



## PBDE flame retardants and PCBs in migrating Steller sea lions (*Eumetopias jubatus*) in the Strait of Georgia, British Columbia, Canada

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### ABSTRACT

Polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) were measured in blubber biopsy samples from 22 live-captured Steller sea lions (*Eumetopias jubatus*) that had just entered the Strait of Georgia, British Columbia, Canada, for their overwintering feeding season.  $\Sigma$ PBDE ranged from 50  $\mu\text{g kg}^{-1}$  (lipid weight) in adult females to 3780  $\mu\text{g kg}^{-1}$  in subadult individuals.  $\Sigma$ PCBs ranged from 272  $\mu\text{g kg}^{-1}$  in adult females to 14280  $\mu\text{g kg}^{-1}$  in subadult individuals. While most PBDE and PCB congeners were transferred through milk to pups, PCBs with  $\log K_{OW} > 7.0$  (PCBs 206, 207, 208 and 209) appeared constrained, resulting in a lighter mixture in pups compared to adult females. The ratio of individual PCB congeners by metabolic group (Groups I, II, III, IV and V) to PCB-153 regressed against length of males suggested poor biotransformation of these compounds (slopes did not differ from zero,  $p > 0.05$ ). PBDE congeners 49, 99, 153 and 183 appeared bioaccumulative (slopes of ratio BDE/PCB 153 versus length were higher than zero,  $p < 0.05$ ), but the dominance of the single congener, BDE-47 (64% of total PBDEs), likely due in part to debromination pathways, reduced our ability to explore congener-specific dynamics of PBDEs in these pinnipeds. With 80% of our Steller sea lions exceeding a recent toxicity reference value for PCBs, the fasting-associated mobilization of these contaminants raises concerns about a heightened vulnerability to adverse effects during annual migrations.

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### 1. Introduction

Persistent organic pollutants (POPs) represent a threat to marine mammals due to their recalcitrance, bioaccumulative nature and toxicity. Polychlorinated biphenyls (PCBs) are legacy industrial POPs that were banned during the late 1970s in North America and are today subject to the terms of the Stockholm Convention. More recently, polybrominated diphenyl ethers (PBDEs) have emerged as a significant concern, having been extensively used as flame retardants in foams, textiles, coatings, furniture, construction materials, electronic devices, plastics and paints since the 1970s (de Boer et al., 1998; de Wit, 2002; Alaei et al., 2003).

There are three primary commercial PBDE products, including the penta-BDE, octa-BDE and deca-BDE formulations (La Guardia

et al., 2006). In Europe and North America, production of two PBDE products (penta- and octa-BDE formulations) ceased in 1998 and 2004, respectively. The third (deca) formulation was recently banned in Europe and Canada, and is subject to some state-based bans in the US and increasing voluntary restrictions (La Guardia et al., 2006; Birnbaum, 2009; de Boer, 2009; Ross et al., 2009). The tetra, penta, hexa and heptabromodiphenyl mixtures are currently classified as POPs under the terms of the Stockholm Convention, and the octa BDE formulation may be added eventually to the list of banned POPs (de Boer, 2009).

Despite having been banned, PCBs are still found at high concentrations in some marine biota of northern hemisphere (Hall and Thomas, 2007; Kelly et al., 2007). The NE Pacific Ocean is no exception, with very high PCB concentrations having been observed in killer whales, *Orcinus orca* (Ross et al., 2000; Ylitalo et al., 2001), and to a lesser extent harbor seals, *Phoca vitulina* (Ross et al., 2004). PBDEs have also been detected in marine mammals from the NE Pacific Ocean, although at lower concentrations than the PCBs (Rayne et al., 2004; Krahn et al., 2007; Ross et al., 2012).

High levels of POPs have been implicated in adverse effects on immune and endocrine systems of marine mammals, with the PCBs, in particular, being of concern (Ross et al., 1996; De Guise

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et al., 1998; Tabuchi et al., 2006; Mos et al., 2006). While many of the measured endpoints are considered sub-lethal, the fitness of individuals is also being affected. High levels PCBs have been associated with a high prevalence of neoplasms and carcinoma, causing mortality in California sea lions, *Zalophus californianus* (Ylitalo et al., 2005). PCBs have been widely implicated in reduced reproduction in pinnipeds in the North and Baltic Seas (Helle et al., 1976; Reijnders, 1986). While less is known about the toxicity of PBDEs, this flame retardant has been implicated in carcinogenicity and the disruption of steroid and thyroid hormones (Meerts et al., 2000, 2001; Hallgren and Darnerud, 2002).

The Steller sea lion (*Eumetopias jubatus*) is a piscivorous pinniped that inhabits the Pacific coastal waters of Canada, the USA and Asia. There are two populations, with the Eastern and Western stocks being genetically distinct and geographically separated at approximately 145° W longitude (Bickham et al., 1996). While the eastern stock is considered stable, the western stock has declined by 80% during the last 30 years across its entire range (National Research Council (2003). In addition to the hypotheses involving nutritional stress and shifts in ocean-climate, which might explain this decline (Rosen and Trites, 2000; Trites et al., 2007), contaminants have also been suggested as a possible contributing factor (Barron et al., 2003). Low to moderate concentrations of PCBs have been observed in Steller sea lions from both the declining western stock (Varanasi et al., 1992; Lee et al., 1996; Krahn, 1997, Krahn et al., 2001) and the stable eastern stock (Krahn, 1997, Krahn et al., 2001). To date, there have been no studies on PCBs or PBDEs in Steller sea lions from British Columbia and adjacent southern coastal US states.

The total Steller sea lion population in British Columbia during the breeding season is estimated to be approximately 20000 individuals, with an overall growth rate of 3.5% per year (Olesiuk, 2008). Of these, approximately 3000 individuals migrate into the waters off southern Vancouver Island and into the Strait of Georgia (Olesiuk, 2004; A. Trites, pers. comm.). Although the British Columbia population has been increasing, Steller sea lions are listed as "Special Concern" under the terms of the Species at Risk Act (SARA) because of human disturbance, risk of oil spills and environmental contaminants (COSEWIC, 2003; Olesiuk, 2008).

As part of a larger effort to characterize the feeding ecology of Steller sea lions frequenting the Strait of Georgia, British Columbia, 22 animals were live-captured and telemetry devices attached prior to release (Jeffries et al., 2004; North Pacific Universities Marine Mammal Research Consortium, 2006). This capture provided a valuable opportunity to measure contaminants and to characterize this potential conservation threat.

## 2. Materials and methods

### 2.1. Capture and sampling

Steller sea lions were live-captured at Norris Rocks (49°29'00"N, 124°39'00"W) in the Strait of Georgia, British Columbia, Canada, in February 2005 and January 2006, using a floating mobile trap described elsewhere (Jeffries et al., 2004). After capture, sea lions were moved into a transfer cage and weighed, and then moved into a squeeze cage, where they were physically restrained. Valium was administered (0.02 to 0.11 mg kg<sup>-1</sup>, mean dosage of 0.06 mg kg<sup>-1</sup>) via intramuscular injection in the shoulder area to those individuals upon which telemetry devices were being attached. Valium was given 10–20 min prior to general anesthetic using isoflurane, administered via a cone over the head. Intubation of the stomach was performed if a stomach sensor was to be inserted. Monitoring was done with a Heska G2Digital pulse-oximeter and temperature probe.

Blubber biopsy samples were collected from 22 individuals, including a freshly aborted fetus, pups ( $n = 3$ ), subadults ( $n = 10$ ), adult females ( $n = 6$ ) and adult males ( $n = 2$ ). Blubber samples were obtained with a 6 mm-biopsy punch from a cleansed (betadine and isopropyl alcohol) site 20 cm lateral to the spinal column and anterior to the pelvis as described elsewhere (Tabuchi et al., 2006; Mos et al., 2006). These samples were wrapped in hexane-rinsed aluminum foil, placed in plastic bags, and temporarily stored on wet ice in the field. After transfer to the laboratory, samples were frozen within 4 h at  $-80^{\circ}\text{C}$  at the Institute of Ocean Sciences (Fisheries and Ocean Canada) until further analysis.

No teeth were extracted for age determination, such that sea lions in this study were grouped by age class for interpretation of contaminant concentrations. We defined a nursing pup as an individual with an estimated age of 0–1.5 years (one deceased pup was a known-age 1.5 year individual, WDFW0206-01, which had milk in its stomach at the time of sampling). Nursing dependency can last up to three years in Steller sea lions (Pitcher and Calkins, 1981). Sampling data, including dates, age and sex categories, morphometrics and lipid content are reported in Table 1.

### 2.2. Contaminant analyses

Blubber samples were analyzed by AXYS Analytical Services Ltd. (Sidney, BC, Canada), using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) as described elsewhere (Christensen et al., 2005). Briefly, blubber samples (ranging 75–440 mg wet weight) were analyzed using an Ultima HRMS equipped with a Hewlett–Packard 5890 GC and a DB-5 Durabond capillary column (60 m  $\times$  0.25 mm, 0.10  $\mu\text{m}$  film). Percent of lipid in samples was determined at using the gravimetric lipid determination by weight of extract method with dichloromethane (DCM).

Briefly, samples were spiked with <sup>13</sup>C-labeled surrogate standards ( $n = 12$  PBDEs;  $n = 29$  PCBs) and then ground with anhydrous sodium sulfate. Samples were transferred to a Soxhlet thimble, surrogate standard was added, and samples were refluxed for 16 h with DCM. The extract was eluted through a gel permeation column with 1:1 DCM:hexane. The extract was applied to a partially deactivated Florisil column and eluted with hexane followed by 15:85 DCM:hexane. Elutes were then combined and eluted with 1:1 DCM:hexane and each fraction was concentrated. Included with each batch of samples was a procedural blank. The lab blank had concentrations above detectable levels ( $<25\text{ pg g}^{-1}$ ) for 20 PBDE congeners, while for most of the PCB congeners the lab blank had concentrations above  $<5\text{ pg g}^{-1}$ .

Limits of detections (LODs) for PBDE congeners generally ranged from  $<10$  to  $<60\text{ pg g}^{-1}$  wet weight, with exception of BDE-209 which had LODs ranging from 66.2 to 2480  $\text{pg g}^{-1}$ . For PCBs, the LODs were in general  $<10\text{ pg g}^{-1}$ , and, in most cases,  $<5\text{ pg g}^{-1}$ . For PBDEs, a total of 34 individual PBDE congener peaks ranging from dibromodiphenyl ethers through decabromodiphenyl ether and six co-eluting bands (each composed of two congeners) were identified and quantified in the blubber samples, constituting a data set of 40 congeners overall: BDE-7, -8/11, -10, -12/13, -15, -17/25, -28/33, -30, -32, -35, -37, -47, -49, -51, -66, -71, -75, -77, -85, -99, -100, -105, -116, -119/120, -126, -128, -138/166, -140, -153, -154, -155, -181, -183, -190, -203, -206, -207, -208, -209.

### 2.3. Data treatment and statistical analysis

Concentrations of PBDEs and PCBs were calculated and reported as the sum of the concentrations congeners (i.e.,  $\sum\text{PBDE}$  and  $\sum\text{PCB}$ ) that were detectable in at least 15 out of 22 individual sea lions ( $\geq 68\%$  of samples). When congeners were not detected, detection limit substitutions were made using half the limit of

**Table 1**

Life history and collection data of Steller sea lions captured at Norris Rock, Strait of Georgia, British Columbia, Canada.

Code ID	Weight (kg)	Length (cm)	Girth (cm)	Sex	Age class	Age (years)	Blubber sample size (mg)	Lipid %
WDFW 0206-02	2	NR	NR	M	Fetus <sup>a</sup>	<0.0	158	8.51
WDFW 0206-01	91	NR	NR	F	Pup <sup>b</sup>	1.5	441	83.4
EJ 05-07	115	171	127	F	Pup	1.5	80	24.3
EJ 05-03	101	162	117	M	Pup	1.5	49	27.0
EJ 05-04	221	214	153	M	Subadult	3.5	74	27.0
EJ 05-05	233	222	155	M	Subadult	4.5–5.5	89	9.44
EJ 05-09	164	207	148	M	Subadult	2.5	68	1.94
EJ 05-13	214	219	137	M	Subadult	3.5	92	30.5
EJ 05-14	270	242	164	M	Subadult	5.5	112	13.5
EJ 05-15	166	207	132	M	Subadult	2.5–3.5	84	18.5
EJ 05-17	195	208	139	M	Subadult	3.5–4.5	108	15.6
EJ 06-02	216	209	143	M	Subadult	3–4	88	15.8
EJ 06-08	298	242	157	M	Subadult	6.5	100	62.7
EJ 06-10	140	188	116	F	Subadult	2.5–3.5	74	55.4
EJ 05-01	278	227	162	F	Adult	NR	117	46.5
EJ 05-18	223	217	142	F	Adult	3.5–4.5	106	18.4
EJ 06-03	200	203	138	F	Adult	3–5	78	11.4
EJ 06-07	187	211	142	F	Adult	3.5–5.5	109	44.8
EJ 06-09	155	197	124	F	Adult	3.5–4.5	103	51.6
EJ 06-11	332	233	167	F	Adult	8–15	83	43.3
EJ 06-01	479	278	184	M	Adult	7–10	98	6.81
EJ 06-05	385	247	169	M	Adult	6.5–8.5	75	15.0

NR = No reported.

<sup>a</sup> Aborted.<sup>b</sup> Fresh dead animal found inside cage.

detection. Where less than 15 animals had detectable concentrations of an analyte (<68% of samples), 0 ng kg<sup>-1</sup> was substituted for non-detect concentrations. Contaminants were not reported if there were low non-detectable ranges (NDRs) in combination with non-detectable levels (<LOD) in all sea lion samples. PBDE concentrations were calculated as the sum of the concentrations of 11 congeners, including co-eluting congeners, that were detectable in ≥68% of samples ( $\sum$ PBDEs = BDE -7/25, -28/33, -47, -49, -66, -99, -100, -153, -154, -155 and -183).

For all PBDE and PCB congeners, reported concentrations were adjusted on the basis of their respective recoveries, as well as concentrations found in the laboratory blank (i.e., the blank concentration was subtracted from the actual concentration measured in the sample). The method of detection limit (MDL) was calculated as the standard deviations of the two procedural blanks analyzed with the samples times three (i.e.,  $3 \times SD_{\text{blanks}}$ ), and then compared to the blank-subtracted concentrations of samples. Samples with concentrations above the MDL were reported. Corrected concentrations of contaminants were lipid normalized by dividing wet weight concentrations by the lipid content ( $\mu\text{g kg}^{-1}$  lipid) of each sample.

Morphometric data were log-transformed to meet the assumption of normality prior to statistical analyses. Analysis of variance (ANOVA) followed by a Tukey–Kramer multiple comparison post hoc test were used to make inter-group comparisons of weight, length and lipid content among subadults, adult females and adult males. The fetus was not included in the ANOVA as only one individual was available.

Inter-group comparisons of PBDEs and PCBs were conducted using ANOVA, with Tukey–Kramer multiple-comparison post hoc test. When the variances of each group were significantly different or unequal (Bartlett test  $p < 0.05$ ), a Welch analysis of variance (ANOVA) was carried out, followed by a Tukey–Kramer multiple-comparison test. The possible confounding effects of life history parameters were assessed by plotting the log transformed data of PBDE and PCBs for subadult and adult males versus length (used in this case as a proxy for age). Adult females were not included in this exercise owing to the influence of the onset of sexual maturity and reproduction, including gestation, parturition and nursing.

Thus, analysis of linear regression was used for each contaminant class to determine if length has a significant influence in contaminants concentration.

Statistical analysis was carried out with JMP 7.0 (SAS Institute Inc.; Cary, NC, USA, 2007) at a level of significance of  $\alpha = 0.05$ . Data were presented as the mean plus or minus the Standard Deviation of the mean (SDM).

#### 2.4. Congener-specific metabolism

The role of biotransformation of individual PBDE and PCB congeners was explored to explore the possible roles of CYP1A and CYP2B metabolic induction in male sea lions. First, the relative presence of each PBDE congener, expressed as a percent of the recalcitrant PCB 153 (i.e., PBDEx/PCB 153 ratios) was calculated and regressed against length (again, a proxy for age) to assess accumulation and metabolism of PBDEs by individuals over time.

In order to explore PCB metabolism, the five structure-activity groups described on the basis of the position of vicinal H atoms and number of chlorine substitutions in the *ortho* position (Boon et al., 1997) were used. Group I is comprised of congeners without any vicinal hydrogen atoms on carbons of either phenyl ring (PCBs 111, 133, 146, 153/168, 162, 165, 167, 172, 175, 178, 180/193, 187, 189, 191, 194, 196, 198/199, and 201–209). Group II is comprised of congeners with vicinal H atoms exclusively at *ortho*- and *meta*-carbons in combination with two or more *ortho*-chlorine atoms (PCBs 44/47/65, 83/99, 85/116/117, 128/166, 129/138/160/163, 130, 137, 158, 170, 177, 190, and 195). Group III congeners have *ortho*- and *meta*-hydrogen pairs with less than two *ortho*-chlorines (PCBs 20/28, 37, 60, 61/70/74/76, 63, 66, 68, 77, 105, 114, 118, 123, and 156/157). Group IV congeners have *meta*- and *para*-hydrogen pairs with two or fewer *ortho*-chlorines (PCBs 21/33, 26/29, 31, 49/69, 52, 59/62/75, 64, 86/87/97/108/119/125, 90/101/113, 92, 109, and 110/115). Group V congeners have *meta*- and *para*-hydrogen pairs with more than two *ortho*-chlorines (PCBs 93/95/98/100/102,135/151/154, 136, 144, 147/149, 174, 176, 179, and 197/200).

The ratio of individual congeners within each group relative to PCB-153 were calculated (PCBx/PCB153), and then regressed

against length. PCB-153 was used as a reference congener to assess the biotransformation of PCBs in sea lions because this congener is one of the most recalcitrant and bioaccumulative congeners in marine mammals (Tanabe et al., 1988; Boon et al., 1997; Wolkers et al., 2004). The following criteria were used to examine metabolism: (a) Poor biotransformation was inferred when the slope of PBDEx/PCB 153 or PCBx/PCB153 ratios versus length = 0 (i.e., slope does not differ significantly from zero); (b) Little or no biotransformation was inferred when a slope of >0 for a positive relationship between the ratios versus length was observed (i.e., the chemical accumulates due to a lack of metabolism); and (c) Metabolism or biotransformation was inferred when the slope <0 or if a significant negative relationship existed (i.e., the chemical decreases due to elimination). The latter supported a role for CYP1A and CYP2B metabolic induction (Boon et al., 1997).

### 2.5. Health risk assessment

The toxic equivalency quotient (TEQ) was calculated for PCBs in Steller sea lions based on toxic equivalency factors (TEFs) established for dioxin-like PCBs, including the planar (non-ortho) PCBs ( $\Sigma$ PCBs 77, 81, 126, and 169) and mono-ortho PCBs ( $\Sigma$ PCBs 105, 114, 118, 123, 156, 157, 167 and 189) (Van den berg et al., 2006). For the  $\Sigma$ TEQ calculations, substitutions using half of detection limits were made when congener-specific PCBs (i. e. planar, non-ortho PCBs) were not detected. The TEQs were then compared to the TEQ threshold levels, including the no observable adverse effect level (NOAEL) and the lowest observable adverse effect level (LOAEL) for dioxin-like PCBs, derived from immunotoxic action and endocrine disruption endpoints assessed in harbor seals (Ross et al., 1995; Kannan et al., 2000). In addition, we used a normal probability density function of log PCB concentrations (i.e., relative frequency) in the animals, which was compared to the recent PCB threshold effect concentration of 1300  $\mu\text{g kg}^{-1}$  lipid for harbor seals (Mos et al., 2010).

## 3. Results and discussion

### 3.1. Biological influences on contaminant levels

Morphometrics varied among Steller sea lion age classes, with average body weights of  $432 \pm 66.5$  kg for adult males,  $229 \pm 65.0$  kg for adult females,  $212 \pm 48.5$  kg for subadults, and  $102 \pm 12.0$  kg for pups (Table 1). Weight and length were

significantly correlated in all Steller sea lions ( $r = 0.94$ ,  $p < 0.0001$ ), as well as in the age categories of subadults ( $r = 0.89$ ;  $p = 0.00004$ ) and adults ( $r = 0.98$ ;  $p = 0.00001$ ). Although male subadults and female adults did not differ in weight and length, the two male adults were heavier (ANOVA,  $p = 0.0052$ ; Tukey–Kramer multicomparisons test,  $p < 0.05$ ) and longer (ANOVA,  $p = 0.0058$ ; Tukey–Kramer multicomparisons test,  $p < 0.05$ ) when compared to male subadults and female adults (Table 1).

Despite some morphometric differences (Supporting Information; Table 1) among age and sex classes, concentrations of  $\Sigma$ PBDEs did not differ among age categories of Steller sea lions (ANOVA,  $F = 0.4883$ ;  $df = 3$ ;  $p = 0.6950$ ; Table 2). There was no correlation between  $\Sigma$ PBDEs and length in male subadults and adults ( $r = 0.31$ ;  $p = 0.3476$ ). In contrast,  $\Sigma$ PBDEs decreased with increasing length of female adults ( $r = 0.83$ ;  $p = 0.0386$ ). This may have been due to the reproductive loss of these fat-soluble contaminants with increasing age (Addison and Smith, 1974; Addison and Brodie, 1987; Aguilar et al., 1999; Ross et al., 2000).

As with PBDEs, no differences in  $\Sigma$ PCB concentrations were observed among age or sex classes (ANOVA,  $F = 0.6095$ ;  $df = 3$ ;  $p = 0.6179$ ; Tukey–Kramer multicomparisons test,  $p > 0.05$ ; Table 3).  $\Sigma$ PCB did not correlate with length in the combined male subadult and adult category ( $r = 0.36$ ;  $p = 0.2796$ ). However,  $\Sigma$ PCBs decreased with increasing length of adult females ( $r = 0.85$ ;  $p = 0.0314$ ), again likely due to age-related loss of these contaminants with female reproduction. The small sample size and lack of detailed age information may have precluded a full exploration of these factors in this study.

### 3.2. PBDE and PCB concentrations and patterns

A total of 11 out of 40 PBDE congeners sought were detected in at least 68% of Steller sea lion samples.  $\Sigma$ PBDEs measured as the sum of these 11 congeners ranged from 50 to 3776  $\mu\text{g kg}^{-1}$  lipid (Table 2), with a geometric mean of 464  $\mu\text{g kg}^{-1}$  lipid (lower value geometric SD = 149  $\mu\text{g kg}^{-1}$  lipid; upper value geometric SD = 1445  $\mu\text{g kg}^{-1}$  lipid). The pattern of PBDE congeners in subadults is shown in Fig. 1a, as an example. Overall, BDE-47 (2,2',4,4'-tetrabromodiphenyl ether) was the dominant congener in all samples, representing  $64.4\% \pm 1.7\%$  of the  $\Sigma$ PBDE concentrations. Other major congeners were BDE-100 (2,2',4,4',6-pentabromodiphenyl ether) and BDE 99 (2,2',4,4',5-pentabromodiphenyl ether), which made up  $16.5\% \pm 0.5\%$  and  $10.5\% \pm 1.2\%$  of  $\Sigma$ PBDEs, respectively (Fig. S1 in Supplementary material).

**Table 2**  
Lipid content and concentration means (range) for the top six PBDEs and the top six PCB congeners ( $\mu\text{g kg}^{-1}$  lipid) detected in blubber samples of Steller sea lions. Data are arranged by age/sex categories.

	Fetus male (n = 1)	Pups (n = 3)	Subadults (n = 10)	Adult females (n = 6)	Adult males (n = 2)
Lipid %	8.51	45.0 (24.0–83.0)	25.0 (1.90–63.0)	36 (11.0–52.0)	10.9 (6.80–15.0)
BDE-28/33	2.50	12.0 (2.00–20.8)	10.1 (0.93–45.6)	3.80 (1.12–6.25)	4.32 (1.40–7.25)
BDE-47	88.5	558 (57.8–1158)	740 (70.3–2777)	323 (30.5–832)	364 (1.36–593)
BDE-99	20.8	92.7 (9.37–199)	69.2 (11.5–159)	47.8 (5.12–106)	86.9 (64.9–109)
BDE-100	22.3	130 (15.0–275)	171 (13.0–600)	90.1 (6.69–230)	116 (54.2–177)
BDE-153	3.90	13.4 (1.61–29.7)	14.3 (2.69–31.3)	10.6 (1.25–21.4)	15.0 (14.4–15.7)
BDE-154	5.61	19.5 (2.17–42.1)	27.4 (2.78–92.7)	17.3 (2.03–42.5)	19.0 (13.3–24.7)
$\Sigma$ PBDE <sup>a</sup>	151	843 (91–1759)	1049 (110–3776)	503 (49.7–1258)	620 (336–904)
PCB-153/168	82.3	614 (70.1–1328)	967 (90.6–2960)	446 (39.9–1081)	583 (260–906)
PCB-129/138/160/163	62.5	436 (53.2–871)	627 (59.8–1946)	277 (30.0–647)	399 (180–619)
PCB-118	33.2	217 (26.2–432)	325 (36.7–1009)	125 (17.3–308)	172 (75.3–269)
PCB-180/193	30.9	197 (21.4–456)	276 (20.1–771)	140 (11.2–345)	188 (81.9–295)
PCB-83/99	27.8	188 (20.5–378)	288 (28.7–920)	123 (13.8–294)	141 (62.4–219)
PCB-147/149	28.4	110 (16.0–176)	109 (11.0–456)	43.2 (12.8–82.9)	90.0 (30.7–149)
$\Sigma$ PCB <sup>b</sup>	572	3158 (393–6296)	4294 (398–14277)	1895 (272–4386)	2713 (1114–4311)

Dominant congener in coeluting peaks as follows: PCB-153/168; PCB-129/138/160/163; PCB-180/193; PCB-83/99; PCB-147/149.

<sup>a</sup> Total PBDE concentrations included the 11 congeners detected in Steller sea lion blubber samples.

<sup>b</sup> Total PCB concentrations included the 93 congeners detected in Steller sea lion blubber samples.

**Table 3**

Regression statistics for the relationships between the ratios of individual PBDE congeners relative to PCB 153 versus length in male Steller sea lions.

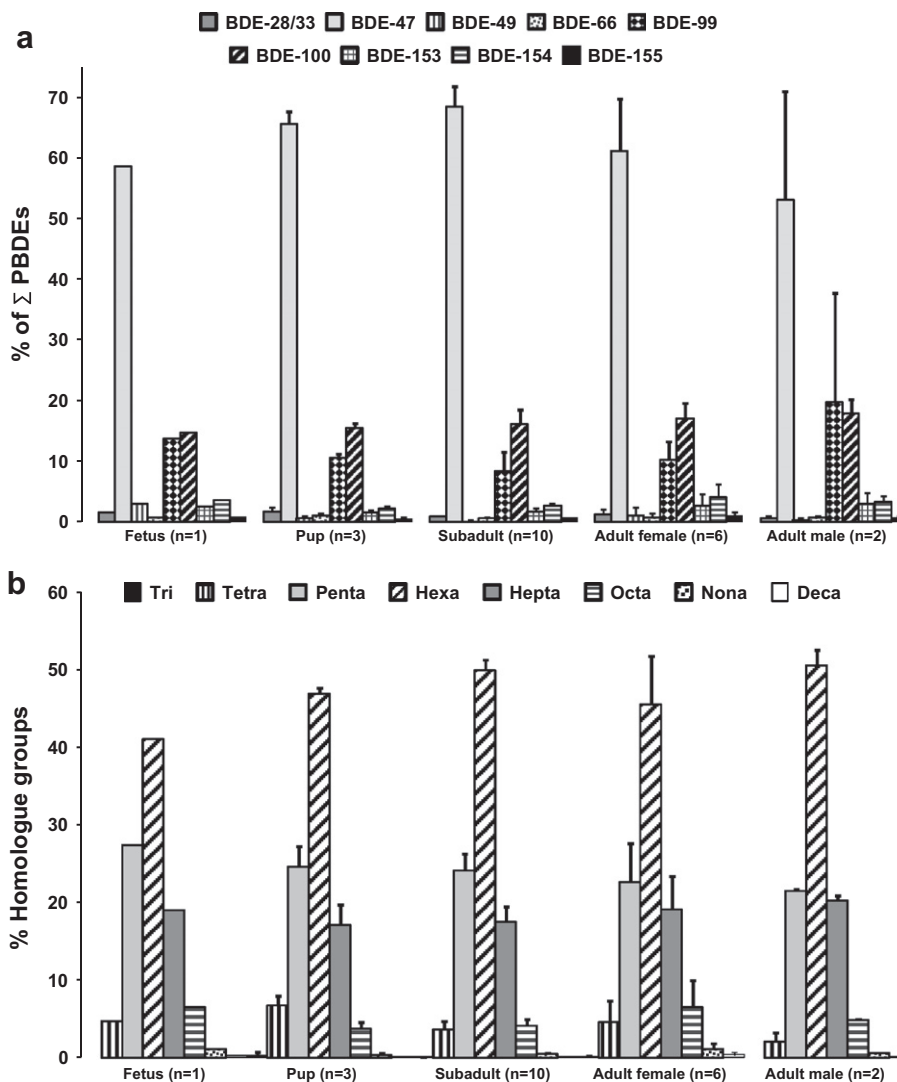
	Slope	$r^2$	$p$ value
<i>PBDEs/PCB 153</i>			
BDE 17/25	$1.5 \times 10^{-05}$	0.193	0.1768
BDE 28/33	$-6.6 \times 10^{-05}$	0.211	0.1550
BDE 47	$-2.9 \times 10^{-03}$	0.129	0.2780
BDE 49	$7.2 \times 10^{-05}$	0.503	0.0146 <sup>a</sup>
BDE 66	$4.8 \times 10^{-05}$	0.274	0.0988
BDE 99	$3.8 \times 10^{-03}$	0.622	0.0039 <sup>a</sup>
BDE 100	$1.8 \times 10^{-04}$	0.008	0.7899
BDE 153	$4.2 \times 10^{-04}$	0.584	0.0062 <sup>a</sup>
BDE 154	$2.2 \times 10^{-04}$	0.249	0.1185
BDE 155	$1.6 \times 10^{-05}$	0.050	0.5080
BDE 183	$1.5 \times 10^{-05}$	0.406	0.0351 <sup>a</sup>

<sup>a</sup> Slope was significantly different from zero.

While the contribution of most congeners (e.g., BDEs-28/33, -66, -100, -153, -154, -183) remained constant among different age classes (ANOVA with Tukey–Kramer test for BDE 17/25, 100 and 183,  $p > 0.05$ ; and Welch ANOVA with Tukey–Kramer test for BDEs-28/33, -47, -49, -66, -99, -153, -154, and -155,  $p > 0.05$ ), the

proportion of BDE-47 was higher in subadults compared to adult males (Tukey–Kramer test,  $p < 0.05$ ;  $t$ -test,  $p = 0.0100$ ) (Fig. 1a; Fig. S1). In addition, the BDE-99 fraction was lower in subadults than in adult males (Tukey–Kramer test,  $p < 0.05$ ;  $t$ -test,  $p = 0.0109$ ). Lipid normalized concentrations of BDE-47 decreased by a factor of 2.0 from subadult animals to adult males, whereas BDE 99 was 1.3-fold greater in adult males compared to subadults (Table 2).

The PBDE composition in the Steller sea lion is similar to the PBDE patterns reported for other marine mammals from the northern hemisphere (Wolkers et al., 2004; Rayne et al., 2004; Tuerk et al., 2005). The importance of BDE-47 at upper trophic levels of the marine food web reflects a combination of the propensity of this congener to biomagnify and/or its generation through debromination pathway of other PBDE congeners (Sellström et al., 1993; Boon et al., 2002; Wolkers et al., 2004; Stapleton et al., 2004a; Kelly et al., 2008). BDE-209 was not detected in our sea lions, likely as a result of its preferential binding to the particle phase in the water column and sediments (Johannessen et al., 2008; Ross et al., 2009) and the subsequent lack of biomagnification of this high  $\log K_{OW}$  congener in aquatic food webs (Wolkers et al., 2004; Kelly et al., 2008). Studies have also demonstrated that BDE-209 is also debrominated to lower brominated congeners



**Fig. 1.** Patterns of PBDEs and PCBs by age class of Steller sea lion sampled in British Columbia, Canada (fetus, pups, subadults, females, and males): (a) PBDE congener composition; (b) PCB homologue group patterns. Results are expressed as mean  $\pm$  Standard Deviation.

(BDE-154, -155) in fish (Stapleton et al., 2004b, 2006a) and gray seals (*Halichoerus grypus*) (Thomas et al., 2005), perhaps also explaining in part our observations.

A total of 93 PCB congeners were detected out of the 159 analytes sought in at least 68% of Steller sea lion samples. The concentration of  $\sum$ PCBs in Steller sea lions ranged from 272 to 14,277  $\mu\text{g kg}^{-1}$  lipid, with a geometric mean of 1893  $\mu\text{g kg}^{-1}$  lipid (lower value geometric SD = 618  $\mu\text{g kg}^{-1}$ ; upper value geometric SD = 5797  $\mu\text{g kg}^{-1}$  lipid; Table 2). The coeluting PCB congeners 153/168 accounted for 21% of  $\sum$ PCBs, followed by PCBs 129/138/160/163 (14.1%), PCB 118 (7.0%), PCB 180/193 (6.5%) and PCBs 83/99 (6.1%) (Fig. S2 in Supplementary material). Hexachlorobiphenyls, followed by penta and hexa homologue groups, dominated the PCB composition in the Steller sea lion, as observed in subadult individuals (Fig. 1b).

While PCB homologue group patterns in subadult and adult individuals (females and males) were similar, and were dominated by the more heavily chlorinated PCB homologue groups, the PCB composition in the fetus and pups was slightly lighter (Fig. S3 in Supplementary material). The PCB pattern in the Steller sea lions is similar to that observed in marine mammals in the Northeastern Pacific Ocean (Ross et al., 2000, 2004), and reflects the distribution and fate of this contaminant class in marine food webs.

$\sum$ PBDEs and  $\sum$ PCBs were correlated ( $r = 0.99$ ;  $p < 0.0001$ ; Fig. S3), indicating that despite some differences in physical-chemical properties, both PBDEs and PCBs biomagnify in marine food webs and bioaccumulate in Steller sea lions. However,  $\sum$ PCB concentrations were four times higher than  $\sum$ PBDE concentrations in Steller sea lions, underscoring the continued and widespread contamination of the marine environment by these legacy chemicals.

### 3.3. Lipid content and PBDE and PCB concentrations

Lipid content in Steller sea lions varied considerably among blubber biopsy samples (from 1.94% to 83.4%), with a mean of  $28.7\% \pm 4.52\%$  (Table 1). The lipid fraction of the blubber samples was less than 50% in about 80% of the Steller sea lions (Table 1), suggesting that animals underwent a fasting period during their migration into the Strait of Georgia. In California sea lions, a lipid content <50% indicates that an animal is nutritionally stressed or fasting (Stapleton et al., 2006b). No differences in lipid content were found among age groups (Kruskal Wallis Test;  $\chi^2 = 8.8904$ ;  $df = 6$ ;  $p = 0.1798$ ; Table 2).

The notion that these sea lions had undergone a fasting period was supported by reduced or normal blood urea nitrogen (BUN) values (range 10–35  $\text{mg dL}^{-1}$  or 4–12.5  $\text{mmol L}^{-1}$ ), which is indicative of protein utilization in subadult and adult individuals (D. Lambourn, unpublished data). These values are consistent with those reported for fasting-adapted pinnipeds (Bossart et al., 2001), and indicate that energy was primarily being obtained from the catabolism of lipid body stores rather than from body protein catabolism.

Food consumption by free-ranging Steller sea lions fluctuates seasonally in response to changes in energy requirements (Winship et al., 2002), coupled with activities such as breeding (Pitcher and Calkins, 1981), periods of growth (lean tissues and energy reserves) and molting (Lager et al., 1994; Costa, 1995). Sea lions spend proportionally more time feeding at sea during winter and spring compared to summer (Merrick and Loughlin, 1997; Trites and Porter, 2002). Foraging time for lactating females is also longer in winter than in summer, suggesting a greater effort is required to obtain sufficient food in winter due to dispersed fish distribution and/or increased energy needs (Merrick and Loughlin, 1997; Trites and Porter, 2002).

Fasting presumably mobilizes PBDEs and PCBs from depleted blubber lipid into the bloodstream, resulting in a consequent

increase in lipid-based concentrations in circulation (De Swart et al., 1995) and blubber (Hall et al., 2008). While we did not measure circulating concentrations of PBDEs or PCBs in blood, we did not detect a relationship between lipid normalized concentrations of PCBs or PBDEs versus lipid content (PCBs:  $r = 0.05$ ,  $p > 0.05$ ; PBDEs:  $r = 0.02$ ,  $p > 0.05$ ; Fig. S4 in Supplementary information). This may be due in part to small sample sizes within different age categories, the wide large range of lipid content and condition, as well as possible variations in individual feeding locally or on route. Since fasting has been implicated in increased POP concentrations in the blubber of otariids (Hall et al., 2008), our observations might also partly reflect recent and localized feeding within the semi-urbanized Strait of Georgia.

### 3.4. Maternal transfer of PBDEs and PCBs

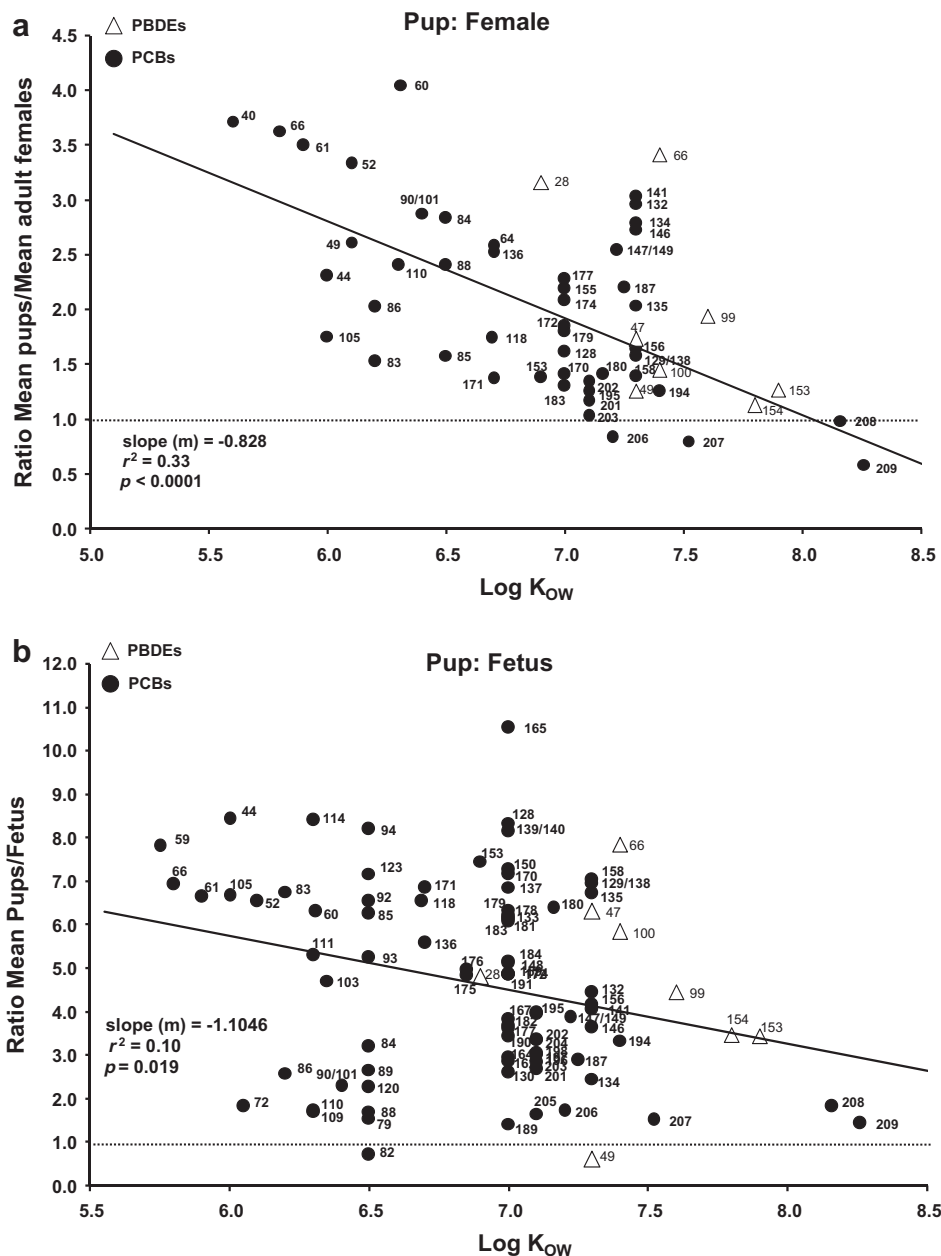
As we were unable to match the aborted fetus to its mother, it was not possible to conduct a specific assessment of contaminant transfer from mother to her fetus. However, a significant linear relationship in the average ratio of PBDE and PCB congeners in pups ( $n = 3$ ) relative to adult females ( $n = 6$ ) versus  $\log K_{OW}$  ( $r = -0.60$ ,  $p < 0.0001$ ; Fig. 2a) allowed us to characterize the putative transfer of contaminants via milk to nursing pups.  $\sum$ PBDE and  $\sum$ PCB concentrations in nursing pups were both 1.7 times higher than those in adult females. For PBDEs, all ratios were higher than 1.0, with BDE-28 and BDE-66 exhibiting ratios above 3.0. While most ratios for PCB congeners were above 1, PCBs -206, -207, -208, and -209 had ratios below 1. In general, lower ratios for PCBs are observed above a  $\log K_{OW}$  of 7.5 in marine mammals (Greig et al., 2007; Desforgues et al., 2012), indicating that the  $\log K_{OW}$  is the major constraining factor for the lactational transfer of PCBs, and PBDEs, in Steller sea lions.

Interestingly, the  $\sum$ PBDE and  $\sum$ PCB ratios of pups relative to the fetus (5.6 and 5.5, respectively) indicate that a large amount of the transfer of contaminants takes place after birth (Fig. 2b), with the exception of PCB-82 and PBDE-49, which exhibited ratios <1. Transplacental and lactational transfer of persistent organic contaminants are thought to be governed by the physico-chemical properties of the congener in question (Addison and Smith, 1974; Addison and Brodie, 1987). Moreover, low concentrations of PCB congeners relative to other tissues were found in the Steller sea lion placenta, underscoring some transfer of PCBs from the mother to pups and indicating a maternal fetal barrier in the uterus (Wang et al., 2011). In cetaceans, however, the maternal transfer of organochlorines (i.e., PCBs and DDT) to offspring during lactation was found to deliver as much as 60–95% of the mother's burden (Borrel et al., 1995; Hickie et al., 2007). However, some feeding on prey by the older pups in our study may confound the transfer assessment of PCBs and PBDEs.

Differences were also evident for homologue group patterns between fetus, pups and adult females for PCBs, but not PBDEs (Table 2; Fig. 1a and b). For example, PCB profiles were dominated by lighter homologue groups (e.g., pentachlorobiphenyls) in fetus relative to adult females, while pups had similar PCB and PBDE patterns compared to adult females.

### 3.5. Contaminant metabolism and accumulation

Metabolism also plays an important role in shaping the POP composition in the tissues of marine mammals. Pinnipeds are able to metabolize most PCB congeners with *meta* and *para* vicinal-H atoms and two *ortho*-chlorines because of their induction of CYP1A and CYP2B cytochrome P450 enzymes (Tanabe et al., 1988; Boon et al., 1997; Routti et al., 2008). Although less studied, similar enzymatic induction and metabolic pathways have been proposed and/or observed for PBDEs (de Wit, 2002; Hallgren and Darnerud, 2002).



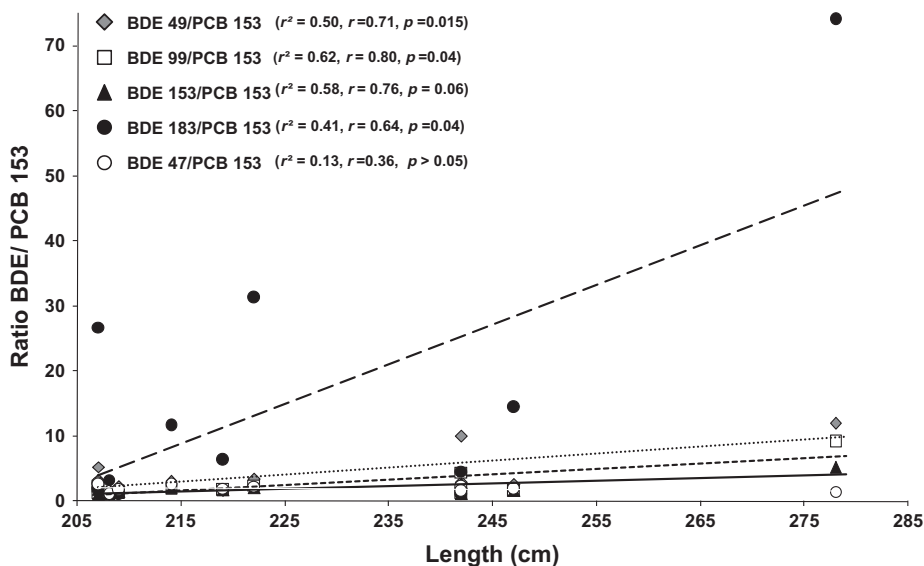
**Fig. 2.** Assessment of maternal transfer of PBDEs and PCBs: a) Ratios of the mean of PBDE (BDE -28, -47, -49, -66, -99, -100, -153, -154) and PCB congeners measured in pups ( $n = 3$ ) relative the mean concentrations detected in adult females ( $n = 6$ ) versus the Log  $K_{ow}$  PBDE and PCB congeners; and, b) Ratios of the mean of PBDE and PCB congeners measured in pups ( $n = 3$ ) relative to PBDE and PCB concentrations measured in fetus. Dotted line represents equal partitioning between pups and females or pup or fetus. Log  $K_{ow}$  values for PCBs and PBDEs were obtained from Mackay et al. (1992) and from Kelly et al. (2008), respectively.

The regression between the ratios of individual PBDE congeners to the recalcitrant PCB-153 versus length showed positive relationships (i.e., slope  $> 0$ ) for four PBDE congeners (BDEs -49, -99, -153 and -183), suggesting bioaccumulation of these congeners relative to PCB-153 (Fig. 3). The slopes for PBDE congeners -17/25, -28/33, -47, -66, -100, -154 and -155 did not differ from zero (Table 3), suggesting a lack of metabolism of these compounds. However, the small sample size and the overwhelming contribution of BDE-47 relative to  $\Sigma$ PBDEs renders it difficult to fully explore the metabolic vulnerability of different PBDEs.

The regression slopes for individual PCB congeners within each metabolic group (I, II, III, IV and V) did not differ from zero ( $p > 0.05$ ) (Table S1 in Supplementary information), except for PCB-137 of Group IV which had a positive slope which was greater

than zero ( $r^2 = 0.57$ ,  $p = 0.007$ ). This appears to suggest that Steller sea lions possess poor biotransformation capabilities and/or a limited induction of cytochrome P450 enzymes in these study animals.

Low uptake and slow excretion rates of PBDEs may also contribute to the bioaccumulation of PBDEs in the Steller sea lion through its food web. In this way, some congeners (e.g., BDE-47) might require longer time periods to reach a steady state, while others PBDE congeners may exhibit a relatively rapid rate of depuration through debromination and/or cytochrome P450 enzyme mediated oxidative metabolism (McKinney et al., 2006; Kelly et al., 2008). Measurements of PBDE and PCB metabolites (e.g., hydroxylated PCBs and PBDEs: OH-PCBs and OH-PBDEs) in sea lions would substantiate these possible scenarios.



**Fig. 3.** Relationship between the ratios of selected PBDE congeners (BDE-47, BDE-49, BDE-99, BDE-153, and BDE-183) relative to PCB-153 versus length in male Steller sea lions (juveniles and adults). Ratios were corrected to the minimum value of initial regressions. Ratio BDE-183/PCB-153 versus length (black circle and dashed line); ratio BDE-49 versus length (grey diamond and round dotted line); ratio BDE-99/PCB-153 versus length (white square and square dotted line); ratio BDE-153/PCB-153 versus length (black triangle and solid black line); and, ratio-BDE 47/PCB-153 (white circle; regression line is not shown as the slope was not significantly different from zero).

### 3.6. PBDE- and PCB- related health risks

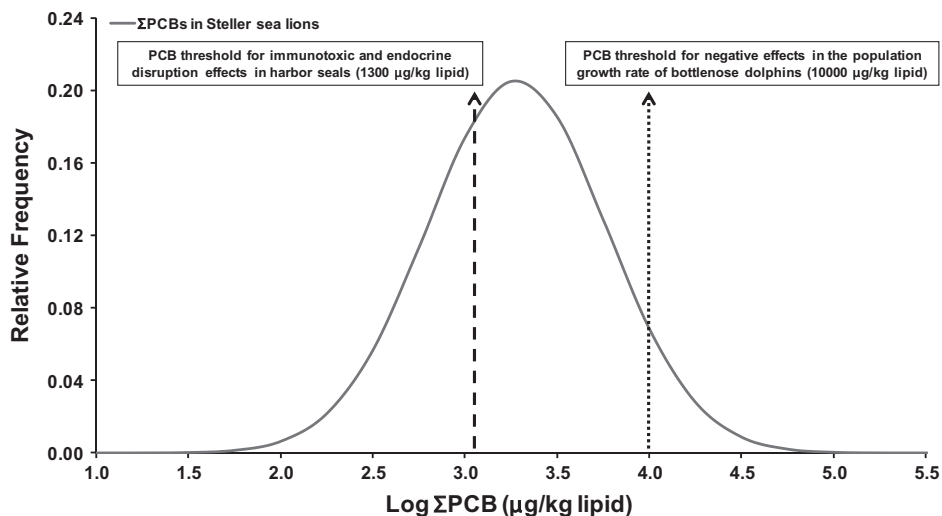
Total toxic equivalents ( $\sum$ TEQ) for non-ortho and mono-ortho (planar)  $\sum$ PCBs in the Steller sea lions ( $10.2 \pm 2.23$  ng TEQ  $\text{kg}^{-1}$  lipid) are below the No Observable Adverse Effects Level (NOAEL-TEQ) thresholds of 90 ng TEQ  $\text{kg}^{-1}$  and 209 ng TEQ  $\text{kg}^{-1}$  for immunotoxic effects reported in harbor seals (Ross et al., 1995). PCB concentrations in 80% of our study animals exceeded the most recent PCB toxicity reference value (i.e. 1300  $\mu\text{g kg}^{-1}$  lipid) for immunotoxicity and endocrine disruption in harbor seals (Mos et al., 2010), while ~8% were above the threshold (i.e. 10000  $\mu\text{g kg}^{-1}$  lipid) associated with a decrease in the population growth rate of bottlenose dolphins (Hall et al., 2006; Fig. 4).

While PBDEs are also of concern due to their potential to disrupt the endocrine and immune systems (Meerts et al., 2001; Hallgren and Darnerud, 2002; Hall and Thomas, 2007), the lack of estab-

lished thresholds preclude an analysis of the risks related to PBDEs in these Steller sea lions.

### 3.7. Comparisons with other marine mammals and regional trends

Concentrations of PBDEs in Steller sea lions were lower or comparable to those in harbor seals from San Francisco (She et al., 2002), California sea lions from coastal California (Stapleton et al., 2006b) and harbor seals from Puget Sound (Ross et al., 2012); Table S2 in Supplementary material), but similar to those observed in resident and transient killer whales from the North-eastern Pacific Ocean (Rayne et al., 2004; Krahn et al., 2007). While PCB concentrations in Steller sea lions were lower than those reported for individuals from the western stock (Krahn, 1997; Krahn et al., 2001; Table S3 in Supplementary material) and from non-migratory harbor seals in the Strait of Georgia (Ross et al., 2004),



**Fig. 4.** Normal probability density curve showing the frequency distribution of PCB concentrations measured in Steller sea lion. The dashed line represents the revised harbor seal toxicity threshold (Mos et al., 2010) and the dotted line represents the PCB threshold for bottlenose dolphins (Hall et al., 2006). The relative frequency of the population sampled, here expressed as the normal probability density distribution function of the log lipid normalized  $\sum$ PCB concentrations measured in Steller sea lions, were plotted against the lipid normalized log values of PCB toxic effect thresholds reported elsewhere (Hall et al., 2006; Mos et al., 2010) to assess what proportion of the animals (i.e., frequency) exceed target threshold PCB concentration.



they were higher than PCB concentrations detected in Southern elephant seals (Miranda-Filho et al., 2007) and Galapagos sea lions (Alava et al., 2009). The  $\sum$ TEQ in Steller sea lions is higher than the  $\sum$ TEQs reported for Northern elephant seals (*Mirounga angustirostris*) from California (Debier et al., 2005), harbor seals from Queen Charlotte Strait and those inhabiting the Strait of Georgia, British Columbia (Ross et al., 2004), but lower than that reported in harbor seals from Puget Sound, Washington (Ross et al., 2004).

Although PCB bans several decades ago have improved habitat quality for marine mammals in the Pacific, concerns linger about health risks associated with some heavily contaminated populations. While PBDEs increasingly face regulation today for many of the same reasons PCBs were phased out in the 1970s, increasing environmental concentrations, coupled with potentially unstable sediment-bound reservoirs of PBDEs (notably decaBDE), represent an emerging threat (Ross et al., 2009). Our results suggest that migrating Steller sea lions are exposed to contaminants that are amplifying in North Pacific food webs, and that these are readily transferred to offspring.

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### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2012.03.094>.

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