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Declining concentrations of persistent PCBs, PBDEs, PCDEs, and PCNs in harbor seals (*Phoca vitulina*) from the Salish Sea

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ABSTRACT

As high trophic level, non-migratory marine mammals, harbor seals (*Phoca vitulina*) inhabiting the Strait of Georgia, Juan de Fuca Strait and Puget Sound (collectively referred to as the Salish Sea) in northwestern North America provide an integrated measure of coastal food web contamination. We measured congener-specific polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs), polychlorinated diphenylethers (PCDEs) and polychlorinated naphthalenes (PCNs) in blubber biopsies from free-ranging harbor seal pups inhabiting four sites in the Salish Sea in 2003. While legacy PCBs dominated the composition of these contaminants in seals at all sites (PCBs > PBDEs > PCDEs > PCNs), PBDEs were noteworthy in that they averaged as much as 59% of total PCB concentrations. We further evaluated temporal trends in seals sampled at one of these sites (Puget Sound) for PCBs and PBDEs between 1984 and 2009, and for PCDEs and PCNs between 1984 and 2003. PBDE concentrations doubled every 3.1 years between 1984 and 2003, but appeared to decline thereafter. Over the course of the 20 years between 1984 and 2003, PCB concentrations had declined by 81%, PCDEs declined by 71%, and PCNs by 98%. Overall, results suggest that regulations and source controls have noticeably reduced inputs of these contaminants to the Salish Sea, consequently reducing the associated health risks to marine wildlife. We estimate the total mass of these contaminants in the 53,000 harbor seals of the Salish Sea in 2009 to be 2.6 kg PCBs and 1.0 kg PBDEs, compared to just trace amounts of the PCDEs and PCNs.

1. Introduction

While boasting a rich diversity of aquatic species, the Salish Sea, comprising the Strait of Georgia, Juan de Fuca Strait and Puget Sound on the Pacific coast of North America, has been at the receiving end of household, agricultural and industrial contaminants from both within and outside its watershed boundaries. Sources within the watershed include major wood and paper industries, agricultural activities, municipal waste water and urban runoff, petrochemical facilities, and a variety of other industrial activities (Waldichuk, 1983). Pulp and paper mills have, in the past, released large quantities of by-produced polychlorinated dibenzo-*p*-dioxins (PCDDs or dioxins) and polychlorinated dibenzofurans (PCDFs or furans) (Bright et al., 1999; Hagen et al., 1997). Industrial activities have contaminated a number of sites, and contributed to heavy polychlorinated biphenyl (PCB) contamination in Puget Sound, Washington, USA (West et al., 2008). In addition to local contaminant inputs, 'global' contaminants are delivered to the Salish Sea through atmospheric transport (Noël et al., 2009) and biological delivery to coastal food webs by migratory species, including several species of salmon (Christensen et al., 2005; Krummel et al., 2005).

Situated atop coastal food webs, many marine mammals can provide an integrated signal of food web contamination by chemicals considered to be persistent, bioaccumulative and toxic. Killer whales (*Orcinus orca*) of the Northeast Pacific Ocean have been found to be particularly contaminated with Persistent Organic Pollutants (POPs), especially the legacy PCBs (Krahn et al., 2007; Ross et al., 2000a). While the salmon-eating resident killer whales are thought to reflect in part a 'global' POP signal (Cullon et al., 2009), harbor seals (*Phoca vitulina*) provide an indication of more localized food web contamination in the Salish Sea. Harbor seals are relatively abundant, widely distributed, and have a limited home range (~50 km^2) (Cottrell, 1996; Olesiuk, 2009). Previous work ascribed dioxins and furans in harbor seals to pulp and paper processes in the Strait of Georgia, PCBs to a history of industrial inputs into Puget Sound, and a 'global' contribution in seals from remote locations (Ross et al., 2004).

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The response of environmental compartments within the Salish Sea to chemical regulations or source controls have been characterized for a number of POPs, such as PCBs and polybrominated diphenyl ethers (PBDEs) using sediment cores (Johannessen et al., 2008), dioxins and furans in Dungeness crab, *Metacarcinus magister* (Hagen et al., 1997), and PCBs and organochlorine pesticides in the eggs of double-crested cormorants, *Phalacrocorax auritus* (Harris et al., 2005). Despite such improvements, the region's killer whales have some of the highest PCB levels reported among the world's marine mammals, presenting troubling questions about the conservation implications for POPs in marine food webs (Ross, 2006).

PCBs have represented a major toxicological concern for many high trophic level species; they were banned in 1976 and 1977 in the USA and Canada, respectively. PCBs have been associated with reproductive impairment, tumors, immunotoxicity and endocrine disruption in captive and free-ranging marine mammals (De Swart et al., 1994; Mos et al., 2006; Reijnders, 1986; Ross et al., 1996; Ylitalo et al., 2005). PBDEs have been widely used as flame retardants in electronics, furniture and other products; two of the three commercial formulations (penta- and octa-) were withdrawn from the market in 2004, while the third product is facing a North American phase-out in 2013. Less is known about the toxicity of PBDEs to marine mammals, but similarities in structures would suggest similar toxic effects. Studies demonstrate that PBDEs affect the function of white blood cells in harbor seals (Frouin et al., 2010). Polychlorinated diphenyl ethers (PCDEs) have been attributed to impurities in pentachlorophenol (PCP) used as a wood preservative in the lumber industry, as well as in a variety of pesticides and other products (Becker et al., 1991). Polychlorinated naphthalenes (PCNs) were intentionally produced as flame retardants and wood preservatives, but have also been described as impurities in PCBs and as by-products of combustion of chlorine-containing products (Falandysz, 1998; Yamashita et al., 2000).

Approximately 53,000 harbor seals are distributed over the 17,000 km^2 of the Salish Sea, feeding on Pacific hake (*Merluccius productus*), Pacific herring (*Clupea pallasi*), Pacific tomcod (*Microgadus proximus*) and a variety of other pelagic and bottomfish species (Cullon et al., 2005; Lance et al., 2012; Olesiuk, 1993). They therefore provide an integrated contaminant signal from the numerous fish and invertebrate prey species upon which they rely, and provide insight into contamination hotspots and emerging contaminants of concern (Cullon et al., 2005).

In this study, we evaluated spatial variation in PCB, PBDE, PCDE and PCN concentrations and patterns in harbor seals inhabiting sites throughout the transboundary waters of the Salish Sea. In addition, we evaluated temporal trends in the concentrations of PCBs and PBDEs in harbor seals from southern Puget Sound during the period 1984–2009, and for PCDEs and PCNs for the period 1984–2003.

2. Material and methods

2.1. Live-capture and sampling of seals for spatial assessment

We collected blubber biopsies from live-captured harbor seal pups from two locations in British Columbia, Canada (Hornby Island and the Fraser River estuary in Vancouver), and two locations in Washington State, USA (Smith Island and Gertrude Island) (Fig. 1). Harbor seals were captured using one of two techniques. At the rocky Hornby Island site, individual seals were captured by hand and using a fish landing net following a rapid yet careful approach by a 3.5 m aluminum outboard boat (Cottrell et al., 2002). At the three remaining sites, which consisted of sandy haul-out areas, multiple seals were captured at one time using a rapidly deployed beach seine net (Jeffries et al., 1993). In all cases, body weight, body length, girth, and sex were determined for each

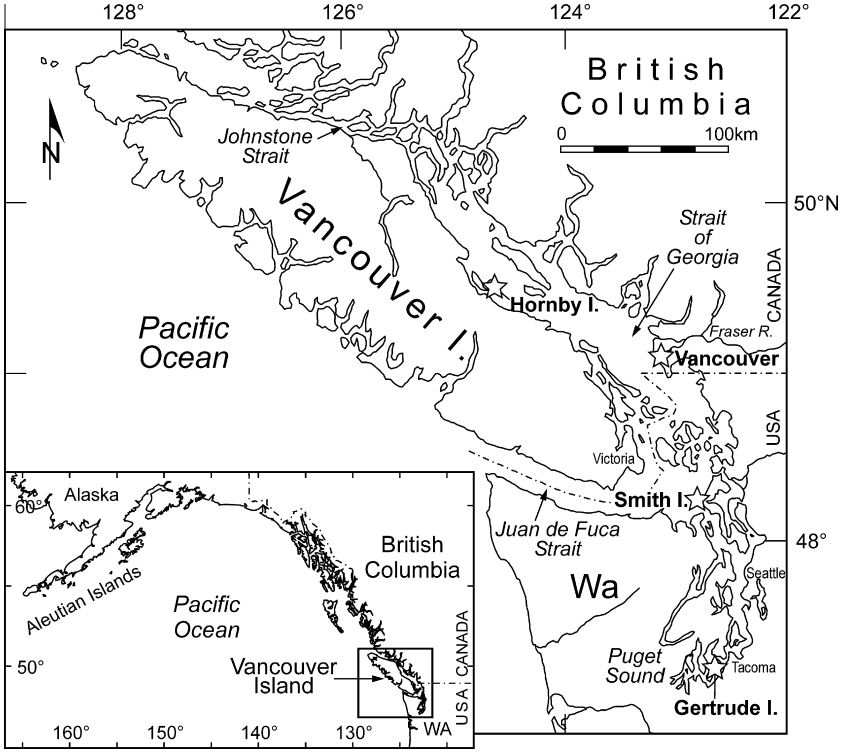

Fig. 1. Harbor seals were live-captured at four sites in 2003, including Hornby Island and Vancouver (Strait of Georgia, British Columbia, Canada), Smith Island (Juan de Fuca Strait, Washington State, USA) and Gertrude Island (Puget Sound, Washington State, USA). Harbor seals were sampled during the period 1984–2009 from Gertrude Island for temporal trend analyses.

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seal captured, while age was estimated on the basis of tooth eruption, pelage, alertness, umbilicus state and general condition.

Blubber samples were collected using an Acu-Punch 6 mm (diameter) biopsy sampler (Acuderm, Ft Lauderdale, FL, USA) following site cleansing and application of a local anesthetic as described elsewhere (Simms et al., 2000). Samples were wrapped in hexane-rinsed aluminum foil, placed in cryovials, and stored at −20 °C (field) and later at −80 °C (laboratory) until analysis. All procedures were carried out under the auspices of the respective animal care committees and scientific research permits for researchers in British Columbia (Fisheries and Oceans Canada Animal Care Committee, using guidelines from the Canadian Council on Animal Care; Scientific Research Permit) and Washington State (United States Marine Mammal Protection Act Research Permits Nos. 835, 782-1812 and 13430). In all circumstances, capture stress and holding time was minimized, and seals were released following sampling 20–90 min after capture.

2.2. Harbor seals for temporal trend measurements

In addition to the study on spatial contaminant trends in 2003, we obtained blubber samples from harbor seals to provide an overview of temporal trends for PCBs, PBDEs, PCDEs and PCNs from one site (Gertrude Island, Puget Sound). This included samples from 1984 ($n = 10$), 1990 ($n = 10$), 1993 ($n = 11$), 1997 ($n = 10$), and 2009 ($n = 7$). Samples from 1993, 1997 and 2009 were obtained by biopsy from live-captured seal pups, while samples from 1984 and 1990 were obtained from freshly deceased seal pups which were found stranded (i.e. no decomposition and deemed to be otherwise in good condition based on blubber thickness measurements; results not shown). Individual seal samples were analyzed for PCBs for all sampling years, for PBDEs for years 2003 and 2009, and for PCDEs and PCNs for 2003. For other years, individual samples were pooled prior to analysis. PCDEs and PCNs were not determined in 2009 samples owing to their extremely low concentrations in 2003.

2.3. Determination of PCB, PBDE, PCDE and PCN concentrations

Harbor seal blubber samples were analyzed for congener-specific (peaks including co-eluting) PCBs ($n = 183$), PBDEs ($n = 40$), PCDEs ($n = 45$), and PCNs ($n = 50$) using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS).

Extraction and clean-up procedures, instrumental analysis and conditions, and quality assurance/quality control criteria used for PCBs by the Laboratory of Expertise for Aquatic Chemical Analysis (LEACA, Fisheries and Oceans Canada, Sidney BC) are described elsewhere (Ikonomou et al., 2001; Ross et al., 2000a). The sample batch for PCBs included a procedural blank, a replicate, and either a certified reference material (CRM; herring) or a standard reference material (SRM; harbor seal) sample (Ikonomou et al., 2001). Reference material data met established laboratory criteria of less than ±two standard deviations before batch data was deemed acceptable.

PBDEs, PCDEs, and PCNs were analyzed by AXYS Analytical Services, Ltd. (Sidney, BC, Canada) according to their laboratory procedures and criteria. The PBDE method is based on the U.S. Environmental Protection Agency (EPA) draft analytical methods and procedures no. 1614 (USEPA Office of Science and Technology, 2003). The PCDE and PCN analyses were carried out using an in-house GC/HRMS method with isotope dilution or internal standard quantification. The sample batch for PBDEs, PCDEs and PCNs included a procedural blank and standard reference material (SRM), wherein batch data were deemed acceptable when the SRM results were within a percentage recovery of 80 and 120.

Briefly, samples for PBDEs, PCDEs, and PCNs were spiked with ^{13}C-labeled surrogate standards 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 dichloromethane (DCM). The extract was eluted through a gel permeation column with 1:1 DCM:hexane. The extract was applied to a partially deactivated Fluorisil column and eluted with hexane followed by 15:85 DCM:hexane. Eluates were then combined and eluted with 1:1 DCM:hexane and each fraction concentrated. Samples were analyzed using an Ultima HRMS (Micromass Inc., Manchester, UK) equipped with a Hewlett Packard 5890 GC (Agilent Technologies, Wilmington, USA) and a DB-5 Durabond capillary column (60 m × 0.25 mm, 0.10 μm film). Percent lipid in samples was determined using the gravimetric lipid determination by weight of extract method with dichloromethane.

Mono- and di-BDE data were not used for interpretation as surrogate recoveries were less than 10%. Since the isotope dilution method of quantification produces data that are recovery corrected, the slight variances from the method acceptance criteria are deemed not to affect the quantification of these analytes. Included with each batch of samples was a procedural blank.

Many congeners were not detectable in samples of harbor seal blubber. Total concentrations for PCBs, PBDEs, PCDEs and PCNs are calculated as the sum of the concentrations of the peaks that were detectable in at least 70% of the seal samples from all sites. Where congeners were undetectable, the detection limit was substituted. Congeners that were detected in less than 70% of the samples were not included in calculations. All results are expressed on a lipid weight (lw) basis.

2.4. Mass balance calculation

The mass of PCBs, PBDEs, PCDEs and PCNs was estimated for the harbor seal population inhabiting the Strait of Georgia (Hornby Island and Vancouver sites), Juan de Fuca Strait (Smith Island) and Puget Sound (Gertrude Island). First, an age-specific population data matrix for males and females was established using harbor seal abundance estimates for these areas (Jeffries et al., 2003; Olesiuk, 1993, 2009). This matrix was based on a maximum life expectancy of 20 years for males and 29 years for females (Olesiuk, 2009), and was adjusted using demographic trends observed in Puget Sound and Juan de Fuca Strait (Olesiuk, 1993).

Second, relationships between PCBs and age in males and females were developed from empirical measurements in seals (pups, juveniles and adults) sampled in the period 1997–2001 at Boundary Bay and in the Puntledge River estuary, British Columbia ($n = 4$, 10 and 15). For this, we first adjusted 1997 PCB concentrations to the year 2001 using a correction factor of 0.745. Age was estimated on the basis of body weight and by visual assessment of tooth, gum and eye condition, and the presence of scarring around the neck and on the flippers. PCB concentrations were measured in blubber biopsies using HRGC/MS, and equations were developed using lines-of-best fit for the data:

$$[\mathrm{PCB}_{\mathrm{juveniles}}] = 0.853 \times \mathrm{age} + 0.963 \text{ for ages} \leqslant 4 \text{ years}$$

$$[\mathrm{PCB}_{\mathrm{males}}] = 0.731 \times \mathrm{age} + 1.221 \text{ for adult males} > 4 \text{ years}$$

$$[\mathrm{PCB}_{\mathrm{females}}] = -0.897 \times \ln(\mathrm{age}) + 4.241 \text{ for adult females} > 4 \text{ years}$$

For each year class, the total mass of PCBs was calculated as follows:

$$\sum \mathrm{PCB\ mass}_{\mathrm{year\ class}} = \mathrm{Body\ mass}_{\mathrm{seal}} \times \mathrm{lipid}\%_{\mathrm{seal}} \times N_{\mathrm{year\ class}} \times [\mathrm{PCB}_{\mathrm{mg/kg\ Equation\ 1,2,\ or\ 3}}] \times \mathrm{Correction\ Factor}$$

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where body mass for each year class was obtained elsewhere (Olesiuk, 1993). Correction factors for each study year were developed using temporal trends observed in Puget Sound seal pups, as follows: 1984 = 2001 × 3.506; 1990 = 2001 × 2.252; 1997 = 2001 × 1.805; 2003 = 2001 × 0.862; 2009 = 2001 × 1.009. This assumes that changes in other age classes corresponded to those observed in pups and that changes across sites were similar to those observed in Puget Sound. Total body lipid (31.84%) was derived from an average of 28.5% observed in a study of Alaska harbor seals (Pitcher, 1986) and adjusted upwards to include an extra 10.5% lipid content as reported for the viscera of another phocid seal, the harp seal (*Phoca groenlandica*) (Beck et al., 1993).

The cumulative totals for all age classes and both sexes yielded the total PCB burden in the seal populations inhabiting the three bodies of water in the Salish Sea, namely the Strait of Georgia, Juan de Fuca Strait and Puget Sound.

Age and sex-based models were generated for the other three contaminant classes by adjusting these formulae by the concentrations of PBDEs, PCDEs and PCNs relative to the PCB concentrations measured in the seal pups from each study year for the three bodies of water within the Salish Sea (Supplementary Table 1). We assumed a similar age and sex relationship as observed in the PCBs, based on similar age and sex relationships for PCBs and PBDEs reported for ringed seals, *Phoca hispida* (Rigét et al., 2006). No detailed reports exist for PCDEs and PCNs vs age in pinnipeds, so we assumed a similar relationship based on similar chemical structures among all of these contaminants.

3. Results and discussion

3.1. PCBs, PBDEs, PCDEs and PCNs in harbor seals

Our study animals consisted primarily of healthy, nearly-weaned free-ranging individuals of comparable age (3–5 weeks), reflecting our intent to eliminate the confounding influence of age and sex on the concentrations of the POPs measured in this study. Body burdens for the contaminants measured in these pups would largely reflect those acquired via transplacental transfer and via mother's milk, as pups had not yet been weaned. Body weights differed slightly among sites for the spatial study carried out in 2003 (ANOVA, $p = 0.013$; Hornby Island 17.9 ± 1.1 kg, Vancouver 19.6 ± 1.0 kg, Smith Island 22.9 ± 1.1 kg, Gertrude Island 18.2 ± 2.5 kg), with this due to a difference between seals from Hornby and Smith islands (Tukey's HSD, $p = 0.005$). Nonetheless, this did not influence comparability among sites, as there were no relationships between the concentrations of any of the four contaminants and body weight among sites for the 2003 study year ($p > 0.05$; results not shown). Body weights of seals sampled for the temporal trend analysis included samples from both freshly deceased individuals in 1984 and 1990 and live-biopsied samples in subsequent years. Earlier pups were smaller than those sampled in later years, averaging 11.9 ± 2.1 kg and 11.3 ± 3.3 kg, respectively, compared to 24.5 ± 4.1 kg in 1993 and 21.3 ± 3.9 kg in 1997 (ANOVA; $p = 0.003$). Analyses of pooled samples for PBDEs, PCDEs and PCNs for earlier years precluded statistical evaluation of a relationship between these contaminant concentrations and body weight, although there was no relationship between PCB concentrations and body weight over time ($p = 0.481$).

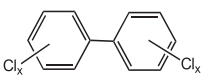
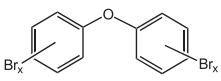
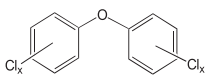
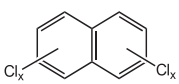
We report here on spatial and temporal trends in harbor seals from the Salish Sea for the relatively well studied PCBs and PBDEs, and the poorly documented PCDEs and PCNs. Of the theoretically possible 702 congeners for these four classes, our study targeted 366 individual analytes (Table 1). Of these, 43 congeners were detected in all seal samples in 2003, while a further 111 were detected in at least 70% of all seals. The majority (263) of congeners were detected in some, but not all, samples.

The number of PCB congeners detected was positively related to the total PCB concentrations in seals ($y = \ln(103.8 + 14.2x)$; $r^2 = 0.42$; $p < 0.001$), ranging from a minimum of 96 at the more remote Hornby Island to a maximum of 137 at Gertrude Island in Puget Sound. The number of PCN congeners detected in seals was also positively correlated with total PCN concentrations ($y = 1.91x + 11.47$; $r^2 = 0.35$; $p < 0.001$), although the fewest congeners were detected at Gertrude Island and the most at Vancouver (ANOVA followed by Tukey's HSD; $p = 0.001$). There was no significant relationship between the number of congeners detected and the total concentration of either PBDEs ($p = 0.089$) or PCDEs ($p = 0.051$).

Of the total contaminant concentration (PCB + PBDE + PCDE + PCN), the legacy PCBs dominated in seals at all four sampling locations, averaging 64.1% of the total, but the PBDEs followed at a surprisingly close 35.5%, followed by minor contributions of PCDEs at 0.57% and PCNs at 0.07% (Fig. 2; Table 2). PCBs were 200 times higher than PBDEs in European harbor seals in 2008 (Weijs et al., 2009), indicating that the PBDEs represent a more important contaminant concern in the Salish Sea.

Total flame retardant concentrations in these harbor seals were similar to total organochlorine pesticide (OCP) concentrations reported previously in Salish Sea seals (Mos et al., 2010), highlighting the mix of POPs to which this high trophic level species is exposed in the region (47.7% OCPs vs 53.3% for PCBs, PBDEs, PCDEs and PCNs). Polychlorinated dibenzo-*p*-dioxin and PCDF concentrations were low in harbor seals sampled in 1996, likely reflecting pulp and paper mill regulations in 1989, reductions in pentachlorophenol (PCP) use as a wood preservative, and short half-life of dioxin-like compounds in harbor seals (Ross et al., 2004).

Table 1
Detection of congeners in samples varied among the polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated diphenylethers (PCDEs) and polychlorinated naphthalenes (PCNs). Number of congeners detected out of the total number of congeners for which analytical quantification was possible (and theoretical total) is based on 32 samples collected from free-ranging harbor seals in 2003 from four sites in the transboundary Salish Sea (British Columbia, Canada and Washington State, USA).

	PCBs	PBDEs	PCDEs	PCNs
				
Range Log $K_{o/w}$	3.26–8.26	4.21–10.33	4.45–8.16	3.9–8.3
0% detected	27	10	6	17
>0 and <70%	93	14	15	30
⩾70 and <100%	64	8	18	21
100%	22	12	7	2
Number of congeners measured (theoretical total possible)	206 (209)	44 (209)	46 (209)	70 (75)

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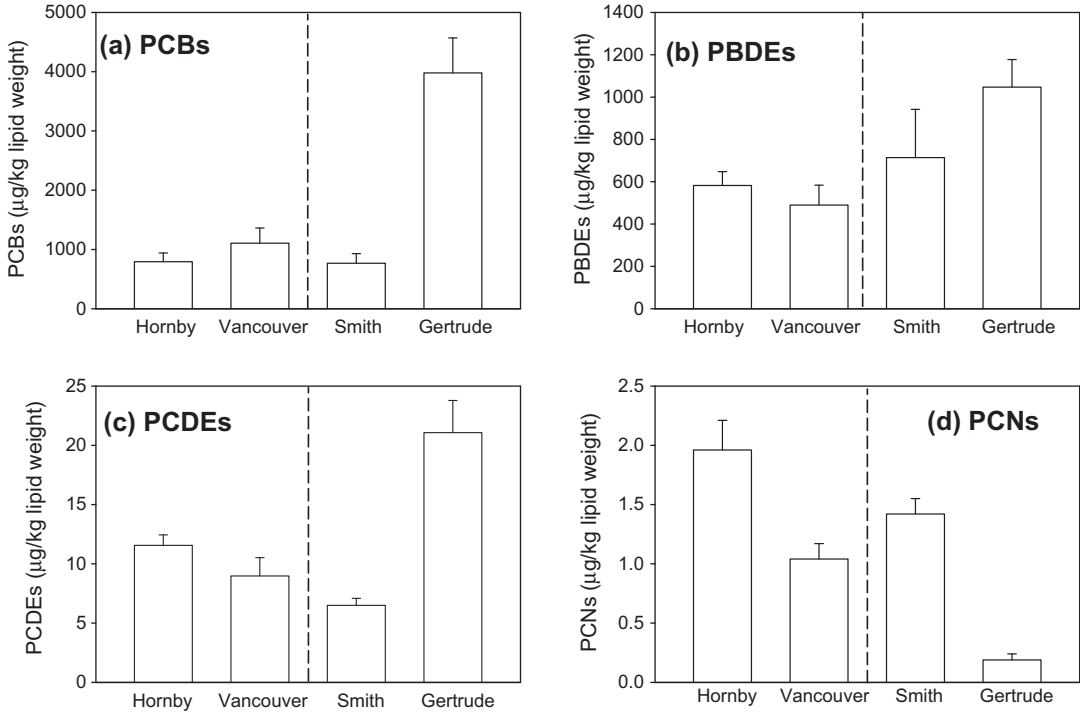

Fig. 2. Blubber biopsies from healthy, free-ranging harbor seal pups in 2003 from four sites in British Columbia (BC, Canada) and Washington State (WA, USA) were analyzed for (a) polychlorinated biphenyls (PCBs; sum of 86 congeners detected in > 70% of samples), (b) polybrominated diphenylethers (PBDEs; sum of 20 congeners), (c) polychlorinated diphenylethers (PCDEs; sum of 25 congeners), and (d) polychlorinated naphthalenes (PCNs; sum of 23 congeners). Sites included Hornby Island and Vancouver (Strait of Georgia, British Columbia, Canada), Smith Island (Juan de Fuca Strait, Washington State, USA) and Gertrude Island (Puget Sound, Washington State, USA). The dotted vertical line represents the international boundary between Canada and the USA.

Table 2
Concentrations (µg/kg lipid weight) of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated diphenylethers (PCDEs) and polychlorinated naphthalenes (PCNs) in blubber biopsies collected from 32 live-captured harbor seals in 2003 from four sites in the Salish Sea (except for Hornby and Vancouver sites, where PCB values were from 2001).

	Hornby Island (A)		Vancouver (B)		Smith Island (C)		Gertrude Island (D)		ANOVA *p* value	Tukey's HSD
∑PCBs	793.3 ± 147.1		1106.0 ± 257.1		768.4 ± 160.6		3979.5 ± 590.3		0.000	AD, BD, CD
	153	180.8 ± 39.6	153	189.0 ± 37.0	153	161.4 ± 37.5	153	680.8 ± 110.5		
	138	129.6 ± 27.3	138	154.7 ± 23.8	138	138.6 ± 29.8	138	596.5 ± 92.9		
	99	47.7 ± 10.1	83	74.6 ± 7.4	99	67.4 ± 11.5	99	268.4 ± 39.4		
	101	41.7 ± 6.8	180	74.034.6	101	44.5 ± 6.7	101	222.1 ± 30.4		
	187	34.4 ± 6.7	187	67.8 ± 29.8	180	42.6 ± 9.2	180	171.4 ± 31.5		
	149	29.8 ± 3.5	90	55.8 ± 4.9	118	29.9 ± 4.6	187	167.5 ± 25.0		
∑PBDEs	582.6 ± 64.9		489.8 ± 93.9		713.9 ± 228.3		1046.9 ± 130.2		0.031	BD
	47	481.3 ± 54.5	47	406.6 ± 80.0	47	649.9 ± 220.3	47	805.6 ± 99.0		
	99	50.1 ± 8.5	99	41.6 ± 9.3	99	31.5 ± 6.3	99	138.5 ± 20.4		
	100	30.7 ± 1.8	100	24.5 4.6	100	14.2 ± 1.7	100	58.8 ± 7.1		
	153	5.9 ± 1.2	153	5.0 ± 1.2	153	4.6 ± 0.8	153	15.2 ± 2.0		
	28 + 33	4.6 ± 0.3	28 + 33	3.7 ± 0.8	49	4.6 ± 1.8	49	11.1 ± 1.6		
	49	3.5 ± 0.3	49	2.8 ± 0.5	28 + 33	2.4 ± 0.2	28 + 33	8.01.2		
∑PCDEs	11.6 ± 0.9		8.9 ± 1.5		6.5 ± 0.6		21.07 ± 2.72		0.000	AD, BD, CD
	153	3.9 ± 0.4	153	2.6 ± 0.6	153	2.3 ± 0.2	153	8.6 ± 1.3		
	171	1.5 ± 0.3	171	1.4 ± 0.3	171	0.7 ± 0.1	99	2.2 ± 0.3		
	154	1.3 ± 0.1	154	0.9 ± 0.2	99	0.7 ± 0.1	154	2.2 ± 0.3		
	99	1.0 ± 0.1	99	0.8 ± 0.1	154	0.6 ± 0.1	171	1.8 ± 0.3		
	138	0.9 ± 0.1	85	0.7 ± 0.5	138	0.5 ± 0.1	138	1.7 ± 0.2		
	184	0.5 ± 0.1	47	0.5 ± 0.1	184	0.3 ± 0.2	184	1.3 ± 0.2		
∑PCNs	1.96 ± 0.25		1.04 ± 0.13		0.84 ± 0.11		0.19 ± 0.05		0.000	AB, AC, AD, BD, CD
	50 + 51	1.3 ± 0.2	50 + 51	0.5 ± 0.1	50 + 51	0.6 ± 0.1	64 + 68	0.03 ± 0.01		
	58	0.1 ± 0.02	38 + 40	0.07 ± 0.01	58	0.04 ± 0.01	50 + 51	0.03 ± 0.01		
	57	0.1 ± 0.01	58	0.04 ± 0.01	57	0.04 ± 0.01	58	0.02 ± 0.01		
	52 + 60	0.08 ± 0.01	57	0.04 ± 0.01	52 + 60	0.03 ± 0.01	53 + 55	0.02 ± 0.01		
	53 + 55	0.07 ± 0.01	28 + 29 + 43	0.03 ± 0.01	53 + 55	0.02 ± 0.01	57	0.01 ± 0.01		
	38 + 40	0.07 ± 0.01	52 + 60	0.03 ± 0.01	38 + 40	0.02 ± 0.01	42	0.01 ± 0.01		

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3.2. Spatial trends for contaminants in 2003

Harbor seals from Gertrude Island in southern Puget Sound in 2003 were 4–5 times more PCB-contaminated than their counterparts at other Salish Sea sites (Fig. 2a; Table 2). This confirms our previous observation that seals in south Puget Sound are heavily exposed to PCBs, although the greater difference in 1996 between seals in Puget Sound and those in the Strait of Georgia (sevenfold) suggests that the distribution and burial of this legacy contaminant over time may be resulting in a more even distribution throughout the Salish Sea (Ross et al., 2004). Herring, an important prey item for harbor seals, support the notion that Puget Sound is relatively contaminated by PCBs compared to other parts of the Salish Sea (West et al., 2008).

The top six PCB congeners accounted for 52.7 ± 2.2 to 62.5 ± 6.9% of the total PCB concentration in seals (Table 2; Supplementary Table 2). PCB 153 (17.4 ± 1.5 to 21.8 ± 1.2% of total PCBs) and PCB 138 (14.5 ± 1.4 to 16.3 ± 0.5% of total PCBs) were consistently the top two congeners at all sites, with some variation across sites for the remaining four top congeners. Variation in PCB patterns in seals at our study sites reveal some of the spatial differences, and is consistent with our previous observation (Fig. 3). A 'heavy' PCB pattern in Puget Sound seals sampled in 1996 was attributed to a near-source profile in a more confined basin, while 'lighter' PCB patterns in seals from sites to the north were attributed to fewer sources, greater sedimentation and burial associated with Fraser River discharge, and the increasing influence of a global 'background' signal (atmospheric and other delivery) in remote food webs (Ross et al., 2004).

Harbor seals from Gertrude Island also had the highest PBDE concentrations among sites, although spatial variation was less pronounced than for the PCBs (Fig. 2b; Table 2). For example, while PCBs were 4.6 times higher at Gertrude than the other sites, PBDE concentrations were only 1.8 times higher. This may reflect the more current use profile for the partially regulated PBDEs, compared to the PCBs that have been banned for more than three decades. Higher PBDE levels in harbor seals in the Strait of Georgia compared to the more remote west coast of Vancouver Island were attributed to proximity to urban centers in the former (Ikonomou and Addison, 2008). PBDE concentrations in Dungeness crab in British Columbia coastal waters were highest near urban sites, followed by pulp mill sites and remote sites (Ikonomou et al., 2006). PBDEs in Salish Sea seals in our study (averaging 490–1047 μg/kg) were lower than those reported in live stranded harbor seals from the southern California coast (2,430 μg/kg; Meng et al., 2009), central California (averaging 660–1100 μg/kg; Greig et al., 2011) or San Francisco Bay (1730 μg/kg; She et al., 2002). Proximity to municipal and industrial sources explained inter-species differences in PBDE concentrations in pinnipeds sampled in the St. Lawrence estuary, Quebec (Frouin et al., 2011).

PBDE patterns in seals were very simple, and except for BDE-99, did not vary among the four study sites (Fig. 3). The contribution of BDE-99 was higher ($p = 0.005$) in Gertrude seals (12.9 ± 1.0%) than those at the three other sites (6.5 ± 1.5 to 8.8 ± 1.4%). BDE-47 dominated PBDE patterns in seals, ranging from 77.2 ± 1.1 to 87.3 ± 2.3% of total PBDE concentrations (Table 2; Supplementary Table 2). The dominant contribution of BDE-47 to total PBDEs has been reported in marine mammals from other parts of the world, including grey seals (*Halichoerus grypus*) in the North Sea at 72% (Kalantzi et al., 2005), ringed seals from East Greenland at 75%, polar bears (*Ursus maritimus*) from different sites in the Arctic at 65–82% (Muir et al., 2006; Rigét et al., 2006), and humpback dolphins (*Sousa chinensis*) from Hong Kong at ~59% (Ramu et al., 2005).

PCDE concentrations were 2–3 times higher in seals from Gertrude Island compared to the three other sites, but were much lower than those observed for PCBs or PBDEs (Fig. 2c; Table 2). The presence of elevated PCDE concentrations at Gertrude Island may

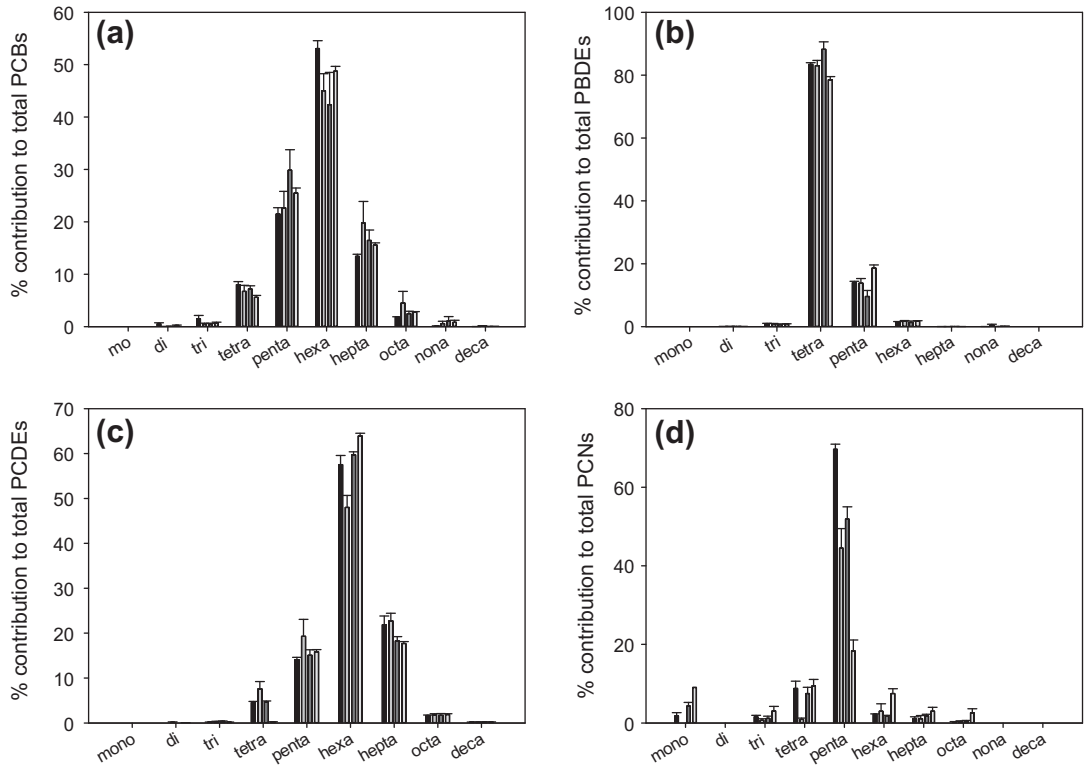

Fig. 3. Homologue group totals for (a) PCBs, (b) PBDEs, (c) PCDEs and (d) PCNs from four sites in coastal British Columbia, Canada (Hornby Island and Vancouver Harbour) and Washington State, USA (Smith Island and Gertrude Island).

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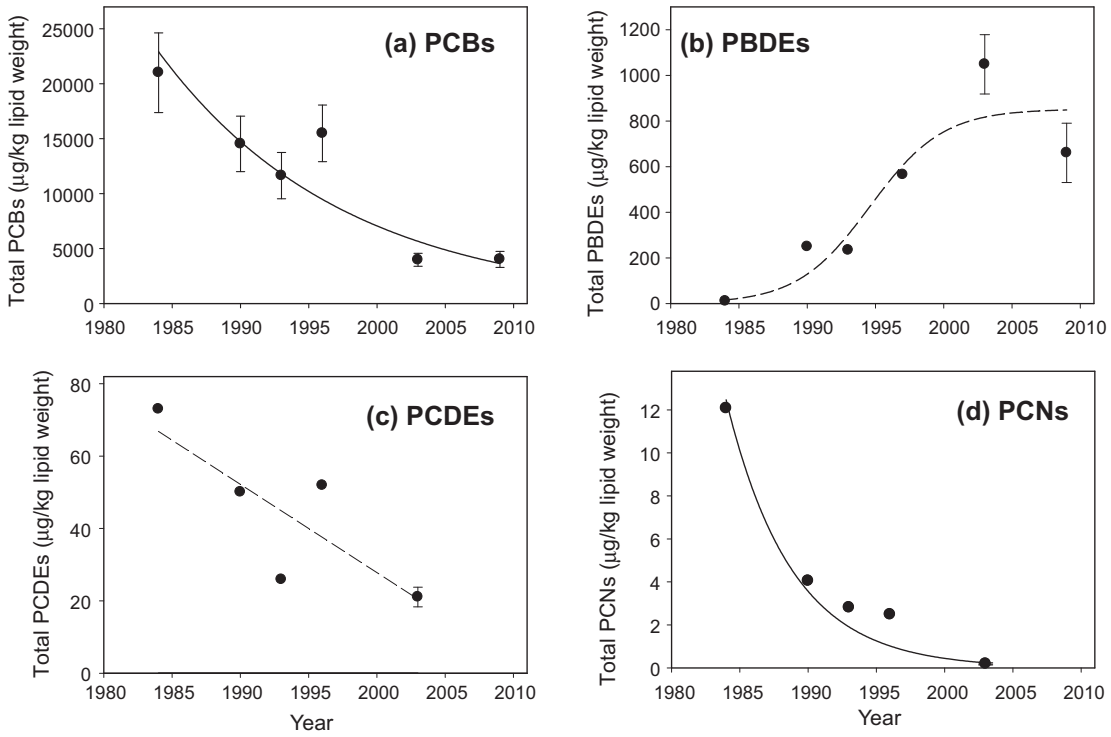

Fig. 4. Temporal trends (1984–2003) are described for total (a) polychlorinated biphenyls (PCBs), (b) polybrominated diphenyl ethers (PBDEs), (c) polychlorinated diphenylethers (PCDEs), and (d) polychlorinated naphthalenes (PCNs) in harbor seal pups from Gertrude Island in south Puget Sound, Washington State, USA. Significant trends are indicated by solid lines, and non-significant trends are indicated by dotted lines as follows: (a) PCBs (exponential decay): $f = a * \exp(-b * x)$; $r^2 = 0.8118$, $p = 0.0142$; (b) PBDEs (sigmoidal): $f = a/(1 + \exp(-(x - x_0)/b))$; $r^2 = 0.8423$, $p = 0.0626$; (c) PCDEs (linear regression): $f = y_0 + a * x$; $r^2 = 0.66$, $p = 0.0947$; (d) PCNs (exponential decay): $f = a * \exp(-b * x)$; $r^2 = 0.9598$, $p = 0.0035$.

be due to the presence of wood treatment facilities in Puget Sound which, prior to the implementation of regulations, would have released these compounds as impurities in PCP and other products.

The top six PCDE congeners comprised 74.1 ± 10.5 to 84.2 ± 3.0% of total concentrations at the four sites, with PCDE-153 dominating at all sites (27.5 ± 2.0 to 40.4 ± 1.2% of total; Table 2; Supplementary Table 2).

Among the flame retardants measured in harbor seals, PCNs were detected at the lowest concentrations, representing less than 1% of total PCB concentrations in the same seals. While PCB, PBDE and PCDE concentrations were highest in Gertrude Island seals, PCNs were lowest (Fig. 2d; Table 2). This may be due to a localized release of these contaminants as by-products of an important pulp and paper processing sector in the Strait of Georgia. Very low levels of PCNs (1–3 orders of magnitude lower than PBDEs) were reported in the region's killer whales (Rayne et al., 2004). As in our study, similar patterns of PCNs, with a dominance of PCN 50/51, were observed in these killer whales. Alternatively, lower concentrations at Gertrude Island may be partly due to a dose-dependent increase in metabolic elimination of these compounds (Boon et al., 1997) in the generally more POP-contaminated seals at this site. A lack of PCN biomagnification from a harbor seal 'food basket' (Cullon et al., 2005) to harbor seals in our study (Biomagnification Factor of 0.03–0.86 in Strait of Georgia) supports the notion that metabolism plays a role in our observations (Supplementary Table 2).

3.3. Temporal trends for contaminants 1984–2009

The 81% drop observed in PCB concentrations in Gertrude Island harbor seals during the 25-year period 1984–2003 indicates that regulations enacted in the USA in 1976 and in Canada in 1977 have reduced PCB inputs into coastal food webs in the Salish Sea (Fig. 4a; Supplementary Table 3). Our calculated half-life for total PCBs in Gertrude Island harbor seals was 10.1 years (Supplementary Table 4). These results continue the downward trend observed in Puget Sound harbor seals which began in the early 1970s (Calambokidis et al., 1984, 2005; Cullon et al., 2005).

The top six PCB congeners accounted for 54.2 ± 1.4 to 63.5 ± 1.7% of the total PCB concentration in seals over this period. PCBs 153, 138 and 99 consistently ranked as the top three congeners over time, while the rankings of PCBs 101, 180 and 187 varied slightly among 4th, 5th and 6th positions. The contribution of the dominant PCB-153 to total PCB concentration varied little over time (averaging 22%), except in 2003, when it dropped to 17.0 ± 0.91% of total (Supplementary Tables 3 and 5).

PBDE concentrations in harbor seals at Gertrude Island increased exponentially during the period 1984–2003, but appeared to drop in 2009 (Fig. 4b; Supplementary Tables 3 and 5). We estimate a doubling time of 3.1 years for PBDEs in seals during the period 1984–2003 (Supplementary Table 4), underscoring the rapid emergence of this flame retardant as a concern in coastal food webs. The apparent drop in 2009 PBDE levels in seals may reflect the withdrawal of the penta- and octa-formulations from the market in 2004, with consequent reductions in their release into coastal environments. Additional sampling into the future will provide further insight into this trend.

A decline in PBDE concentrations in great blue heron (*Ardea herodias*) and double-crested cormorant (*P. auritus*) eggs after peaking in the mid-1990s also points to reduced inputs into the Salish Sea (Elliott et al., 2005), although a delayed response in harbor seals may reflect their long lifespan (up to 30–40 years) and higher trophic level compared to these seabirds. Increases in PBDEs in St. Lawrence River beluga whales (*Delphinapterus leucas*) were ob-

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served during the period 1988–2007 (Lebeuf et al., 2004), but appeared to level off in the latter phase of that period (2000–2007) (Raach et al., 2011). PBDEs in several marine mammal species in Japan appeared to peak in the late 1990s (Kajiwara et al., 2004; Tanabe, 2008). A similar reversal in PBDE trends was observed in harbor porpoises (*Phocoena phocoena*) in northern Europe following the removal of penta-BDEs from the market prior to its ban in 2004 (Law et al., 2010). However, the production and sale of the deca-BDE formulation until at least 2013 in North America will continue to contribute to large environmental reservoirs of congeners which may breakdown into lighter, more bioaccumulative congeners over time (Ross et al., 2009). Most PBDE congeners in lake trout from Lake Ontario peaked in the mid-1990s, but BDE-209 continued to increase thereafter (Ismail et al., 2009). Further monitoring of harbor seals will help to substantiate spatial and temporal trends and document responses to recent regulations.

The top six PBDE congeners accounted for 97.3–99.9% of total PBDE concentrations during the 1984–2003 period, with BDEs 47, 99 and 100 dominating (Supplementary Tables 3 and 5). Pattern variations over time were small, with BDE-47 averaging 70.1 ± 0.4% of total PBDEs during the years 1984–1997, with a slight upward trend to 77.2 ± 1.1% in 2003.

The downward trend for PCDEs in harbor seals was not significant, likely a reflection of small sample size and/or the very low concentrations observed (Fig. 4c). The apparent 3.5-fold reduction between 1984 and 2003 might be substantiated by additional monitoring (Supplementary Table 4). The composition of PCDEs in seals at Gertrude Island varied slightly over time, but PCDE-153 remained the top congener throughout the period, accounting for 32.0 to 40.4 ± 1.2% of the total (Supplementary Tables 3 and 5). This contribution appeared to be highest in 2003, when PCDE-153 accounted for 40.4 ± 1.2% of total PCDEs.

Total PCN concentrations declined between 1984 and 2003 in Gertrude Island seals (Fig. 4d; Supplementary Tables 3 and 5), with an estimated half-life of 3.4 years (Supplementary Table 4). While some variation in the contribution of the top six congeners occurred over time, PCN 50/51 dominated at 40.8–54.4% of total, except for the year 2003, when its contribution dropped to a 15.8% contribution (Supplementary Table 3). The top congener in 2003 was PCN 64/68, a co-eluting congener that previously ranked 3rd to 6th.

3.4. Do contaminant results from 2003 reflect favorable conditions for prey?

The sharp drop in PCB concentrations in harbor seals between 1997 and 2003 was not expected, as trends reported in other matrices displayed modest changes during this period (Johannessen et al., 2008). The contribution of PCB-153 to total PCBs also decreased, shifting towards a pattern resembling that in seals from other sites that same year. PBDE concentrations continued on a trend upwards, but composition changed, with an increase in the contribution of BDE-47 from 70.9 to 77.2 ± 1.1% of total, and a drop in the contribution of BDE-99 from 20 to 12.9 ± 1.0% of total. Lighter PCB signatures have been observed in seals and fish from more remote areas of the Salish Sea and the NE Pacific Ocean, consistent with a global 'background' (Cullon et al., 2005; O'Neill and West, 2009; Ross et al., 2004; West et al., 2008).

Favorable survival conditions for herring towards the turn of the 21st Century led to the highest estimated abundance of herring since 1955 in the Strait of Georgia, estimated at 100,000 tonnes in 2002 and 150,000 tonnes in 2003 (Fisheries and Oceans Canada, 2002, 2004). The spawning biomass for herring in the adjacent Puget Sound was 51% higher in 2002 and 16% higher in 2003 compared to the running 25-year average (calculated from Stick and Lindquist, 2009). With resident fish species in Puget Sound exhibiting higher PCB and PBDE concentrations and heavier signatures than elsewhere in the Salish Sea (Cullon et al., 2005; West et al., 2008), the lower concentrations and lighter signatures observed in Puget Sound seals in 2003 could conceivably reflect a shift toward more herring, both migratory and resident. When available, herring represents a preferred prey item for harbor seals, with its high energy content (Cullon et al., 2005; Olesiuk, 1993). The effects of climate change on food web structure can alter the ways in which persistent contaminants move through the environment and amplify in food webs, and must be considered by contaminant monitoring programs over time and space (Macdonald et al., 2003).

3.5. Mass balance of persistent contaminants in harbor seals

While contaminant concentrations in harbor seals of the Salish Sea changed over time, so too did the number of seals, which increased 2.5 times between 1984 and 2003 in the Salish Sea (Olesiuk, 2009; S. Jeffries, unpublished data). Consequently, the total contaminant burdens in the ~53,000 harbor seals in the Salish Sea (39,190 for the Strait of Georgia, 11,036 for Juan de Fuca Strait, and 2657 for Puget Sound) reflect a combination of the concentrations in the seals and their population size (Table 3). We estimate that there was a total of 2.56 kg of PCBs in harbor seals from the Salish Sea in 2009, a substantial drop from the estimated 9.80 kg in 1997. These seals also had an estimated 0.95 kg of PBDEs in 2009, 31% lower than the peak year (2003), which followed two decades of exponential increases. PCDE and PCN burdens in the Salish Sea seal population were very low after steady decreases from 1984 to 2009; PCDE totals approached zero, while PCN levels amounted to a mere 2 g. The much smaller population of highly contaminated southern resident killer whales ($N = \sim 88$ individuals), that also ply the waters of the Salish Sea, was estimated to have 4.7 kg of PCBs based on 1993–96 biopsy samplings (Ross et al., 2000a).

3.6. Health risks

Health effects associated with persistent environment contaminants have been observed in long-lived, high trophic level marine mammals. These include immunotoxicity (Lie et al., 2005; Mos et al., 2006; Ross et al., 1996), endocrine disruption (Tabuchi et al., 2006), and reproductive impairment (Helle et al., 1976; Reijnders, 1986). While causal underpinnings may not be clear in the case of marine mammal populations exposed to complex contaminant mixtures, POPs have been implicated in reduced reproduction and increased mortality associated with persistent contaminants have been observed in some populations (Hall et al., 2006a; Jepson et al., 1999; Ylitalo et al., 2005). Since logistical, technical, legal and ethical constraints largely preclude mechanistic toxicological research in marine mammals, a weight of evidence compiled from field, semi-field, and laboratory-based *in vitro* and surrogate species approaches provides guidance on the toxicity of POPs (Ross, 2000; Ross and Birnbaum, 2003). While multiple chemicals within a complex mixture have likely contributed to some of the field observations, PCBs have emerged as a pre-eminent concern for marine mammals in the northern hemisphere (Ross et al., 2000b). In the Salish Sea, a risk-based evaluation of 13 POPs identified the PCBs as the major toxicological concern in harbor seals (Mos et al., 2010).

PCB toxicity reference values (TRVs) for marine mammals have been derived, thereby providing benchmarks for conservation, mitigation and/or risk management. These include levels of (i) 17 mg/kg lw, the concentration associated with immunotoxicity (reduced natural killer cell function, T-cell function and *in vivo* responses to immunization) and endocrine disruption (reduced plasma vitamin A) in captive harbor seals fed relatively contaminated herring from

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Table 3
Total mass of PCBs, PBDEs, PCDEs and PCNs (kg) were estimated for the free-ranging populations of harbor seals inhabiting the Salish Sea (Strait of Georgia, Juan de Fuca Strait and Puget Sound) using a life history-based contaminant model. Seal abundance estimates were combined with a demographically-based model for the four flame retardants to generate total contaminant burdens for each of the three main bodies of water in the Salish Sea.

Year:	1984	1990	1993	1997	2003	2009
Number of seals in Salish Sea:	21,175	37,081	52,522	54,983	52,883	52,883
PCBs						
Strait of Georgia	2.876	4.043	4.515	6.288	1.612	1.695
Juan de Fuca	1.059	0.811	1.354	1.704	0.368	0.387
Puget Sound	1.882	2.058	1.240	1.809	0.459	0.483
Total PCBs in Salish Sea seals	*5.817*	*6.911*	*7.110*	*9.801*	*2.439*	*2.564*
PBDEs						
Strait of Georgia	.0023	.0687	.0903	.2515	.9190	0.631
Juan de Fuca	.0008	.0138	.0271	.0682	.3420	0.235
Puget Sound	.0005	.0350	.0248	.0724	.1194	0.082
Total PBDEs in Salish Sea seals	*.0036*	*.1175*	*.1422*	*.3921*	*1.380*	*0.948*
PCDEs						
Strait of Georgia	.0101	.0141	.0099	.0151	.0114	Nd
Juan de Fuca	.0037	.0028	.0030	.0058	.0031	Nd
Puget Sound	.0066	.0072	.0027	.0062	.0024	Nd
Total PCDEs in Salish Sea seals	*.0204*	*.0241*	*.0156*	*.0271*	*.0169*	*Nd*
PCNs						
Strait of Georgia	.0017	.0011	.0011	.0002	.0024	.0017
Juan de Fuca	.0006	.0002	.0003	.0003	.0007	.0005
Puget Sound	.0011	.0006	.0002	.0003	.0001	.0000
Total PCNs in Salish Sea seals	*.0034*	*.0019*	*.0016*	*.0008*	*.0032*	*.0022*

Nd: Not done as estimated 2009 numbers from temporal regression were negative.

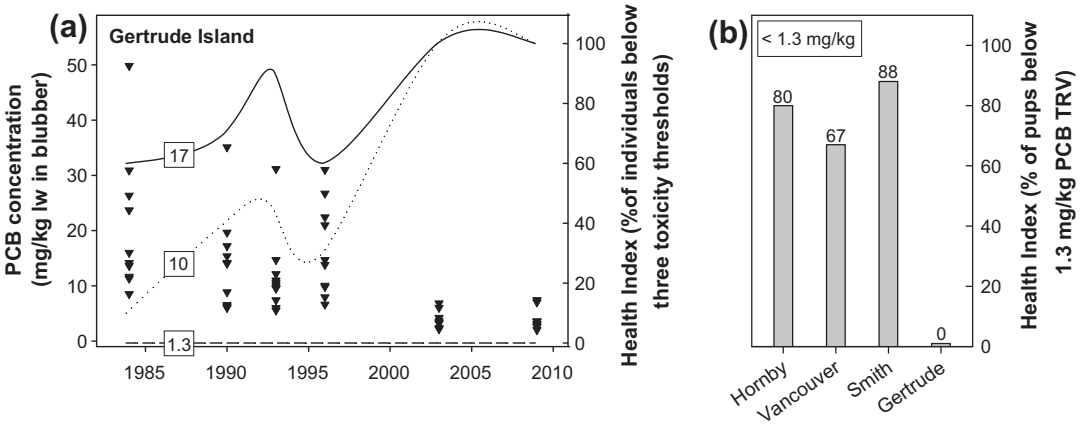

Fig. 5. A) Declining PCB concentrations in harbor seals from Gertrude Island in Puget Sound, Washington, USA (left vertical axis; triangles representing each sampled individual) are described in terms of an improving 'Health Index' [right vertical axis; lines reflect percent of the sampled seals falling below each of three established toxicity reference values (TRVs): 17 mg/kg PCBs for immunotoxicity in captive harbor seals (Ross et al., 1996); 10 mg/kg for EC_{50} for bottlenose dolphin calf mortality (Hall et al., 2006b); and 1.3 mg/kg PCBs EC_5 for immune and endocrine disruption in free-ranging harbor seals (Mos et al., 2010)]. (B) The PCB-related Health Index for the most conservative TRV of 1.3 mg/kg was lowest in seals from the industrialized south Puget Sound (Gertrude Island), and relatively high for seals from the other three sites. This is denoted here as a percentage of the sampled seals falling below the more protective 1.3 mg/kg TRV for immunotoxicity and endocrine disruption (Mos et al., 2010).

the Baltic Sea (De Swart et al., 1996; Ross et al., 1996); (ii) 10 mg/kg lw, the median effective concentration (EC_{50}) at which bottlenose dolphin (*Tursiops truncatus*) calf mortality was predicted to be 50% (Hall et al., 2006b); and (iii) 1.3 mg/lg lw, the 5% tissue residue concentration in free-ranging harbor seals which exhibited dose-related alterations in immune function, endocrine function (vitamin A and thyroid hormones), and gene expression (Aryl hydrocarbon, retinoic acid, and thyroid hormone receptors) (Mos et al., 2010).

The application of these TRVs to our results reveals a reduction in risk of adverse health effects, displayed as an improving 'health index' in Gertrude Island seals between 1984 and 2009 (Fig. 5a). Substantial portions of the seals exceeded all three TRVs prior to 2003, but during 2003 and 2009, 100% of seal pups fell below the two more liberal thresholds (10 and 17 mg/kg). This indicates that health risks in harbor seals of the industrialized Puget Sound declined following regulations. Despite the improving trend, a comparison of seals sampled from the four Salish Sea sites in 2003 reveals that all Gertrude Island seals exceeded the more conservative 1.3 mg/kg TRV, whereas seals from the other sites had relatively good health indices (Fig. 5b). A lack of inferred or demonstrated toxicity reference values for PBDEs, PCDEs and PCNs precludes similar exercises for these contaminants in seals. Nevertheless, low and declining PCDE and PCN levels in Salish Sea seals would indicate that these contaminants likely present a low risk to the health of seals, while the dominant PCBs and the emergent PBDEs warrant further scrutiny.

4. Conclusions

As high-trophic level, non-migratory animals, harbor seals provide an integrated overview of the degree to which the Salish Sea food web is contaminated with persistent, bioaccumulative and toxic compounds from countless local and international sources

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and activities. The lingering health risks associated with relatively high concentrations of PCBs found in harbor seals and other high trophic level species in the Salish Sea are testimony to the persistence of these compounds in the environment. However, our results indicate that the concentrations of several contaminants of concern have declined in harbor seals over time, underscoring an ecosystem level benefit arising from the implementation of national regulations.

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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.pocean.2013.05.027.

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