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Modulation in Persistent Organic Pollutant Concentration and Profile by Prey Availability and Reproductive Status in Southern Resident Killer Whale Scat Samples

Jessica I. Lundin,[*,†] Gina M. Ylitalo,[‡] Rebecca K. Booth,[†] Bernadita Anulacion,[‡] Jennifer A. Hempelmann,[‡] Kim M. Parsons,[‡,§] Deborah A. Giles,[∥] Elizabeth A. Seely,[†] M. Bradley Hanson,[‡] Candice K. Emmons,[‡] and Samuel K. Wasser[†]

[†]Center for Conservation Biology, Department of Biology, University of Washington, Seattle, Washington 98195, United States

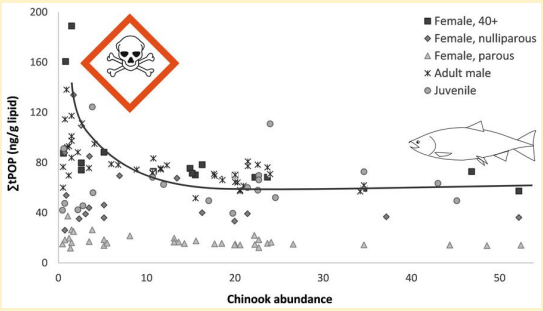
[‡]Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington 98112, United States

[§]Alaska Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Seattle, Washington 98115, United States

[∥]Center for Whale Research, Friday Harbor, Washington 98250, United States

Supporting Information

ABSTRACT: Persistent organic pollutants (POPs), specifically PCBs, PBDEs, and DDTs, in the marine environment are well documented, however accumulation and mobilization patterns at the top of the food-web are poorly understood. This study broadens the understanding of POPs in the endangered Southern Resident killer whale population by addressing modulation by prey availability and reproductive status, along with endocrine disrupting effects. A total of 140 killer whale scat samples collected from 54 unique whales across a 4 year sampling period (2010−2013) were analyzed for concentrations of POPs. Toxicant measures were linked to pod, age, and birth order in genotyped individuals, prey abundance using open-source test fishery data, and pregnancy status based on hormone indices from the same sample. Toxicant concentrations were highest and had the greatest potential for toxicity when prey abundance was the lowest. In addition, these toxicants were likely from endogenous lipid stores. Bioaccumulation of POPs increased with age, with the exception of presumed nulliparous females. The exceptional pattern may be explained by females experiencing unobserved neonatal loss. Transfer of POPs through mobilization of endogenous lipid stores during lactation was highest for first-borns with diminished transfer to subsequent calves. Contrary to expectation, POP concentrations did not demonstrate an associated disruption of thyroid hormone, although this association may have been masked by impacts of prey abundance on thyroid hormone concentrations. The noninvasive method for measuring POP concentrations in killer whales through scat employed in this study may improve toxicant monitoring in the marine environment and promote conservation efforts.



INTRODUCTION

Persistent organic pollutants (POPs) are lipophilic compounds that are resistant to degradation. As apex predators, odontocete (toothed) whales are susceptible to pronounced biomagnification of POPs.[1,2] The endangered Southern Resident killer whale (SRKW; *Orcinus orca*) population, consisting of three family groups, or pods, referred to as J, K, and L, experienced an unexplained 20% decline in their population in the late 1990s. Exposure to POPs associated with deleterious physiologic effects,[3] particularly polychlorinated-biphenyls (PCBs), polybrominated diphenylethers (PBDEs), and dichloro-diphenyl-trichloroethanes (DDTs), as well as decreased prey availability, were proposed as major risk factors to this population in the Recovery Plan,[3] and remain current threats.[4]

The SRKW population forage almost exclusively on salmonids between late spring and fall, particularly Fraser River Chinook salmon (*Oncorhynchus tshawytscha*).[5−7] Diet has been highlighted as the most likely source of exposure to POPs for SRKWs.[8,9]

POPs readily bioaccumulate in adipose tissue. The concentration of these toxicants generally increase with age, particularly the POPs more resistant to biotransformation.[10,11] It is hypothesized that systemic concentrations of these

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compounds, and associated bioavailability to target organs, increase when fat-metabolism occurs in response to decreased prey availability.[12] Lactating females also mobilize endogenous free fatty acid and lipoprotein stores during milk production; 2−4 kg of milk converts to 1 kg of calf mass gain,[13] consequently transferring high-levels of toxicants to nursing young. Decreased POP loads have been reported in lactating female killer whales,[14] harp seals (*Phoca groenlandica*),[15] gray seals (*Halochoerus grypus*),[16−18] and polar bears (*Ursus maritimus*).[19]

Blubber biopsy samples previously collected on the SRKWs indicate that PCB exposures in this population exceed a toxicity threshold for marine mammals (17 000 ng/g lipid) extrapolated from studies of immunologic and reproductive effects in seals, otters, and mink.[14,20−22] POPs can disrupt endocrine systems through interference with cellular messaging systems (i.e., hormones) responsible for the regulation of developmental processes, metabolism, immune function, and reproduction.[23] Additionally, risk assessments of other cetacean populations suggest that current levels of contamination may impair immune[24−28] and reproductive systems.[29−34]

The ability to test hypotheses relating to temporal trends in bioaccumulated toxins in this endangered SRKW population has been constrained by low sample acquisition of tissue biopsies, or collections from necropsied animals. In this study we capitalized on a novel sample collection approach to monitor trends in toxicant concentrations across seasons and years using killer whale fecal samples.[35,36] Concentration and chemical profile of circulating toxicants were characterized by prey abundance, age-sex class, and reproductive class. Endocrine disruption from toxicant exposure was evaluated using hormone measures[37,38] from the same scat samples.

■ MATERIALS AND METHODS

Sample Collection. A total of 263 SRKW scat samples were collected May through October from 2010 to 2013. Samples were located by detection dogs trained to locate SRKW scat floating on the water's surface.[39−41] Samples were scooped off of the surface of the water, as previously described,[35] and frozen at −20 °C until processed in the lab. Collections occurred from mid-May through mid-October, when the SRKWs appear with regularity in the areas around the San Juan Islands and Puget Sound of Washington state, collectively referred to as the Salish Sea. The study period aligned with peak run times for Fraser River Chinook salmon, the preferred seasonal prey of the SRKWs.[7]

Life History Data. Age, sex, family lineage, and reproductive status of whales genotyped in this study were determined using annual population census data collected through photoidentification since 1976 by the Center for Whale Research.[42] Age-sex class[43−45] was defined as juveniles (either sex, < 10 years), adult males (≥10 years), reproductive-age females (≥10−<40 years),[43,44] and postreproductive females (40+ years); age-sex class was also considered by parity (nulliparous and parous) in reproductive-age females. Reproductive status was defined in reproductive-age females as confirmed pregnancy ["Preg (conf)"] (defined below), possible pregnancy ["Preg (poss)"] (defined below), length of lactation (<1 and 1−2 years), and resting mature (neither pregnant nor lactating). Length of lactation was defined as time following estimated date of parturition.

Laboratory Methods. Samples were thawed, homogenized, and subsampled for both genotype analysis, to determine individual identification,[46] and toxicant analysis. Individual identification was linked with the population census data to determine age-sex class, family lineage, and reproductive status.

Hormone Measures. Samples were refrozen at −20 °C, freeze-dried, and 80 mg (dry fecal weight) was extracted in 15 mL of 70% ethanol for hormone analyses using methods described previously.[37,38,41] Fecal hormones measured include: glucocorticoid (GC), thyroid (total triiodothyronine, T3; and total thyroxine, T4), testosterone (T), progesterone (P4), and estrogen (E). Measures of GC, T3, and T4 concentrations in the scat were used to evaluate nutritional stress.[41] (See Supporting Information (SI) for details). Samples from reproductive-age females with a P4 concentration above 2000 ng/g dry fecal weight were considered to be from pregnant females.[47] Pregnancies confirmed by subsequent observation of a live birth ["Preg (conf)"] were distinguished from those that failed to produce a live calf ["Preg (poss)"] within the 18-month gestation period.[43]

Analysis of Persistent Organic Pollutants. Analyses of POPs in killer whale scat samples were performed using two different protocols, one for high-mass[36] and one for low-mass samples.[35] (See SI for details.) All samples collected during a field season (39−80 samples per year) were processed as 80 mg pellets (dry fecal weight). Analysis of all samples for low-mass toxicant concentrations was cost prohibitive; as such, 126 samples were selected based on a priori hypotheses to evaluate seasonal and annual changes in toxicant concentration, and variation based on reproductive status. Sample extraction and purification was performed using modified procedures from EPA Methods 3630C and 1614.[48−50] All sample extracts and standards were analyzed using a gas chromatography coupled with mass spectrometry (GC/MS) system operating in the negative chemical ionization mode. Target analyte selection was based on pilot sample analyses performed on the high-mass samples (described below). Due to the small sample mass, fewer final target analytes were above the limit of detection compared to the high-mass samples. Final target analytes included 4 PCB congeners (PCBs 138, 153, 180, 187), 2 PBDE congeners (PBDEs 47, 100), and *p,p′*-DDE (*p,p′*-dichloro-diphenyl-dichloroethylene; the predominant metabolite of DDT).

Fifty-six samples were large-enough to be subsampled and processed as high-mass samples (2.0 g wet fecal weight). These large samples were less common because much of the scat sample would sink prior to collection. These samples were extracted, purified, and analyzed for POP concentrations using procedures modified from Sloan et al.[36] Samples were analyzed using a GC/MS system. Additional target analytes were above the limit of detection in the high-mass samples. In total, 40 PCB congeners (PCBs 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), 11 PBDE congeners (PBDEs 28, 47, 49, 66, 85, 99, 100, 153, 154, 155, and 183), and six DDT compounds (*o,p′*-DDD, *o,p′*-DDE, *o,p′*-DDT, *p,p′*-DDD, *p,p′*-DDE, and *p,p′*-DDT) were analyzed. POP concentrations are reported as wet weight (ng/g), normalized by percent lipid (lipid adjusted, la). Linear associations of toxicant concentrations in the 39 samples analyzed as both low-mass and high-mass samples were significant; the final analytic data set included toxicant measures on 157 samples. (See SI for details.)

Blubber Biopsy Samples. Blubber samples were collected in the United States and Canada between May 2006 and

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Table 1. Toxicant Concentrations in Scat Samples by Year, Season, Pod, Age-Sex Class, Reproductive Status, Number of Calves, and Birth Order: Total Samples, *n* = 140 from 54 Unique Whales; High-Mass Samples, *n* = 56 from 34 Unique Whales

		% lipid	sample count	unique whales	∑4PCBs[a] (ng/g la)			∑40PCBs (ng/g la)			∑2PBDEs[a] (ng/g la)			*p,p′*-DDE (ng/g la)		
					total samples			high-mass samples			total samples			total samples		
		median, sd	total (n)/ high-mass (n)		mean[b]	95% CI		mean[b]	95% CI		mean[b]	95% CI		mean[b]	95% CI	
year	2010	1.0, 0.8	23/17	18/14	17.0	12.0	24.0	45.2	26.3	77.9	5.7	4.5	7.3	34.9	24.1	50.7
	2011	2.6, 4.9	47/15	38/15	15.6	12.0	20.1	26.6	15.6	45.4	7.1	5.9	8.5	33.1	24.9	44.1
	2012	0.9, 1.4	39/11	24/10	17.1	12.8	22.7	48.9	26.8	89.5	7.1	5.8	8.6	32.6	23.8	44.8
	2013	1.2, 2.3	31/13	24/10	13.6	9.9	18.5	59.5	32.9	107.6	7.0	5.6	8.7	26.6	18.9	37.3
season[c]	spring	1.4, 5.3	33/12	23/9	20.3	14.9	27.5	73.9	39.6	137.7	8.3	6.7	10.3	41.0	29.4	57.2
	summer	1.0, 2.2	45/19	32/15	16.1	12.2	21.1	32.0	18.3	56.0	6.8	5.6	8.2	33.6	24.8	45.4
	fall	1.5, 2.1	62/25	33/18	13.3	10.4	17.0	36.3	22.6	58.1	6.1	5.1	7.2	26.5	20.1	34.8
pod	J	1.3, 3.8	67/28	19/15	17.4	12.8	23.7	48.1	27.3	84.6	8.0	6.5	9.8	24.6	17.2	35.2
	K	1.5, 2.3	32/13	13/7	16.1	10.9	23.7	33.2	15.0	73.4	6.3	4.8	8.1	39.4	25.3	61.4
	L	1.0, 1.7	41/15	22/12	14.1	10.4	19.1	38.0	21.2	68.1	6.1	5.0	7.5	35.8	25.3	50.5
age-sex class[d]	juvenile	2.2, 8.0	22/5	12/3	17.2	11.7	25.1	59.0	23.2	149.8	7.9	6.2	10.1	36.9	23.8	57.4
	female, nulliparous	0.9, 1.4	15/7	4/4	15.0	8.7	26.0	45.3	20.1	102.2	9.1	6.6	1.3	22.7	11.6	44.4
	female, parous	1.0, 1.6	37/17	17/12	6.1	4.5	8.3	9.8	6.0	16.0	3.7	3.1	4.5	8.7	6.0	12.5
	female, 40+	1.6, 2.7	25/11	7/6	23.4	15.2	36.1	53.9	27.9	104.0	8.3	6.4	10.7	54.9	32.4	92.9
	adult male	1.3, 2.2	41/16	16/9	20.7	15.1	28.3	61.8	36.0	106.0	7.5	6.1	9.0	47.5	32.7	68.9
reproductive status[e] (females, age 10–40)	preg (conf)	1.3, 0.4	8/4	5/3	5.1	2.5	10.3	10.0	2.6	37.4	3.2	1.9	5.4	7.9	3.7	16.8
	preg (poss)	0.9, 1.5	13/5	6/3	5.9	2.9	12.0	13.7	2.3	81.9	4.7	2.7	7.9	7.9	3.7	16.7
	lact, <1 yr	0.4, 0.9	4/4	4/4	4.8	2.2	10.4	12.1	3.9	37.3	2.5	1.4	4.4	6.5	3.1	13.8
	lact, 1–2 yrs	3.5, 3.0	3/3	3/3	2.3	1.0	5.3	6.3	1.8	21.9	1.8	1.0	3.5	3.6	1.6	8.1
	resting mature	1.3, 0.9	24/8	12/6	7.8	5.5	11.3	11.7	4.7	29.2	5.2	3.9	6.7	12.2	8.2	18.4
number of calves[f] (females, age 10–40)	0	0.9, 1.4	15/7	4/4	15.2	9.1	25.4	46.0	26.0	81.7	9.1	6.1	13.7	23.3	11.7	46.5
	1	0.6, 2.6	10/5	6/3	10.6	6.3	18.0	16.6	8.6	32.0	4.4	2.9	6.6	16.8	9.1	31.1
	2	0.7, 0.8	8/3	4/2	6.9	3.8	12.7	21.1	9.0	49.1	4.6	2.9	7.4	7.0	3.4	14.5
	3	1.0, 0.7	7/4	3/3	3.7	1.9	7.1	6.5	3.1	13.6	2.7	1.6	4.5	4.2	1.8	9.6
	4	1.0, na	4/1	2/1	4.9	2.0	11.5	5.3	1.2	22.9	4.1	2.1	8.0	11.2	3.8	32.8
	5	2.5, 0.7	6/3	2/2	5.9	2.7	12.7	4.6	1.9	11.2	3.3	1.8	6.0	8.8	3.2	24.0
	6	1.8, na	2/1	1/1	3.4	1.0	11.2	6.6	1.5	29.4	3.6	1.4	9.0	6.1	1.4	26.7
birth order (juveniles, age <10)	1	0.8, 0.2	9/2	5/1	32.2	22.3	46.3	199.5	na	na	9.5	6.4	14.2	90.6	48.5	169.0
	2	na, na	3/0	2/0	19.2	9.2	40.3	na	na	na	10.8	6.9	16.9	37.8	12.9	110.3
	3	2.6, na	3/1	1/1	15.1	8.4	27.3	65.9	na	na	7.4	3.6	15.2	35.1	12.3	100.1
	4	10.8, 12.1	7/2	4/1	7.6	4.5	12.6	24.2	na	na	6.0	4.0	8.9	11.6	5.4	24.9

[a]∑4PCBs = PCB138, PCB153, PCB180, PCB187; ∑2PBDEs = PBDE47, PBDE100. [b]All geometric means adjusted for Julian day and age-sex class, when not a main effect, and repeat samples from individual whale. [c]Julian day did not improve the fit of the model and was not included. [d]Two whales changed category over 4 year study (K34, L100). [e]Also adjusted for number of calves; one whale changed category over 4 year study (L103). [f]One whale changed category over 4 year study (J28).

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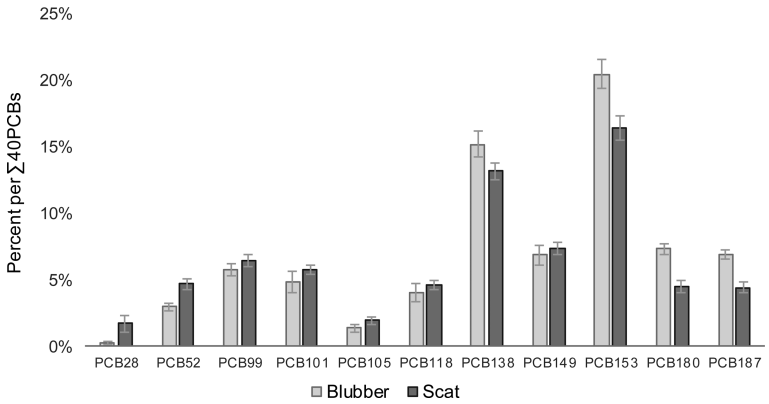

Figure 1. A comparison of blubber biopsy and scat samples, by PCB congener; n = 18 individual whales with matched samples, relative concentrations statistically associated for all congeners evaluated (p-value < 0.05; PCB180, p-value < 0.10). Bars represent mean of matched samples by matrices.

January 2013 using documented sampling techniques.[51] All samples were analyzed for the same target analytes as the high-mass scat samples using similar techniques, described elsewhere.[36] Blubber biopsy samples from SRKWs were matched with scat samples from the same individual, restricted to adult whales that did not give birth between collection of blubber and fecal sample. The average period of time between blubber and scat sample collection was 3 years, 2 months (range: 1 month to 7 years, 2 months).

Statistical Analysis. Years of POP accumulation was defined as age for juveniles, males, and nulliparous females. For parous females, POP accumulation was calculated as years since last calf minus 2 years for lactation. Toxicant concentration was evaluated by number of calves in reproductive-age females. Analysis by birth order was restricted to juvenile whales to ensure the measured concentrations were more reflective of maternal burden offload than subsequent accumulation through diet.[31] Toxicant concentration and potential endocrine disruption was evaluated using T3, T4, T3/T4, GC, P4, E, and T as the predictors. Models with reproductive hormones (P4, T, and E) were also stratified by sex. Toxicant measures from blubber and scat samples from the same individual were compared by relative contribution of individual PCB and PBDE congeners.

Albion test fishery data was used to estimate the number of Chinook salmon returning to the Fraser River watershed (henceforth referred to as "FR Chinook").[52] The FR Chinook salmon collectively consist of three runs: Spring run, before July 15; Summer run, between July 15 and September 1; and Fall run, after September 1.[53] Catch data were smoothed using a general linear model to create a continuous predictor variable by year (SI Figure S1). The Albion test fishery is an estimated 140 km from the west side of San Juan Island. To account for fish travel time between the whales' primary feeding area, where the majority of our samples were collected, and the test fishery, fish run data were lagged by 12-days.[41] (See SI for details.)

The evaluation of toxicant concentration and prey abundance was performed using FR Chinook as the main effects predictor. Toxicant measures were incorporated by class of compound, ∑(cumulative)4PCBs, ∑40PCBs, ∑2PBDEs, and p,p'-DDE, and by total POPs (tPOPs; defined as the summation of the individual classes). A subanalysis was conducted using Principal Component Analysis (PCA) factors from congener- and metabolite-specific data. Toxicant values were normalized as percent per ∑40PCBs, ∑11PBDEs, or ∑6DDTs. All values were log-transformed to achieve normal distribution and entered into the PCA with varimax (orthogonal) rotation. Components needed to have an eigenvalue >1.0 to be retained. (See SI for details.) Covariates of interest included FR Chinook, year of sample collection, season, pod, age-sex class, years of accumulation, birth order, fecal thyroid (T3, T4, and T3/T4 ratio) levels, and fecal GC levels. Four samples were excluded because they fell outside of the range of dates with associated prey abundance data, after lagging. Three additional samples were excluded due to missing hormone (covariate) data. The final analytic data set contained 133 samples. The main effects were tested for linearity, as well as interactions with the retained covariates.

All models used mixed effects multiple linear regression. Whale identity was included as a random effect to account for repeat sampling of individuals. Final model selections were based on the smallest value of AICc (ΔAICc > 2). All statistical analyses were performed in SAS v9.3 (SAS Institute Inc., Cary, NC).

RESULTS

∑4PCBs, ∑2PBDEs, and p,p'-DDE were measured in 157 samples, with confirmed genetic identity on 140 samples from 54 unique whales. Twenty-three of the samples used for the toxicant analysis were collected in 2010, 47 in 2011, 39 in 2012, and 31 samples in 2013. Samples represented all three SRKW pods, with 35% of samples from unique members of J pod (19/54), 24% from K pod, and 40% from L pod (Table 1). Likewise, age-sex class was well represented with 22% of samples from unique juvenile whales (12/54), 39% from reproductive-age females, 13% from postreproductive females, and 30% from adult males. There was no statistical difference in ∑4PCBs, ∑40PCBs, ∑2PBDEs, or p,p'-DDE concentrations between year or pod (Table 1). Samples were collected across seasons; season was not statistically different for any class of toxicant compounds (Table 1). Lipid levels in scat increased with toxicant concentration (p < 0.05), indicating fat-soluble toxicants were excreted with the lipids. (See SI for details.)

Eighteen individual whales had toxicant measures on both high-mass scat samples and blubber biopsy samples. Previous analyses demonstrated significant linear associations between measures of p,p'-DDE, ∑4PCBs, and ∑2PBDEs in blubber and scat samples matched by individual.[35] In the present study,

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Table 2. tPOP, ∑4PCBs, ∑40PCBs, ∑2PBDEs, and *p,p′*-DDE (ng/g la) in Scat with Respect to FR Chinook Abundance

metric (ng/g la)	*n*	estimated slope	standard error	95% CI	*p*-value
∑4PCBs	133	−0.102	0.049	−0.199, −0.005	0.019
∑40PCBs	53	−0.163	0.106	−0.376, 0.050	0.132
∑2PBDEs	133	−0.088	0.033	−0.153, −0.022	0.052
p,p′-DDE	133	−0.087	0.046	−0.158, −0.016	0.022
tPOP	133	−0.107	0.045	−0.196, −0.017	0.014

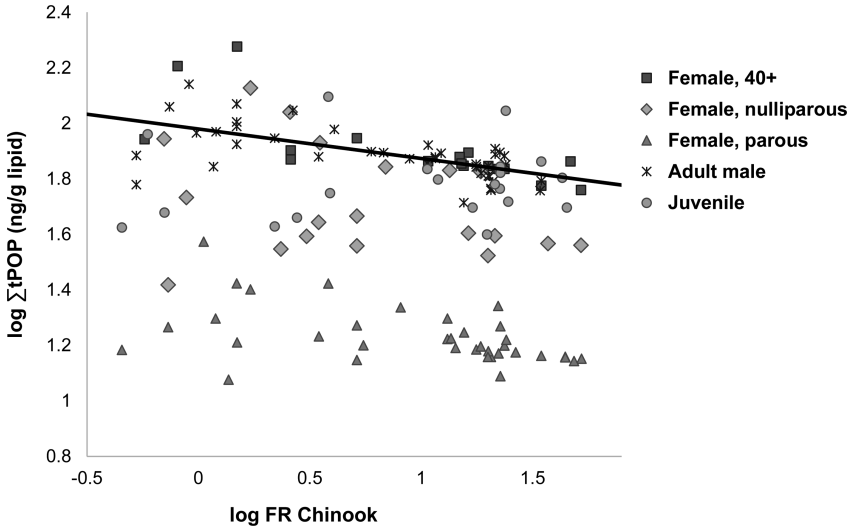

Figure 2. tPOP (ng/g lipid) in scat with respect to FR Chinook abundance; line represents linear trend for adult males.

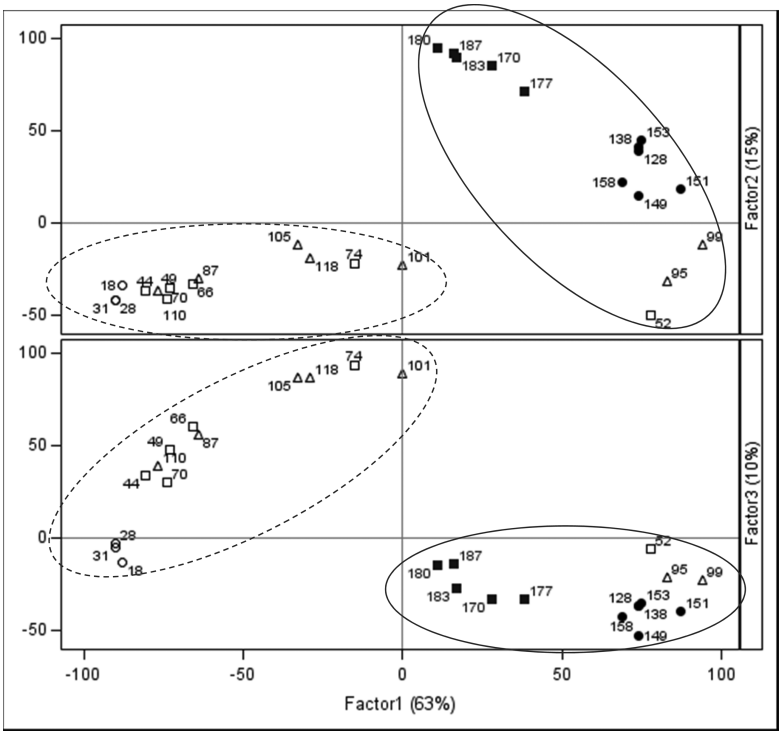

Figure 3. Rotated Factor Solution from PCA for PCB congeners measured in scat samples of Southern Resident killer whales; *n* = 56 samples. Number of chlorines: ○ = 3, □ = 4, Δ = 5, ● = 6, ■ = 7. Solid line circle denotes Persistent PCBs. Dashed circle denotes Readily Metabolized PCBs.

congener-specific analyses comparing relative concentrations in scat and blubber samples collected from the same whale demonstrated significant linear associations (*p*-value < 0.05) for all PCBs evaluated, PCBs 28, 52, 99, 101, 105, 118, 138, 149,

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153, and 187, with the exception of PCB180 (p-value < 0.10), as well as PBDEs 47 and 99 (PCBs, Figure 1; PBDEs, SI Figure S2). Relative concentrations of PBDEs 28, 100, 153, and 154 were not significantly associated between the matrices (p-values > 0.10). Inclusion of years between blubber and scat sample collection from the same whale did not improve the fit of the data to the model. (See SI for details).

The concentration of tPOPs in scat samples decreased as prey abundance increased (slope estimate, −0.107; 95% Confidence Interval [CI], −0.196 to −0.017) (Table 2; Figure 2). Individual models of ∑4PCBs, ∑2PBDEs, and p,p'-DDE also demonstrated significant inverse estimations (all metrics, p < 0.05) (Table 2). The PCA (high-mass samples only; n = 56) demonstrated 63.1% of the total variance in the PCB data set was accounted for by Factor 1. Factor 1 readily differentiated PCBs by structural group; positive loadings included all PCBs in groups 1, 2, and 5 ("Persistent PCBs"; resistant to metabolism and degradation) and negative loadings included all PCBs in groups 3 and 4 ("Readily Metabolized PCBs") (Figure 3).[10,11] (See SI for details). Regression model estimates for PCA Factor 1 with respect to FR Chinook abundance was −0.552 (95% CI, −1.061 to −0.044) indicating a change in PCB profile across a gradient of prey abundance (Table 3).

Table 3. PCA Factors 1, 2, and 3 for PCB Congeners with Respect to FR Chinook Abundance

	estimated slope	standard error	95% CI	p-value
factor 1	−0.552	0.252	−1.061, −0.044	0.034
factor 2	0.232	0.422	−0.679, 1.082	0.586
factor 3	0.107	0.341	−0.580, 0.795	0.754

Factors 2 and 3 accounted for 14.6% and 10.0% of the total variance in the data set, respectively, and distinguished congeners by number of chlorine substituents. These factors were not significantly associated with prey abundance when run as conditional response variables (Table 3). In similar analyses, PCA factors for PBDEs and DDTs were also unassociated with prey abundance (data not shown).

Total POPs increased with years of accumulation in parous females (tPOPs: slope estimate, 0.048; 95% CI, 0.014 to 0.082) and postreproductive females (0.007; 0.003 to 0.010) (p-value < 0.05 for both) (Figure 4; SI Table S1). There was a suggested (nonsignificant) increase of tPOP and years of accumulation for adult males (slope estimate, 0.019; 95% CI, −0.005 to 0.043), and a suggested decrease in juveniles (−0.069; −0.159 to 0.021) and nulliparous females (−0.027; −0.084 to 0.030) (Figure 4; SI Table S1). Similar patterns were found when individual models were run for ∑4PCBs, ∑2PBDEs, and p,p'-DDE (SI Table S1). The pattern for ∑40PCBs demonstrated a suggested increase for juveniles and decrease for adult males. This reasons for this are unknown at this time, but may be related to sample size (n = 56).

∑4PCB levels were lower for parous females [mean, 95% CI; 6.1, 4.5−8.3] compared to all other age-sex class groups (juveniles, 17.2, 11.7−25.1; females, nulliparous; 15.0, 8.7−26.0; females, 40+, 23.4, 15.2−36.1; and adult males, 20.7, 15.1−28.3) (p-values < 0.001, = 0.035, < 0.001, and < 0.001 respectively) (Table 1). Similar findings were evident for p,p'-DDE, ∑40PCBs, and ∑2PBDEs. The decrease in toxicant concentration with each successive calf in parous females was monotonic, with a significant trend for both ∑40PCBs ng/g la [coefficient, −0.179; p-value < 0.001] and percent Persistent PCBs (−0.036; 0.029) (n = 24) (SI Table S2; data not shown). The statistical trend was maintained for ∑4PCBs, ∑2PBDEs, and p,p'-DDE (p < 0.015) (SI Table S2). There was also a monotonic decrease in toxicant concentration by birth order with significant trends (∑4PCBs, ∑2PBDEs, p,p'-DDE; all p < 0.033) (n = 22) (SI Table S3).

In reproductive-age females, ∑40PCBs ng/g la concentrations were similar in samples from whales characterized as possibly pregnant mean, 13.7; 95% CI, 2.3−81.9), lactating less than 1 year (12.1; 3.9−37.3), resting mature (11.7; 4.7−29.2), and confirmed pregnant (10.0; 2.6−37.4); concentration in females lactating between 1 and 2 years was lower (6.3; 1.8−21.9), but not significant (Table 1). The contaminant profile demonstrated a nonsignificant but notable increase in percent Persistent PCBs in whales classified as possibly pregnant (73%; 95% CI, 61−85) compared to other reproductive groups (range, 43−56%) (Figure 5). All samples from possibly pregnant whales were from three nulliparous females (J31, J32, L90). The parous females were either confirmed pregnant (J16, J28, L55), lactating (J17, J28, J35, K12, L54), or were

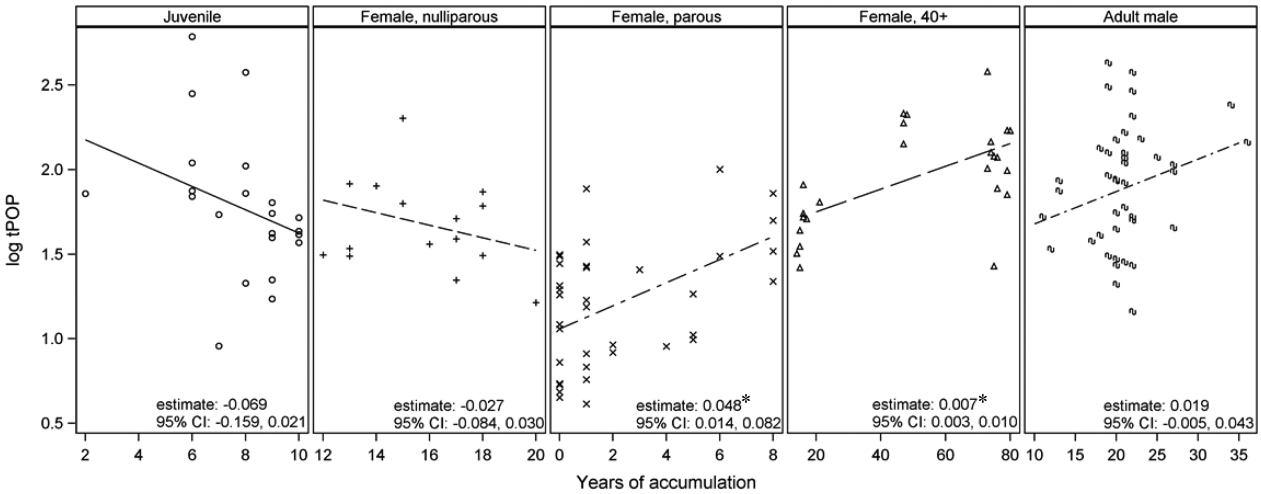

Figure 4. tPOP (ng/g la) in scat with respect to years of accumulation by age-sex class; n = 140 samples; * p-value < 0.05.

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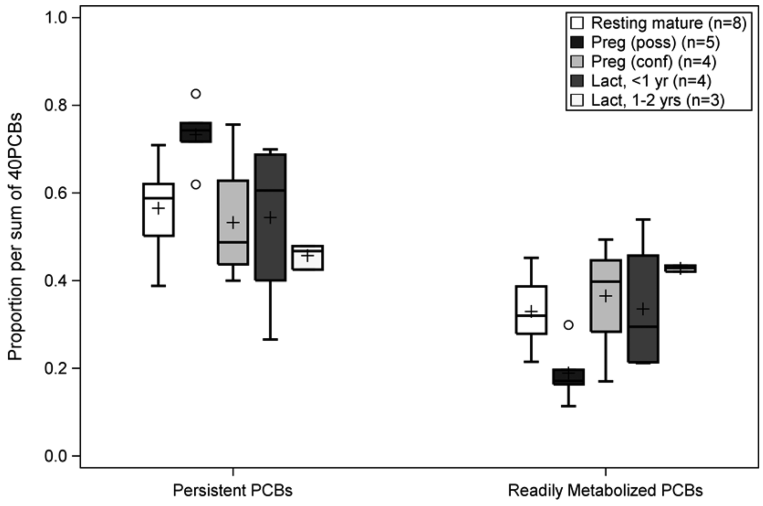

Figure 5. Proportion of PCB structural group (per ∑40PCBs) in scat by reproductive status in reproductive-age females; n = 24 scat samples from 16 unique whales.

neither pregnant nor lactating (resting mature; J14, J19, K14, K16, L82) at the time of sampling.

Fortuitously, repeat samples were collected from two nursing whales, J35 nursing her first calf, J47, and K12 nursing her fifth calf, K43. Samples from J35 were collected on lactation days 223 and 663, and samples from K12 were collected on lactation days 302 and 640. For both whales, the concentration of ∑40PCBs ng/g la decreased across lactation days; the primiparous J35 decreased from 33.4 to 5.6 (an 83% difference), and multiparous K12 decreased from 3.6 to 3.3 (an 8% difference) (data not shown). The percent Persistent PCBs decreased from 70% to 48% in J35 and 54% to 47% in K12, across lactation days (Figure 6). Similarly, the decrease in ∑6DDTs and ∑11PBDEs ng/g la across lactation days was 80% and 84% in J35, and 20% and 14% in K12, respectively (data not shown).

The associations of hormone (T3, T4, T3/T4, GC, P4, E, and T) and toxicant (∑4PCB, ∑2PBDE, and *p,p′*-DDE) concentrations were evaluated, adjusted for prey abundance (FR Chinook) and age-sex class. ∑40PCB was not included because the restricted sample size (n = 56) reduced analysis power. A Bonferroni correction was used to account for multiple comparisons (dividing the critical value, 0.05, by the number of models, n = 21), resulting in a critical value of 0.002. Using this conservative critical value, only the inverse association between estrogen and ∑2PBDE was significant (slope estimate, −0.167; p-value < 0.001) (SI Table S4).

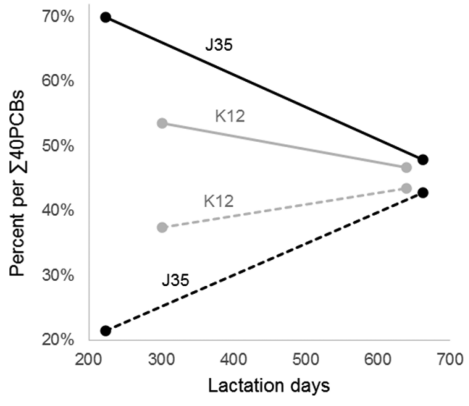

Figure 6. Percent of PCB structural group (per ∑40PCBs) in scat by days of lactation for repeat scat samples collected from a lactating primiparous female (J35) and a lactating multiparous female (K12). Solid line, Persistent PCBs. Dashed line, Readily Metabolized PCBs.

DISCUSSION

Toxicant measures from the 140 scat samples analyzed in this study demonstrate the influence of age-sex class, and reproductive factors such as pregnancy and lactation, on contaminant accumulation and mobilization in Southern Resident killer whales. The patterns are consistent with previous findings using measures from blubber biopsy samples, such as decreased toxicant concentrations in parous females.[14,54] Unique to this study is the demonstration of toxicant concentration and profile modulation by prey abundance. The implications of increased toxicant exposures during nutritional shortage emphasize the importance of salmon recovery in killer whale conservation efforts.

Decreased prey (Chinook salmon) abundance has been associated with increased mortality[55] and decreased fecundity[45] in this population of killer whales. Toxicant exposures may exacerbate these outcomes, particularly the elevated levels of "Persistent PCBs" during nutritional shortage. Persistent PCBs induce bioactivating enzymes related to deleterious end points such as reproductive impairment and compromised immune function.[25,28,30,31,56,57] The Persistent PCBs are more resistant to metabolic breakdown, determined by the organization of hydrogen and chlorine atoms on the biphenyl ring and described in previous studies on seals and cetaceans,[10,11] and are more lipophilic (log octanol:water partition coefficient (log K_{ow}): 6.7−7.1) than the Readily Metabolized PCBs (log K_{ow}: 5.9−6.3). These characteristics indicate a larger capacity to accumulate in the fat-rich tissues of the killer whales.[58] An increase in Persistent PCBs when prey abundance is low (Table 3; PCA Factor 1: slope estimate, −0.552; 95% CI, −1.061 to −0.044) indicates the source of the exposure is likely from internal lipid stores that are metabolized for energy.

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Our analysis assumes exposure to a consistent toxicant source throughout the season. However, the FR Chinook salmon are divided into distinct spawning time periods in Spring, Summer, and Fall.[53] Our findings could reflect change in toxicant profiles of FR Chinook across the different stocks, such as migration-related metabolism leading to a decrease in the more Readily Metabolized (i.e., less chlorinated) PCB congeners.[8] Our findings could also be influenced by change to an alternative prey source, such as chum salmon (*O. keta*), coho salmon (*O. kisutch*), or Pacific halibut (*Hippoglossus stenolepis*), that may have a different contamination profile.[7,50] Further study evaluating the toxicant profiles of FR Chinook salmon and other potential prey sources includes calculation of a metabolic index that would quantify relative bioaccumulation in the food chain, indicating whether the toxicants are from a prey or blubber source.[59] Elevated concentrations of POPs were identified in the Spring season compared to the Summer and Fall (Table 1). However, our annual sampling began before the Spring FR Chinook run peak and ended before the Fall run began to decline. Therefore, a FR Chinook abundance related effect on toxicant measures may not have been captured in the Fall. Additional sampling later in the Fall run, as well as in the winter season, would also be informative. Lastly, environmental degradation may alter toxicant profiles.[60] However, all samples were collected shortly after defecation (estimated 30 minutes), thus minimizing the exposure of the sample to environmental conditions (e.g., UV exposure, ocean salts, etc.). Additionally, correlations of scat and matched blubber toxicant concentrations were significant ($p < 0.01$)[35] increasing confidence that any weathering that may have occurred would not substantially modify the results.

The increase in toxicant concentration with age, excluding toxicant offloading due to placental and lactational transfer, was expected based on known bioaccumulation patterns and previous studies on wild cetacean populations (Figure 4; SI Table S1).[14,21,31,33,54,61] The suggested inverse association with age for juveniles was expected based on growth dilution of toxicants in calves.[2,14] The suggested inverse association in nulliparous females, on the other hand, was unanticipated. This pattern may be due to toxicant offloading from undocumented fetal or neonate loss, continued growth dilution effect (whales reach full size around age 20),[62] or orphan-induced lactation (a phenomenon reported in *Tursiops*).[13] Years of accumulation and postjuvenile growth dilution was further evaluated with adult male and female whales stratified by 10−20, and greater than 20 years old. No significant associations were found. The slope estimate in adult males, 10−20 years old, was 0.004 (tPOP; 95% CI, −0.033 to 0.041) (data not shown), compared to −0.027 (95% CI, −0.084 to 0.030) in nulliparous females of similar age. As such, although growth dilution may contribute to this effect in nulliparous females, alternative explanations listed above are also likely.

The four nulliparous females in this study ranged in age from 16 to 22 years. Three of these nulliparous females (J31, J32, and L90) had a possible pregnancy (defined above) over the course of this 4 year study. Of these, J32 was found dead with an almost full term fetus in December of 2014.[63] Fertility in this population generally starts around age 10, reaching a maximum between ages 20−22.[45] The delay in producing a successful calf by nulliparous female whales negatively impacts growth and long-term viability of this endangered population. High POP burdens have previously been associated with disruption of reproduction in marine mammals.[32,34,64,65] Further sampling with particular focus on reproductive-age females of known pregnancy status would contribute to the understanding of calf survivorship and POP concentrations in this population.

We found an 83% difference in ∑40PCBs concentrations in J35 over 440 days of nursing her first calf, and an 8% difference in ∑40PCBs concentrations in K12 over 338 days nursing her fifth calf. These results are consistent with the estimated 70−90% lactation transfer of maternal toxicant body burden in primiparous females,[31] based on average values reported previously in other species of delphinids [bottlenose dolphins (*Tursiops truncatus*) and striped dolphins (*Stenella coeruleoalba*)].[66] This trend is reinforced by decreasing toxicant burden with increasing parity in reproductive-age females (tPOP; p-value = 0.004) (SI Table S2), and a decreasing trend of toxicant burden in juvenile whales by birth order (tPOPs: p-value < 0.001) (SI Table S3). Marine mammals closely related to killer whales [Family: Delphinidae; bottlenose dolphin (*Tursiops truncates*), common dolphin (*Delphinus delphis*), and humpback dolphin (*Sousa Chinensis*)] have been documented to produce milk that is 10−30% fat, largely derived from the mobilization of endogenous lipid reserves.[13,67] As fat reserves are depleted, dietary fats may become a greater contributor of lipids for milk production. The contamination patterns of J35 and K12 supports that assertion with an increase in Readily Metabolized PCBs over the course of lactation days (Figure 6). By the second year of lactation both nursing mothers had a similar percentage of Readily Metabolized PCBs (43% and 43%, respectfully) suggesting that milk production was less dependent on endogenous lipids.

PBDEs and PCBs share a structural similarity to thyroid hormone, and have been demonstrated to disrupt thyroid hormone levels.[68−71] Specifically, consistent evidence has shown an associated decrease in thyroxin (T4), the precursor hormone of triiodothyronine (T3),[71] and a PCB-related increase in thyroid hormone receptor α mRNA has been identified in blubber biopsy samples from killer whales.[72] As such, PBDE and PCB concentrations were expected to modify thyroid hormone concentrations in any given sample. This was not found, but could have been masked by effects of prey abundance on thyroid hormone (a marker of nutritional status[41]) and toxicant concentrations. Further evaluation with a larger sample size may highlight this distinction, if it exists. Previous associations of toxicants and modified GC, P4, E, and T levels have been demonstrated, but have been inconsistent.[73] Our finding of a significant inverse association between PBDEs and estrogen (slope estimate, −0.167; p-value < 0.001) was not entirely surprising based on previous demonstrations of PBDE interacting with estrogen receptors.[71] PBDE exposure has specifically been associated with decreased serum estradiol concentrations in females rats,[74] and their offspring.[74,75] There is also supporting evidence for an association of PCB exposure and modified estrogens levels,[72,76,77] which was significant in this study without the multiple comparison adjustment (slope estimate, −0.225; p-value < 0.050) (SI Table S4).

The effect of diminishing salmon populations is consistently being demonstrated as a predominant contributor to the decline of the SRKWs.[41,45,55] Salmon are an endangered[78] keystone species with a biologic foundation that ensure nutritional preservation of innumerable wildlife species from birds to bears and orcas as well as commercial, tribal, and recreational fishing communities. The Transient ecotype of killer whale share summer foraging grounds with Southern Resident killer whales and have concentrations of POPs that far

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exceed the concentrations of the SRKWs,[21] yet their population has been steadily growing for at least four decades.[79] The consistent prey base of pinnipeds, cetaceans, and seabirds[6] has kept Transient killer whales from experiencing the physiologic consequence of nutritional shortage. Further study comparing these two populations may help elucidate how the interaction between prey shortage and exposure to lipophilic POPs impacts SRKW population recovery and survival.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b00825.

Text includes additional details of fecal hormone measures, PCB structural groups, statistical comparison of toxicant measures in scat and blubber biopsy samples, statistical analysis of lipid excretion, laboratory analysis of toxicant concentration in scat, and data management. Figures include a comparison of scat and blubber toxicant concentrations by PBDE congeners, and smoothed FR Chinook data. Tables include years of toxicant accumulation by age-sex class, toxicant concentration by number of calves (parous females), toxicant concentration by birth order (juvenile whales), and toxicant concentration evaluations with hormone indices (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jlundin2@uw.edu.

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) USEPA, United States Environmental Protection Agency. *The Foundation for Global Action on Persistent Organic Pollutants: A United States Perspective*, 2002.

(2) Hickie, B. E.; Ross, P. S.; Macdonald, R. W.; Ford, J. K. Killer whales (Orcinus orca) face protracted health risks associated with lifetime exposure to PCBs. *Environ. Sci. Technol.* **2007**, *41* (18), 6613–9.

(3) National Marine Fisheries Service. *Recovery Plan for Southern Resident Killer Whales (Orcinus orca)*; National Marine Fisheries Service: Northwest Region, Seattle, WA. 2008.

(4) NOAA Fisheries Killer Whale (Orcinus Orca). http://www.nmfs.noaa.gov/pr/species/mammals/whales/killer-whale.html (accessed February 25, 2015).

(5) Ford, J. K. B.; Ellis, G. M. Selective foraging by fish-eating killer whales Orcinus orca in British Columbia. *Mar. Ecol.: Prog. Ser.* **2006**, *316*, 185–199.

(6) Ford, J. K. B.; Ellis, G. M.; Barrett-Lennard, L. G.; Morton, A. B.; Palm, R. S.; Balcomb, K. C., III Dietary specialization in two sympatric populations of killer whales (Orcinus orca) in coastal British Columbia and adjacent waters. *Can. J. Zool.* **1998**, *76* (8), 1456–1471.

(7) Hanson, M. B.; Baird, R. W.; Ford, J. K. B.; Hempelmann, J. A.; Van Doornik, D. M.; Candy, J. R.; Emmons, C. K.; Schorr, G. S.; Gisborne, B.; Ayres, K. L.; Wasser, S. K.; Balcomb, K. C.; Balcomb-Bartok, K.; Sneva, J. G.; Ford, M. J. Species and stock identification of prey consumed by endangered southern resident killer whales in their summer range. *Endangered Species Research* **2010**, *11*, 69–82.

(8) Cullon, D. L.; Yunker, M. B.; Alleyne, C.; Dangerfield, N. J.; O'Neill, S.; Whiticar, M. J.; Ross, P. S. Persistent organic pollutants in chinook salmon (Oncorhynchus tshawytscha): implications for resident killer whales of British Columbia and adjacent waters. *Environ. Toxicol. Chem.* **2009**, *28* (1), 148–61.

(9) Alava, J. J.; Ross, P. S.; Lachmuth, C.; Ford, J. K.; Hickie, B. E.; Gobas, F. A. Habitat-based PCB environmental quality criteria for the protection of endangered killer whales (Orcinus orca). *Environ. Sci. Technol.* **2012**, *46* (22), 12655–63.

(10) Boon, J. P.; Oostingh, I.; van der Meer, J.; Hillebrand, M. T. A model for the bioaccumulation of chlorobiphenyl congeners in marine mammals. *Eur. J. Pharmacol., Environ. Toxicol. Pharmacol. Sect.* **1994**, *270* (2–3), 237–51.

(11) Wolkers, H.; Corkeron, P. J.; Van Parijs, S. M.; Simila, T.; Van Bavel, B. Accumulation and transfer of contaminants in killer whales (Orcinus orca) from Norway: indications for contaminant metabolism. *Environ. Toxicol. Chem.* **2007**, *26* (8), 1582–90.

(12) Aguilar, A.; Borrell, A.; Pastor, T. Biological factors affecting variability of persistent pollutant levels in cetaceans. *J. Cetacean Res. Manage.* **1999** (Special Issue 1), 83–116.

(13) Oftedal, O. T. Lactation in whales and dolphins: evidence of divergence between baleen- and toothed-species. *Journal of mammary gland biology and neoplasia* **1997**, *2* (3), 205–30.

(14) Krahn, M. M.; Hanson, M. B.; Schorr, G. S.; Emmons, C. K.; Burrows, D. G.; Bolton, J. L.; Baird, R. W.; Ylitalo, G. M. Effects of age, sex and reproductive status on persistent organic pollutant concentrations in "Southern Resident" killer whales. *Mar. Pollut. Bull.* **2009**, *58* (10), 1522–9.

(15) Wolkers, H.; Burkow, I. C.; Hammill, M. O.; Lydersen, C.; Witkamp, R. F. Transfer of polychlorinated biphenyls and chlorinated pesticides from mother to pup in relation to cytochrome P450 enzyme activities in harp seals (Phoca groenlandica) from the gulf of St. Lawrence, Canada. *Environ. Toxicol. Chem.* **2002**, *21* (1), 94–101.

(16) Addison, R. F.; Brodie, P. F. Transfer of organochlorine residues from blubber through the circulatory system to milk in the lactating

DOI: 10.1021/acs.est.6b00825
Environ. Sci. Technol. 2016, 50, 6506–6516

gray seal (Halichoerus grypus). *Can. J. Fish. Aquat. Sci.* **1987**, *44*, 782−786.
(17) Debier, C.; Pomeroy, P. P.; Dupont, C.; Joiris, C.; Comblin, V.; Le Boulenge, E.; Larondelle, Y.; Thome, J. P. Quantitative dynamics of PCB transfer from mother to pup during lactation in UK grey seals Halichoerus grypus. *Mar. Ecol.: Prog. Ser.* **2003**, *247*, 237−248.
(18) Pomeroy, P. P.; Green, N.; Hall, A. J.; Walton, M.; Jones, K. C.; Harwood, J. Congener-specific exposure of grey seal (Halichoerus grypus) pups to chlorinated biphenyls during lactation. *Can. J. Fish. Aquat. Sci.* **1996**, *53*, 1526−1534.
(19) Knott, K. K.; Boyd, D.; Ylitalo, G. M.; O'Hara, T. M. Lactational transfer of mercury and polychlorinated biphenyls in polar bears. *Chemosphere* **2012**, *88* (4), 395−402.
(20) Kannan, K.; Blankenship, A. L.; Jones, P. D.; Giesy, J. P. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum. Ecol. Risk Assess.* **2000**, *6*, 181−201.
(21) Ross, P. S.; Ellis, G. M.; Ikonomou, M. G.; Barrett-Lennard, L. G.; Addison, R. F. High PCB concentrations in free-ranging pacific killer whales, Orcinus orca: effects of age, sex and dietary preference. *Mar. Pollut. Bull.* **2000**, *40* (6), 504−515.
(22) Krahn, M. M.; Hanson, M. B.; Baird, R. W.; Boyer, R. H.; Burrows, D. G.; Emmons, C. K.; Ford, J. K. B.; Jones, L. L.; Noren, D. P.; Ross, P. S.; Schorr, G. S.; Collier, T. K. Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from southern resident killer whales. *Mar. Pollut. Bull.* **2007**, *54* (12), 1903−11.
(23) Kavlock, R. J.; Daston, G. P.; DeRosa, C.; Fenner-Crisp, P.; Gray, L. E.; Kaattari, S.; Lucier, G.; Luster, M.; Mac, M. J.; Maczka, C. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. *Environ. Health Perspect.* **1996**, *104* (Suppl 4), 715. 10.1289/ehp.96104s4715
(24) de Swart, R. L.; Ross, P. S.; Vos, J. G.; Osterhaus, A. D. Impaired immunity in harbour seals (Phoca vitulina) exposed to bioaccumulated environmental contaminants: review of a long-term feeding study. *Environ. Health Perspect* **1996**, *104* (Suppl 4), 823−8.
(25) Jepson, P. D.; Bennett, P. M.; Deaville, R.; Allchin, C. R.; Baker, J. R.; Law, R. J. Relationships between polychlorinated biphenyls and health status in harbor porpoises (Phocoena phocoena) stranded in the United Kingdom. *Environ. Toxicol. Chem.* **2005**, *24* (1), 238−48.
(26) Beland, P.; DeGuise, S. Toxic compounds and health reproductive effects in St. Lawrence beluga whales. *J. Great Lakes Res.* **1993**, *19*, 766−775.
(27) Hall, A. J.; Hugunin, K.; Deaville, R.; Law, R. J.; Allchin, C. R.; Jepson, P. D. The risk of infection from polychlorinated biphenyl exposure in the harbor porpoise (Phocoena phocoena): a case-control approach. *Environ. Health Perspect.* **2006**, *114* (5), 704−711.
(28) Lahvis, G. P.; Wells, R. S.; Kuehl, D. W.; Stewart, J. L.; Rhinehart, H. L.; Via, C. S. Decreased lymphocyte responses in free-ranging bottlenose dolphins (Tursiops truncatus) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ. Health Perspect* **1995**, *103* (Suppl 4), 67−72.
(29) Reijnders, P. J. Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* **1986**, *324* (6096), 456−457.
(30) Pierce, G. J.; Santos, M. B.; Murphy, S.; Learmonth, J. A.; Zuur, A. F.; Rogan, E.; Bustamante, P.; Caurant, F.; Lahaye, V.; Ridoux, V.; Zegers, B. N.; Mets, A.; Addink, M.; Smeenk, C.; Jauniaux, T.; Law, R. J.; Dabin, W.; López, A.; Alonso Farré, J. M.; González, A. F.; Guerra, A.; García-Hartmann, M.; Reid, R. J.; Moffat, C. F.; Lockyer, C.; Boon, J. P. Bioaccumulation of persistent organic pollutants in female common dolphins (Delphinus delphis) and harbour porpoises (Phocoena phocoena) from western European seas: geographical trends, causal factors and effects on reproduction and mortality. *Environ. Pollut.* **2008**, *153* (2), 401−415.
(31) Wells, R. S.; Tornero, V.; Borrell, A.; Aguilar, A.; Rowles, T. K.; Rhinehart, H. L.; Hofmann, S.; Jarman, W. M.; Hohn, A. A.; Sweeney, J. C. Integrating life-history and reproductive success data to examine potential relationships with organochlorine compounds for bottlenose dolphins (Tursiops truncatus) in Sarasota Bay, Florida. *Sci. Total Environ.* **2005**, *349* (1−3), 106−19.
(32) Schwacke, L. H.; Voit, E. O.; Hansen, L. J.; Wells, R. S.; Mitchum, G. B.; Hohn, A. A.; Fair, P. A. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (Tursiops truncatus) from the southeast United States coast. *Environ. Toxicol. Chem.* **2002**, *21* (12), 2752−2764.
(33) Hall, A. J.; McConnell, B. J.; Rowles, T. K.; Aguilar, A.; Borrell, A.; Schwacke, L.; Reijnders, P. J.; Wells, R. S. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. *Environ. Health Perspect* **2006**, *114* (Suppl 1), 60−4.
(34) Murphy, S.; Barber, J. L.; Learmonth, J. A.; Read, F. L.; Deaville, R.; Perkins, M. W.; Brownlow, A.; Davison, N.; Penrose, R.; Pierce, G. J.; Law, R. J.; Jepson, P. D. Reproductive Failure in UK Harbour Porpoises Phocoena phocoena: Legacy of Pollutant Exposure? *PLoS One* **2015**, *10* (7), e0131085.
(35) Lundin, J. I.; Dills, R.; Ylitalo, G. M.; Ahmad, J.; Hempelmann, J. A.; Parsons, K. M.; Wasser, S. K. Persistent organic pollutant determination in killer whale scat samples: optimization and validiation of the method, and application to real samples. *Arch. Environ. Contam. Toxicol.* **2016**, *70* (1), 9−19.
(36) Sloan, C. A.; Anulacion, B. F.; Baugh, K. A.; Bolton, J. L.; Boyd, D.; Boyer, R. H.; Burrows, D. G.; Herman, D. P.; Pearce, R. W.; Ylitalo, G. M. *Northwest Fisheries Science Center's Analyses of Tissue, Sediment, And Water Samples for Organic Contaminants by Gas Chromatography/ Mass Spectrometry and Analyses of Tissue for Lipid Classes by Thin Layer Chromatography/Flame Ionization Detection*. In U.S. Department of Commerce, 2014.
(37) Wasser, S. K.; Azkarate, J. C.; Booth, R. K.; Hayward, L.; Hunt, K.; Ayres, K.; Vynne, C.; Gobush, K.; Canales-Espinosa, D.; Rodriguez-Luna, E. Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. *Gen. Comp. Endocrinol.* **2010**, *168* (1), 1−7.
(38) Wasser, S. K.; Hunt, K. E.; Brown, J. L.; Cooper, K.; Crockett, C. M.; Bechert, U.; Millspaugh, J. J.; Larson, S.; Monfort, S. L. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. *Gen. Comp. Endocrinol.* **2000**, *120* (3), 260−75.
(39) Wasser, S. K.; Davenport, B.; Ramage, E. R.; Hunt, K. E.; Parker, M.; Clarke, C.; Stenhouse, G. Scat detection dogs in wildlife research and management: Applications to grizzly and black bears in the Yellowhead Ecosystem, Alberta, Canada. *Can. J. Zool.* **2004**, *82*, 475−492.
(40) Rolland, R. M.; Hamilton, P. K.; Kraus, S. D.; Davenport, B.; Gillett, R. M.; Wasser, S. K. Faecal sampling using detection dogs to study reproduction and health in north Atlantic right whales (Eubalaena glacialis). *J. Cetacean Res. Manage.* **2006**, *8* (2), 121−125.
(41) Ayres, K. L.; Booth, R. K.; Hempelmann, J. A.; Koski, K. L.; Emmons, C. K.; Baird, R. W.; Balcomb-Bartok, K.; Hanson, M. B.; Ford, M. J.; Wasser, S. K. Distinguishing the impacts of inadequate prey and vessel traffic on an endangered killer whale (Orcinus orca) population. *PLoS One* **2012**, *7* (6), e36842.
(42) Center for Whale Research. Official Orca Survey. Naturalist's Family Tree Guide to Orca Whales of the Southern Resident Community. 2013 Fall Edition. http://www.whaleresearch.com/orca_ID_pods.html (accessed December 8, 2015).
(43) Robeck, T. R.; Steinman, K. J.; Gearhart, S.; Reidarson, T. R.; McBain, J. F.; Monfort, S. L. Reproductive physiology and development of artificial insemination technology in killer whales (Orcinus orca). *Biol. Reprod.* **2004**, *71* (2), 650−60.
(44) Center for Whale Research. About killer whales, http://www.whaleresearch.com/#!about-orcas/c1qa8.
(45) Ward, E. J.; Holmes, E. E.; Balcomb, K. C. Quantifying the effects of prey abundance on killer whale reproduction. *J. Appl. Ecol.* **2009**, *46*, 632−640.
(46) Ford, M. J.; Hanson, M. B.; Hempelmann, J. A.; Ayres, K. L.; Emmons, C. K.; Schorr, G. S.; Baird, R. W.; Balcomb, K. C.; Wasser, S. K.; Parsons, K. M.; Balcomb-Bartok, K. Inferred Paternity and Male Reproductive Success in a Killer Whale (Orcinus orca) Population. *J. Hered.* **2011**, *102* (5), 537−53.

DOI: 10.1021/acs.est.6b00825
Environ. Sci. Technol. 2016, 50, 6506−6516

(47) Wasser, S. K.; Lundin, J. I.; Ayres, K. A.; Seely, E.; Giles, D.; Hempelmann, J.; Parsons, K. M.; Booth, R. K. Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident Killer Whales (Orcinus orca). *General and Comparative Endocrinology* **in review**.
(48) Environmental Protection Agency. *Method 1614. Brominated Diphenyl Ethers in Water Soil, Sediment and Tissue by HRGC/HRMS*, EPA-821-R-07-005. 2007.
(49) Environmental Protection Agency. *Method 3630C. Silica Gel Cleanup*, 1996.
(50) Ford, M. J.; Hempelmann, J.; Hanson, M. B.; Ayres, K. L.; Baird, R. W.; Emmons, C. K.; Lundin, J. I.; Schorr, G. S.; Wasser, S. K.; Park, L. K. Estimation of a Killer Whale (Orcinus orca) Population's Diet Using Sequencing Analysis of DNA from Feces. *PLoS One* **2016**, *11* (1), e0144956.
(51) Barrett-Lennard, L. G. *Population Structure and Mating Patterns of Killer Whales as Revealed by DNA Analysis*, Ph.D. Thesis, University of British Columbia: Vancouver, BC, 2000.
(52) Government of Canada, Fisheries and Oceans Canada, Albion Chinook Daily Catch Per Unit Effort, Fraser River at Albion, BC, 2014. http://www.pac.dfo-mpo.gc.ca/fm-gp/fraser/docs/commercial/albionchinook-quinnat-eng.html.
(53) DFO, Fraser River Chinook Salmon. Department of Fisheries and Oceans (DFO): Canada, Stock Status Report D6-11. 1999.
(54) Ylitalo, G. M.; Matkin, C. O.; Buzitis, J.; Krahn, M. M.; Jones, L. L.; Rowles, T.; Stein, J. E. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (Orcinus orca) from Prince William Sound, AK. *Sci. Total Environ.* **2001**, *281* (1−3), 183−203.
(55) Ford, J. K.; Ellis, G. M.; Olesiuk, P. F.; Balcomb, K. C. Linking killer whale survival and prey abundance: food limitation in the oceans' apex predator? *Biol. Lett.* **2010**, *6* (1), 139−142.
(56) McFarland, V. A.; Clarke, J. U. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ. Health Perspect* **1989**, *81*, 225−39.
(57) Kannan, K.; Tanabe, S.; Borrell, A.; Aguilar, A.; Focardi, S.; Tatsukawa, R. Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the western Mediterranean sea. *Arch. Environ. Contam. Toxicol.* **1993**, *25* (2), 227−33.
(58) Erickson, M. D. *Analytical Chemistry of PCBs*, 2nd ed.; Lewis Publishers: Boca Raton, FL, 1997.
(59) Wolkers, H.; van Bavel, B.; Derocher, A. E.; Wiig, O.; Kovacs, K. M.; Lydersen, C.; Lindstrom, G. Congener-specific accumulation and food chain transfer of polybrominated diphenyl ethers in two arctic food chains. *Environ. Sci. Technol.* **2004**, *38* (6), 1667−74.
(60) Urbaniak, M. Polychlorinated biphenyls: sources, distribution and transformation in the environment-a literature review. *Acta Toxicol* **2007**, *15* (2), 83−93.
(61) Yordy, J. E.; Wells, R. S.; Balmer, B. C.; Schwacke, L. H.; Rowles, T. K.; Kucklick, J. R. Life history as a source of variation for persistent organic pollutant (POP) patterns in a community of common bottlenose dolphins (Tursiops truncatus) resident to Sarasota Bay, FL. *Sci. Total Environ.* **2010**, *408* (9), 2163−72.
(62) Fearnbach, H.; Durban, J. W.; Ellifrit, D. K.; Balcomb, K. C., III Size and long-term growth trends of endangered fish-eating killer whales. *Endangered Species Research* **2011**, *13*, 173−180.
(63) DFO Fisheries and Oceans Canada, Necropsy Results: Southern Resident Killer Whale J32. http://www.pac.dfo-mpo.gc.ca/fm-gp/species-especes/mammals-mammiferes/srkw-eprs-j32-eng.html.
(64) Reddy, M. L.; Reif, J. S.; Bachand, A.; Ridgway, S. H. Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. *Sci. Total Environ.* **2001**, *274* (1−3), 171−82.
(65) Murphy, S.; González, Á. F.; Guerra, Á. Assessing the effect of persistent organic pollutants on reproductive activity in common dolphins and harbour porpoises. *Journal of the Northwest Atlantic Fisheries Organization* **2010**, *42*, 153−173.
(66) Mongillo, T. M.; Holmes, E. E.; Noren, D. P.; VanBlaricom, G. R.; Punt, A. E.; O'Neill, S. M.; Ylitalo, G. M.; Hanson, M. B.; Ross, P. S. Predicted polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) accumulation in southern resident killer whales. *Mar. Ecol.: Prog. Ser.* **2012**, *453*, 263−277.
(67) West, K. L.; Oftedal, O. T.; Carpenter, J. R.; Krames, B. J.; Campbell, M.; Sweeney, J. C. Effect of lactation stage and concurrent pregnancy on milk composition in the bottlenose dolphin. *J. Zool.* **2007**, *273* (2), 148−160.
(68) Hallgren, S.; Sinjari, T.; Håkansson, H.; Darnerud, P. O. Effects of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.* **2001**, *75* (4), 200−8.
(69) Hallgren, S.; Darnerud, P. O. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and chlorinated paraffins (CPs) in rats-testing interactions and mechanisms for thyroid hormone effects. *Toxicology* **2002**, *177* (2−3), 227−43.
(70) Brouwer, A.; Morse, D. C.; Lans, M. C.; Schuur, A. G.; Murk, A. J.; Klasson-Wehler, E.; Bergman, A.; Visser, T. J. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. *Toxicol. Ind. Health* **1998**, *14* (1−2), 59−84.
(71) Costa, L. G.; Giordano, G.; Tagliaferri, S.; Caglieri, A.; Mutti, A. Polybrominated diphenyl ether (PBDE) flame retardants: environmental contamination, human body burden and potential adverse health effects. *Acta Biomed* **2008**, *79* (3), 172−83.
(72) Buckman, A. H.; Veldhoen, N.; Ellis, G.; Ford, J. K.; Helbing, C. C.; Ross, P. S. PCB-associated changes in mRNA expression in killer whales (Orcinus orca) from the NE Pacific Ocean. *Environ. Sci. Technol.* **2011**, *45* (23), 10194−202.
(73) Tyler, C. R.; Jobling, S.; Sumpter, J. P. Endocrine disruption in wildlife: a critical review of the evidence. *Crit. Rev. Toxicol.* **1998**, *28* (4), 319−61.
(74) Lilienthal, H.; Hack, A.; Roth-Harer, A.; Grande, S. W.; Talsness, C. E. Effects of developmental exposure to 2,2, 4,4, 5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behavior in rats. *Environ. Health Perspect* **2006**, *114* (2), 194−201.
(75) Talsness, C. E.; Kuriyama, S. N.; Sterner-Kock, A.; Schnitker, P.; Grande, S. W.; Shakibaei, M.; Andrade, A.; Grote, K.; Chahoud, I. In utero and lactational exposures to low doses of polybrominated diphenyl ether-47 alter the reproductive system and thyroid gland of female rat offspring. *Environ. Health Perspect* **2008**, *116* (3), 308−14.
(76) Kraugerud, M.; Zimmer, K. E.; Dahl, E.; Berg, V.; Olsaker, I.; Farstad, W.; Ropstad, E.; Verhaegen, S. Three structurally different polychlorinated biphenyl congeners (Pcb 118, 153, and 126) affect hormone production and gene expression in the human H295R in vitro model. *J. Toxicol. Environ. Health, Part A* **2010**, *73* (16), 1122−32.
(77) Haave, M.; Ropstad, E.; Derocher, A. E.; Lie, E.; Dahl, E.; Wiig, O.; Skaare, J. U.; Jenssen, B. M. Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. *Environ. Health Perspect* **2002**, *111* (4), 431−6.
(78) National Marine Fisheries Service, Endangered and Threatened Marine Species under NMFS' Jurisdiction, 2015 http://www.nmfs.noaa.gov/pr/species/esa/listed.htm#fish.
(79) Allen, B. M.; Angliss, R. P., Alaska Marine Mammal Stock Assessments. Killer Whale (Orcinus orca): West Coast Transient Stock. NOAA-TM-AFSC-277. 2013.

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Environ. Sci. Technol. 2016, 50, 6506−6516