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Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales

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Abstract

"Southern Resident" killer whales include three "pods" (J, K and L) that reside primarily in Puget Sound/Georgia Basin during the spring, summer and fall. This population was listed as "endangered" in the US and Canada following a 20% decline between 1996 and 2001. The current study, using blubber/epidermis biopsy samples, contributes contemporary information about potential factors (i.e., levels of pollutants or changes in diet) that could adversely affect Southern Residents. Carbon and nitrogen stable isotopes indicated J- and L-pod consumed prey from similar trophic levels in 2004/2006 and also showed no evidence for a large shift in the trophic level of prey consumed by L-pod between 1996 and 2004/2006. ∑PCBs decreased for Southern Residents biopsied in 2004/2006 compared to 1993–1995. Surprisingly, however, a three-year-old male whale (J39) had the highest concentrations of ∑PBDEs, ∑HCHs and HCB. POP ratio differences between J- and L-pod suggested that they occupy different ranges in winter.

Keywords: Biopsy sampling; Stable isotopes; Persistent organic pollutants; Brominated diphenyl ethers; *Orcinus orca*

1. Introduction

Three types of killer whales (*Orcinus orca*) have been documented in the temperate coastal waters of the eastern North Pacific and are termed "residents", "transients" and "offshores" (Bigg, 1982; Ford et al., 2000). Resident killer whales forage, primarily for fish, in relatively large groups in coastal areas. Transient killer whales, whose range extends over a broader area, generally hunt marine mammals. Less is known about offshore killer whales, but their range extends from Alaska to California and their prey appears to include high trophic level fish (Krahn et al., 2007). One resident population, the "Southern Resident" killer whales, was listed as "endangered" under the terms of the Species-at-Risk Act (SARA) in Canada in 2001 (Baird, 2001; Ross, 2006) and the US Endangered Species Act (ESA) in 2005. The Southern Residents include three groups or "pods" of killer whales (J-, K- and L-pod) that reside primarily in Puget Sound (Washington State), the Strait of Juan de Fuca (between the United States and Canada), and the Strait of Georgia (British Columbia) during the spring, summer, and fall (Balcomb, 1982; Osborne, 1986). Photo-identification research begun in 1974 has documented that the Southern Resident population has fluctuated considerably—the first census in 1974 counted 71 whales, the highest count was 97 in 1996 and the November 2006 count was 86 individuals (Center for Whale Research, 2006). Although there has been an overall increase in the population since 1974, a steep decline of 20% occurred

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between 1996 and 2001 (from 97 whales to 78), contributing to the ESA listing. This population downturn was accompanied by large differences in survival rates among age classes, sexes, and pods, suggesting external causes, such as environmental conditions (e.g., El Niño events) during which prey availability may be altered (Krahn et al., 2002).

Persistent organic pollutants [POPs; e.g., PCBs, DDTs, hexachlorocyclohexanes (HCHs), chlordanes, hexachlorobenzene (HCB) and polybrominated diphenyl ethers (PDBEs)]—as well as other stressors (e.g., decrease in quantity and quality of prey, increase in marine noise)—have been cited as potential causes for the recent Southern Resident population decline (Baird, 2001; Krahn et al., 2004, 2002). A large body of evidence links POP exposure to a range of deleterious biological effects (e.g., immune and endocrine system disruption) in marine mammals (O'Hara and O'Shea, 2001). For example, immunosuppressive effects were observed in captive harbor seals (*Phoca vitulina*) that were fed herring from the highly contaminated Baltic Sea (de Swart et al., 1994; Ross et al., 1995). In addition, immune dysfunction, thyroid disruption and neurotoxicity were observed in laboratory animals exposed to PBDE congeners (de Wit, 2002; Eriksson et al., 2001, 2002). Furthermore, POP concentrations in free-ranging harbor seals from Puget Sound, WA have been associated with adverse health effects, including immunotoxicity and endocrine disruption (Mos et al., 2006, 2007; Tabuchi et al., 2006), highlighting regional concerns about the conservation risks associated with high contaminant levels in killer whales from this area (Ross, 2006).

A few studies have measured contaminants in eastern North Pacific killer whales (Calambokidis et al., 1984; Hayteas and Duffield, 2000; Jarman et al., 1996; Ross et al., 2000) and high levels of POPs (e.g., PCBs, DDTs) were found in blubber. Nonetheless, the information on contaminant concentrations in Southern Resident killer whales is a decade old (e.g., biopsy samples were collected in the mid-1990s), so information on current levels of the legacy POPs (e.g., PCBs and DDTs), as well as levels of certain emerging contaminants (e.g., the flame-retardant PBDEs), is needed to evaluate the present-day risk to these whales from exposure to POPs.

Marine predators, such as killer whales, incorporate chemicals from their prey to reflect the average trophic level of the diet and the regions from which the prey were taken (Krahn et al., 2007). Thus, stable isotope enrichments of ^{13}C and ^{15}N in the epidermis can be measured to assess the geographic area and trophic position at which marine mammals feed (Kelly, 2000). In addition, POP patterns and ratios can be used to provide insight into regional sources of pollutants transferred to predators from their prey (Calambokidis and Barlow, 1991; Krahn et al., 1999, 2007; Muir et al., 1990). For example, cetacean stocks have been differentiated using patterns of POPs in their blubber (Krahn et al., 1999; Muir et al., 1996), presumably due to differences in POPs in their prey. POPs ratios (e.g., $\sum$DDTs/$\sum$PCBs and $\sum$PBDEs/$\sum$PCBs) have been used to provide insight into killer whale foraging regions for killer whale populations found in Alaska (Krahn et al., 2007). Because DDT was heavily used in California before its ban in the 1970s, $\sum$DDTs/$\sum$PCBs ratios are typically higher in marine species from waters off California (the "California signature") than in comparable species from other eastern North Pacific locations (Brown et al., 1998; Calambokidis and Barlow, 1991; Jarman et al., 1996). Furthermore, the PBDE flame retardants found in urban runoff and sewage effluents have emerged as an important class of toxicants because of their recent exponential increase in marine biota (de Wit, 2002). Thus, investigation of the $\sum$PBDEs/$\sum$PCBs ratio may identify sources of contamination indicative of an urban environment (the "urban signature").

The current study was designed to contribute contemporary information regarding potential risk factors (i.e., levels of pollutants or changes in diet) to Southern Residents through analysis of blubber/epidermis biopsy samples. Nine Southern Resident killer whales (four from J-pod and five from L-pod) were biopsied in 2004/2006. Each sample was analyzed for POPs, carbon and nitrogen stable isotopes and lipids in order to assess possible changes in the Southern Residents POP levels and diet. For example, $\sum$PCBs were compared to levels measured in adult Southern Resident killer whales biopsied about a decade earlier to assess how contaminant levels have changed over time. These data were also used to estimate whether the current levels of these POPs are high enough in Southern Residents to pose a potential risk to the population. In addition, stable isotope values were used to investigate the trophic level at which Southern Residents feed. Finally, ratios of particular POPs provided information on regional foraging areas.

2. Materials and methods

2.1. Killer whale photo-identification and sampling

The killer whales biopsied for this study have been photo-identified through the work of the Center for Whale Research in Friday Harbor, WA (Center for Whale Research, 2006) and are part of a population that has been tracked yearly since 1974. For this study, Southern Resident killer whale blubber/epidermis biopsy samples were collected in Canada during May, 2004 from L-pod ($n = 3$) and in the US (Puget Sound/Georgia Basin) in May and June, 2006 from both J-pod ($n = 4$) and L-pod ($n = 2$) (Table 1). All samples were obtained using documented sampling techniques (Barrett-Lennard, 2000; Hoelzel et al., 1998; Ylitalo et al., 2001) and biopsy tips measuring 0.6×3.5 cm were used in both Canada and the US. Each biopsy sample collected in 2004 (Canada) was wrapped in acetone/hexane-rinsed aluminium foil, placed into cryovials, placed in liquid nitrogen immediately after collection and then stored at -80 °C in the laboratory.

Table 1
Life history data for Southern Resident killer whales sampled by biopsy

Sampling date	Animal ID	Age (years)	Sex
6/15/2006	J19	27	F
5/23/2006	J39	3	M
5/19/2006	J1	55	M
5/19/2006	J27	15	M
5/22/2006	L57	29	M
2004	L71	18	M
2004	L74	18	M
2004	L78	15	M
6/14/2006	L85	15	M

Subsamples taken lengthwise (averaging ~ 100 mg) were subsequently shipped on dry ice to Seattle for contaminant analysis. US biopsy samples were transferred to clean glass vials, stored on ice while in the field and subsequently stored at −80 °C until analyzed. Each biopsy sample collected in 2006 (US) was first split in half lengthwise. Then one-half the sample was cut horizontally to a standardized depth of 2 cm as reported previously (Herman et al., 2005). The remainder of the biopsy sample (about 3/4 of the sample) was archived at −80 °C. Blubber from 2004/2006 biopsy samples was analyzed for POPs and, because epidermis samples were not available from the 2004 biopsies, only 2006 epidermis samples were analyzed for stable isotopes. Epidermis samples (but no blubber) were also available from three Southern Residents from L-pod biopsied in 1996 (Table 2), so stable isotope analyses were also conducted on these samples.

2.2. Analyses for stable isotopes of carbon and nitrogen

Stable isotope analyses of epidermis from killer whales were conducted on lipid-extracted tissues as described previously (Herman et al., 2005). All nitrogen values were referenced to atmospheric nitrogen ($\delta^{15}N$ for atmospheric N_2 is 0‰ exactly) and carbon values were referenced to Vienna Pee Dee Belemnite [i.e., $\delta^{13}C$ of NBS 19 ≡ 1.95‰ (Coplen et al., 2006)]. The daily laboratory standards were calibrated using the following primary standard values: NBS-22 ($\delta^{13}C = -30.03$); IAEA-CH-6 ($\delta^{13}C = -10.45$); IAEA-N-1 ($\delta^{15}N = 0.43$); and IAEA-N-2 ($\delta^{15}N = 20.39$). Precision for isotope analysis was $\leqslant \pm 0.3$‰ for $\delta^{15}N$ and $\leqslant \pm 0.2$‰ for $\delta^{13}C$. A standard reference material (NIST SRM 1946) was processed with every 20 analyses to monitor analytical accuracy.

Table 2
Mean[a](±1 SD) stable isotope values in epidermis from 1996 and 2006 biopsy samples of Southern Resident killer whales

Whale ID	Sex	Age (years)	$\delta^{13}C$	$\delta^{15}N$
Sampled in 2006				
J19	F	27	−15.2 ± 0.1	16.3 ± 0.1
J39	M	3	−15.8 ± 0.02	16.1 ± 0.1
J1	M	55	−15.7 ± 0.5	16.3 ± 0.1
J27	M	15	−16.0 ± 0.1	16.6 ± 0.04
L57	M	29	−16.0 ± 0.1	16.5 ± 0.1
L85	M	15	−15.6 ± 0.03	17.0 ± 0.1
Sampled in 1996				
L11	F	39	−16.1 ± 0.1	16.6 ± 0.4
L41	M	19	−16.0 ± 0.1	17.3 ± 0.5
L77	F	9	−15.7 ± 0.1	17.1 ± 0.7

[a] Mean of 3 analyses of each skin sample.

2.3. Analyses for persistent organic pollutants

Blubber from killer whale biopsies was analyzed for POP concentrations using the procedure of Sloan et al. (2005). Briefly, the method involves: (1) extraction of approximately 0.5–1.0 g of tissue (mixed with sodium and magnesium sulfates to remove water) by Accelerated Solvent Extraction using 50 ml methylene chloride at 100 °C and 2000 psi; (2) clean-up of the entire methylene chloride extract on a single stacked silica gel/alumina column; (3) separation of OCs from the bulk lipid and other biogenic material by high-performance size exclusion liquid chromatography; and (4) analysis on a low resolution quadrupole GC/MS system equipped with a 60-m DB-5 GC capillary column. The instrument was calibrated using a set of 10 multi-level calibration standards of known concentrations. A total of 45 PCB congeners and 24 chlorinated pesticides were determined in the samples. ∑PCBs is the sum of all 45 PCB congeners analyzed; ∑DDTs is the sum of *o*,*p*′-DDD, *p*,*p*′-DDD, *p*,*p*′-DDE, *o*,*p*′-DDE, *o*,*p*′-DDT and *p*,*p*′-DDT; ∑chlordanes is the sum of oxychlordane, *gamma*-chlordane, nona-III-chlordane, *alpha*-chlordane, *trans*-nonachlor, and *cis*-nonachlor; the ∑HCHs is the sum of *alpha*-,*beta*-, and *gamma*-HCH isomers and finally ∑PBDEs is the sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183. Total lipids in killer whale biopsy samples were measured by a TLC-FID method (Ylitalo et al., 2005). All POP concentrations reported in this paper have been lipid-normalized.

As part of a performance-based quality assurance program, quality control samples [i.e., method blank, replicate and standard reference materials (SRMs)] were analyzed with each set of field samples as described by Sloan et al. (2006). Results obtained were in agreement with certified and reference values published by National Institute of Standards and Technology for each SRM; other quality control samples met established laboratory criteria.

2.4. Statistical analyses

All univariate and multivariate analyses were conducted using JMP Statistical Discovery Software (Mac professional edition, version 5.01). Unless indicated otherwise, all univariate comparisons between two group means were significance tested using a two sample Student's *t*-test assuming unequal variances. Significant differences among multiple groups having equal variances were evaluated using a Tukey HSD test ($\alpha = 0.05$).

3. Results and discussion

Decreased quantity and quality of prey have been cited as possible risk factors for the population decline of Southern Resident killer whales (Krahn et al., 2004, 2002). The preferred prey of Southern Residents are reported to be Chinook salmon (*Oncorhynchus tshawytscha*) (Ford and Ellis, 2006), a high trophic level species (Herman et al., 2005). Other potential prey species (e.g., groundfish and other salmon species) are generally of lower trophic levels than those for Chinook (O'Neill et al., 2006), so consumption might provide less nutrition for the whales. Thus, stable isotope values of nitrogen—often used to assess the trophic level at which marine mammals feed (Kelly, 2000)—were investigated in Southern Resident killer whales. Because the stable isotope values of carbon and nitrogen measured in the epidermis of these whales (Table 2) did not differ significantly between J-pod ($n = 4$) and L-pod ($n = 2$), the two pods appear to be consuming prey from the same trophic level. Stable isotope values of carbon and nitrogen were also measured in epidermis samples from three L-pod Southern Residents sampled in 1996 (Table 2). The values did not appear to differ greatly between 1996 ($\delta^{13}C = -15.9 \pm 0.2$ and $\delta^{15}N = 17.0 \pm 0.4$, $n = 3$) and 2006 ($\delta^{13}C = -15.8 \pm 0.2$ and $\delta^{15}N = 16.7 \pm 0.4$, $n = 2$), but the sample size was small. Because of the limited dataset available ($n = 3$ in 1996 and $n = 2$ in 2006), $\delta^{15}N$ values for diet were investigated to see if changes in trophic level of prey could be detected with a small sample size. If the 1996 whales were eating a diet comprising 80% Chinook ($\delta^{15}N = 14.7$) and 20% chum salmon (*Oncorhynchus keta*) ($\delta^{15}N = 12.2$) [stable isotope values from O'Neill et al. (2006)], a change in diet to 50% Chinook and 50% chum salmon in 2006 would lead to statistically detectable differences (i.e., $\delta^{15}N$ mean ± CI would change from 17.7 ± 0.3 to 16.9 ± 0.3; $p < 0.05$). Thus, the limited stable isotope data appear to demonstrate that the trophic level of prey species consumed by L-pod whales in 2006 had not greatly changed when compared to the diet of that pod in 1996.

Concentrations of POPs—∑PCBs, ∑DDTs, ∑PBDEs, ∑chlordanes, ∑HCHs and HCB—were measured in the biopsy blubber of Southern Resident killer whales collected in 2004/2006 (Table 3 and Fig. 1). The 2004/2006 data were used to calculate POP ratios and to estimate whether current ∑PCB levels are high enough to pose a potential risk to the population. In addition, ∑PCBs were compared to levels measured in adult Southern Resident killer whales biopsied about a decade earlier to assess how contaminant levels have changed over time. Ross et al. (2000) reported ∑PCB concentrations (mean ± SEM) of 146,000 ± 32,700 ng/g lipid for males ($n = 4$; approximate ages 18, 37, 40, 44; mean 35) and 55,400 ± 19,300 ng/g lipid for females ($n = 2$; ages 14 and 49; mean 32) biopsied between 1993 and 1996. In the current study, ∑PCB concentrations (mean ± SEM) were 66,000 ± 26,000 ng/g lipid in the adult male Southern Residents ($n = 7$; approximate ages 15, 15, 15, 18, 18, 29, 55; mean 24) and 45,000 ng/g lipid in the single adult female (age = 27), about 45% and 80% of earlier-reported concentrations, respectively. Although the single female Southern Resident biopsied in 2006 was similar in age to the mean age of female whales biopsied between 1993 and 1995, the 2004/2006 males were younger by about 10 years than the males biopsied in 1993–1995. Thus, age may account, in part, for temporal differences in ∑PCBs. In addition, differences in the methods used to calculate ∑PCBs—the current study summed 45 major PCB congeners, whereas Ross et al. (2000) summed 136 "detectable" congeners—could partially account for higher ∑PCBs in 1993–1995 samples due to inclusion of several minor PCB congeners. Finally, because the ban on PCB use in much of the world has resulted in decreased levels of PCBs in the environment (de Wit et al., 2004), it is quite possible that the exposure of Southern Resident killer whales to PCBs has also decreased.

Northern Residents—a population of killer whales occupying British Columbia waters in summer—had POP concentrations that were reported to increase with age in males (Ross et al., 2000). In contrast, concentrations in reproductive females did not increase until senescence (40–45 years), because females transfer a substantial portion of their contaminant burden to their calves (Ross et al., 2000). As a result, POP concentrations in females are generally lower than in males and are also partially

Table 3
Persistent organic pollutants (ng/g lipid) and percent lipid in blubber of biopsy samples from Southern Resident killer whales

Animal ID	Age (years)	Lipid (%)	∑PCBs	∑DDTs	∑PBDEs	∑Chlordanes	∑HCHs	HCB
Female								
J19	27	29.4	45,000	26,000	7500	4300	310	160
Males								
J39	3	40.9	34,000	24,000	15,000	5100	1300	1600
L78	15	15.2	22,000	38,000	2600	7400	630	600
L85	15	24.8	50,000	120,000	2500	7700	530	550
J27	15	30.4	74,000	61,000	6300	7000	580	480
L71	18	9.6	36,000	72,000	2600	13,000	920	730
L74	18	18.0	45,000	86,000	3100	12,000	720	600
L57	29	19.4	56,000	110,000	3300	8600	640	520
J1	55	21.9	180,000	160,000	6800	14,000	820	570

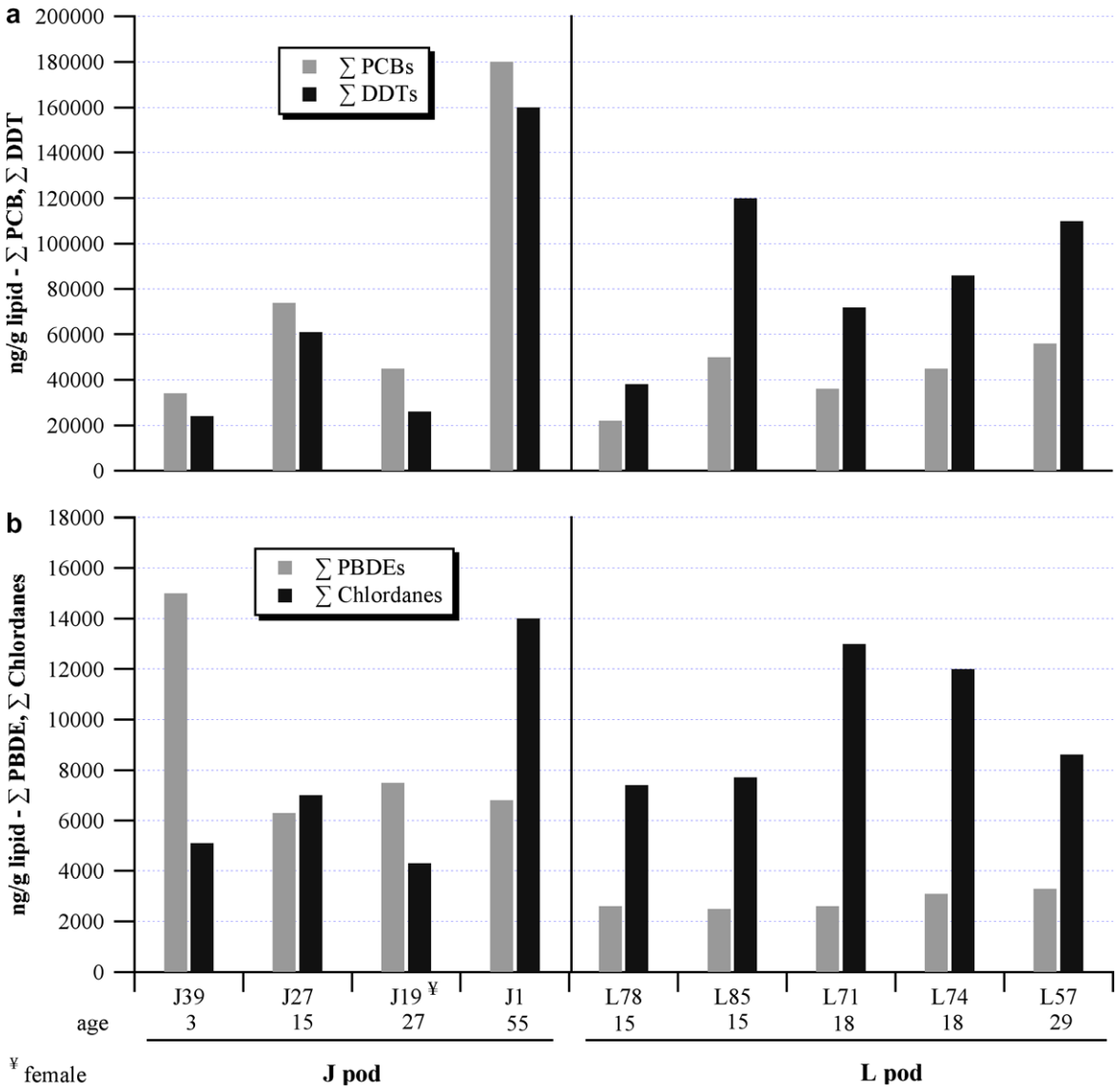

Fig. 1. Concentrations (ng/g lipid) of selected POPs in individual Southern Resident killer whales.

dependent of the number of times they have given birth (Borga et al., 2004; Ross et al., 2000; Ylitalo et al., 2001). To evaluate whether POP concentrations in the male Southern Resident killer whales also increase with age, concentrations of each group of POPs were regressed with age for each male whale ($n = 8$). ∑PCBs, ∑DDTs and ∑chlordanes all increased with age in male Southern Residents (∑PCBs: $r^2 = 0.80$, $P < 0.003$; ∑DDTs: $r^2 = 0.71$, $P < 0.009$; and ∑chlordanes: $r^2 = 0.50$, $P = 0.05$). As expected, the oldest male, J1, had the highest concentrations of these POP groups (Table 3; Fig. 1). Surprisingly, however, the three-year-old male whale (J39) had the highest concentrations of ∑PBDEs, ∑HCHs and HCB (Table 3; Fig. 1). Furthermore, these POPs did not correlate with age (∑PBDEs: $r^2 = 0.045$, $P = 0.62$; ∑HCHs: $r^2 = 0.045$, $P = 0.61$; and HCB: $r^2 = 0.23$, $P = 0.23$). Even when J39 was eliminated from the regression, these POPs still did not correlate with age. The contaminant levels in this young individual may have been influenced by its birth order (Ylitalo et al., 2001), as well as the extent of growth dilution in POP concentrations during its first few years (Hickie et al., 2000).

Although little work exists on toxic endpoints of POPs in cetaceans, some toxic thresholds (e.g., immune and reproductive dysfunction) have been reported, particularly for PCBs in pinnipeds (Boon et al., 1987; Reijnders, 1986; Ross et al., 1996, 1995) and other mammalian species (Brunstrom and Halldin, 2000; Murk et al., 1998; Roos et al., 2001). All Southern Resident killer whales sampled in this study had ∑PCBs that exceeded thresholds for health effects (e.g., 17,000 ng/g lipid for immunosuppression) established in captive studies of harbor seals (Ross et al., 1996, 1995). While caution should be used when making interspecies comparisons (de Wit et al., 2004; Levin et al., 2007, 2005; Mori et al., 2006), the results suggest that these killer whales are highly contaminated with PCBs and

at risk for adverse health effects. Unfortunately, marine mammal health effect thresholds for other POPs (e.g., DDTs, PBDEs, chlordanes, HCHs and HCB) have not been reported in the literature.

In one report, a positive correlation was found between blubber concentrations of ∑PBDEs (61–1500 ng/g, lipid) and alterations in thyroid hormone levels of post-weaned and juvenile grey seals (Hall et al., 2003). Thus, it was particularly troubling to find that blubber of the juvenile whale J39 contained concentrations of ∑PBDEs 10 times higher (15,000 ng/g, lipid) than the highest of those in the grey seals. Even the Southern Resident with the lowest ∑PBDEs (L85; 2500 ng/g lipid) had concentrations higher than those associated with endocrine disruption in the grey seals. Because PBDEs are structurally similar to PCBs, these two groups of chemicals are thought to have similar mechanisms of toxic action (de Wit, 2002). Juvenile animals may be more vulnerable to toxic effects of contaminants than older animals are, because biological systems in young animals are undergoing rapid development, so disruption of the regulating hormones could have serious consequences. For example, in mice, postnatal exposure to PCBs and PBDEs caused developmental neurotoxic effects when present during a critical stage of neonatal brain development (Eriksson et al., 2006, 2002; Viberg et al., 2003). In addition, neurobehavioral effects worsened with age in mice neonatally exposed to both PCB 52 and PBDE 99 (Eriksson et al., 2006). Finally, the PBDE concentrations linked to thyroid hormone disruption in the study by Hall et al. (2003) were much lower than the previously documented thresholds for PCB-associated reproductive and immune dysfunction (Reijnders, 1986; Ross et al., 1996), so further work will be necessary to determine the relative toxicity of PBDEs and PCBs.

As previously noted, the ratios of certain POPs in marine biota can provide insight into regional patterns of pollutants. One interesting observation to emerge from studying ∑DDTs/∑PCBs ratios in Southern Residents, was the difference between J-pod and L-pod. Although the ∑DDTs/∑PCBs ratios within each pod were similar, the ratios for L-pod whales (2.0 ± 0.20) were about 3-fold higher and significantly different ($p < 0.0005$) from those for J-pod (0.75 ± 0.14; Fig. 2). These "legacy" POPs have been acquired over the lifetime of each whale, so the differences in ratios suggest either that they are feeding on different prey or that the areas in which L-pod whales feed are, at times, spatially distinct from those of J-pod whales. The latter scenario is supported by the higher frequency of J-pod sightings in Puget Sound compared to those of the other two pods (Osborne, 1999). Furthermore, K- and L-pods—but not J-pod—have been sighted several times off the coast of California. Together, this provides support for the observation that L-pod's relatively high ∑DDTs/∑PCBs ratio reflects an increased consumption of prey containing higher relative DDTs—a "California signature" (Brown et al., 1998; Calambokidis and Barlow, 1991; Jarman et al., 1996). Conversely, the higher relative PCB content in J-pod is consistent with observations of high PCB concentrations in Puget Sound biota, including "resident" Chinook salmon (O'Neill et al., 2006) and harbor seals (Ross et al., 2004).

Additional support for the "California signature" of prey consumed by L-pod is found by comparing the ∑DDTs/∑PCBs ratio in L-pod ($n = 5$; 1.99 ± 0.20) to those for Chinook salmon from California ($n = 10$; 2.60 ± 0.50), the Columbia River ($n = 10$; 1.17 ± 0.23), the Fraser River ($n = 13$; 0.73 ± 0.21) and Puget Sound (resident "blackmouth"; $n = 26$; 0.24 ± 0.29) (O'Neill et al., 2006). L-pod would likely not have attained the

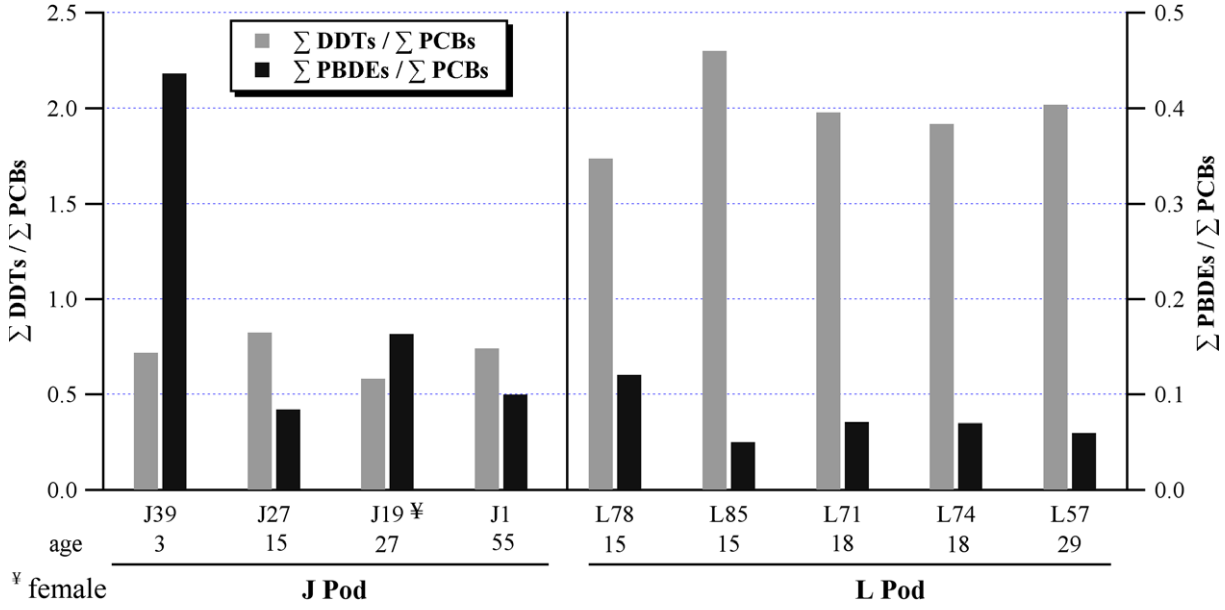

Fig. 2. The ratios ∑DDTs/∑PCBs and ∑PBDEs/∑PCBs in individual Southern Resident killer whales.

relatively high ∑DDTs/∑PCBs ratio by consuming only Puget Sound or Fraser River Chinook. On the other hand, the high ∑DDTs/∑PCBs ratios of Columbia River and California Chinook indicate that these populations are likely included in the L-pod diet. Conversely, J-pod ($n = 4$; 0.75 ± 0.14) could not consume large quantities of California or Columbia River Chinook without its ∑DDTs/∑PCBs ratio showing an increase, but the Fraser River Chinook ratio (O'Neill et al., 2006) is in the correct range to result in J-pod's ∑DDTs/∑PCBs ratio. The three Southern Resident pods share the Puget Sound/Georgia Basin habitat in the warmer months of the year and field observations have indicated these whales consume salmon from a similar source [primarily the Fraser River (Hanson et al., in preparation)]. In contrast, POP ratios suggest that J- and L-pods are consuming prey from different areas at other times of the year, so these pods likely occupy different ranges in winter.

The Juvenile Southern Resident (J39) had a substantially higher ∑PBDEs/∑PCBs ratio than was found for the other whales (Fig. 2), consistent with its relatively low ∑PCBs and high ∑PBDEs concentrations. Because this whale is young and probably nursed for one to two years (Haenel, 1986), a substantial portion of its contaminants were likely obtained from its mother (Hickie et al., 1999). Interestingly, J39's mother (J11) had not been observed with a calf for eight years prior to the birth of J39 when she was 31 years old (Center for Whale Research, 2006). Thus, the contaminant levels of J11 prior to giving birth may have been relatively high, and as a consequence, high amounts of POPs, including ∑PBDEs, were transferred to her offspring, J39. Nonetheless, some provisioning of solid food to calves occurs, e.g., Heyning (1988), thus J39 may have also been feeding on fish and acquiring contaminants directly from prey species. High PBDE levels suggest an "urban" signature, so this whale—and particularly its mother—may have spent a large part of their lives in Puget Sound/Georgia Basin or other areas where the ∑PBDEs/∑PCBs ratio is high in prey species.

4. Conclusions

In conclusion, stable isotope values of carbon and nitrogen indicated the J- and L-pods of Southern Residents consumed prey from similar trophic levels in 2004/2006. These stable isotopes also provided no evidence for a large shift in the trophic level of prey consumed by L-pod between 1996 and 2004/2006, but the statistical power to resolve such differences was low. Mean levels of ∑PCBs decreased for Southern Residents biopsied in 2004/2006 compared to those from 1993 to 1995. Differences in ages in the two groups of males, differences in methods used to calculate ∑PCBs and decreases in environmental levels of PCBs could all be contributors to these temporal differences. Surprisingly, a three-year-old male whale (J39) had the highest concentrations of ∑PBDEs, ∑HCHs and HCB measured in the current study and a substantial portion of its contaminants were likely transferred from its mother. Although the three Southern Resident pods occupy the same waters in warmer months, POP ratio differences between J- and L-pod suggested that these two pods occupy different ranges in winter. Finally, all Southern Resident killer whales sampled in this study had ∑PCBs that exceeded thresholds for health effects established in captive studies of harbor seals. While caution should be used when making interspecies comparisons, the results suggest that these killer whales are highly contaminated with PCBs and at risk for adverse health effects.

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