (O'Neill and West, 2009) and harbor seals (Ross et al., 2013), the puffin's concentrations ( $\sum$ PCBs: 46,852 ng/g lipid wt.;  $\sum$ DDTs: 24,988 ng/g lipid wt.; \(\sumes PBDEs: 3436 ng/g lipid wt.; \) Good et al., unpubl. data) rivaled levels documented in killer whales resident in Puget Sound and the Georgia Basin (Krahn et al., 2007).

Long-term association with areas more impacted by persistent organic pollutants (e.g., Protection Island in Puget Sound) could lead to concentrations of POPs known to affect behavior, reproduction, and immune system function seen in other marine birds (Verreault et al., 2010). The extent to which these rhinoceros auklets disperse or migrate after breeding is unknown, potentially decoupling the individual birds we sampled from the environs surrounding their breeding colonies for some portions of the year. Still, while information on the extent of philopatry is lacking, birds on Protection Island are known to return to the same or nearby burrows year after year (Wilson, 1977). More extensive and systematic sampling from these colonies, which are in different ecosystems and that represent a significant portion of the North American breeding population (Pearson et al., 2013), would shed light on potential impacts of persistent organic pollutants on rhinoceros auklet populations and their prey along the Pacific coast of North America.

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