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Effects of age, sex and reproductive status on persistent organic pollutant concentrations in "Southern Resident" killer whales

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ABSTRACT

"Southern Resident" killer whales (*Orcinus orca*) that comprise three fish-eating "pods" (J, K and L) were listed as "endangered" in the US and Canada following a 20% population decline between 1996 and 2001. Blubber biopsy samples from Southern Resident juveniles had statistically higher concentrations of certain persistent organic pollutants than were found for adults. Most Southern Resident killer whales, including the four juveniles, exceeded the health-effects threshold for total PCBs in marine mammal blubber. Maternal transfer of contaminants to the juveniles during rapid development of their biological systems may put these young whales at greater risk than adults for adverse health effects (e.g., immune and endocrine system dysfunction). Pollutant ratios and field observations established that two of the pods (K- and L-pod) travel to California to forage. Nitrogen stable isotope values, supported by field observations, indicated possible changes in the diet of L-pod over the last decade.

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1. Introduction

"Resident" killer whales (*Orcinus orca*) forage, primarily for fish, in coastal areas from California to Alaska in the eastern North Pacific. The "Southern Resident" population includes stable groups of related individuals, termed "pods" (J-, K- and L-pods), that reside 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; Hauser et al., 2007; Osborne, 1986). Photo-identification research begun in 1974 and continued every year since then (Center for Whale Research, 2008) has documented that the Southern Resident population has fluctuated considerably – the first census in 1974 counted 71 whales and the highest count was 97 in 1996. A steep decline of 20% occurred between 1996 and 2001 (from 97 whales to 78) (Krahn et al., 2004a, 2002). This decline contributed to listing the "Southern Resident" killer whales as "endangered" in Canada in 2001 and the US in 2005. Although the population had rebounded to 91 whales by 2005, the July 2008 count was only 85 individuals (Carretta et al., 2007; Center for Whale Research, 2008). The population decrease in the late 1990s 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 have changed (Krahn et al., 2002). As an outcome of the endangered species listings in the US and Canada, recovery plans for this population have been prepared that define threats to survival and formulate recovery strategies to mitigate the threats (Fisheries and Oceans Canada, 2008; National Marine Fisheries Service, 2008).

Potential causes cited for the Southern Resident population decline included environmental pollutants, as well as other stressors (e.g., decrease in quantity and quality of prey, increase in marine noise) (Baird, 2001; Krahn et al., 2004a, 2002). Persistent organic pollutants (POPs) comprise both chlorinated [e.g., PCBs, DDTs, chlordanes, hexachlorocyclohexanes (HCHs) and hexachlorobenzene (HCB)] and brominated [e.g., polybrominated diphenyl ethers (PBDEs)] environmental contaminants. A large body of evidence links organochlorine contaminant exposure to a range of deleterious biological effects (e.g., immune, reproductive and endocrine system dysfunction, increased risk of infection) in marine mammals (de Swart et al., 1994; Hall et al., 2006; Jepson et al., 2005; O'Hara and O'Shea, 2001; Ross et al., 1995). Furthermore, immune dysfunction, thyroid disruption and neurotoxicity were observed in laboratory animals exposed to PBDE congeners (de Wit, 2002; Eriksson et al., 2001, 2002). High levels of POPs (e.g., PCBs and DDTs) were found in blubber of eastern North Pacific killer whales in a few early studies (Calambokidis et al., 1984; Hayteas and Duffield, 2000; Jarman et al., 1996; Ross et al., 2000). More recently, Krahn et al. (2007b) reported that Southern Resident killer whale blubber contained $\sum$PCBs that exceeded thresholds for health ef-

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fects established in captive studies of harbor seal (Ross et al., 1996, 1995).

Chemical "tracers" from prey are incorporated into the tissues of marine predators (e.g., killer whales) and can provide important diet information (Krahn et al., 2007a). For example, stable isotope values of carbon and nitrogen can be measured in the epidermis of whales to assess the geographic area and trophic position at which these animals feed (Kelly, 2000). In addition, patterns and ratios of POPs can provide insight into regional sources of pollutants (e.g., DDTs from local sources in waters off California) transferred from prey to predators (Calambokidis and Barlow, 1991; Krahn et al., 1999, 2007a; Muir et al., 1990). Among the Southern Residents, L-pod whales showed significantly higher ratios of ∑DDTs relative to ∑PCBs than were found for J-pod (Krahn et al., 2007b). Because POPs are acquired over the lifetime of each whale, the differences in this ratio suggested either that these pods were feeding on different prey species or that the areas in which L-pod whales feed have been, at times, spatially distinct from those of J-pod whales. This chemical tracer evidence was supported by field observations, because L-pod has been sighted several times off the coast of California (Black, Unpublished data), whereas J-pod has not been seen in those waters (Osborne, 1999).

This study extends the information reported by Krahn et al. (2007b), by providing a greater sample size from which to draw statistical conclusions. Twelve Southern Resident killer whales (two from J-pod, five from K-pod and five from L-pod) were biopsied in 2007. 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. Of particular interest was whether the three young whales biopsied for this study had levels of certain POPs as high as those found for the young whale (J39) biopsied in 2006. Furthermore, this study provided the first data on levels of POPs in K-pod whales. In addition, two mother–offspring pairs were biopsied, so relationships between POP levels in these pairs were assessed. Finally, stable isotope values were used to investigate the trophic level at which Southern Residents feed and ratios of particular POPs provided information on regional foraging areas.

2. Materials and methods

2.1. Killer whale photo-identification and sampling

Since 1974, an annual photographic census has been conducted of all individuals in the Southern Resident population by scientists from the Center for Whale Research (Friday Harbor, WA), so age, sex, and reproductive history have been documented for each whale (Center for Whale Research, 2008). Identities of individual Southern Resident killer whales to be biopsied were determined (and confirmed by photographs) before blubber/epidermis biopsy samples were collected during 2007 from whales in J-pod ($n = 2$), K-pod ($n = 5$) and L-pod ($n = 5$) (Table 1). All samples were obtained using documented sampling techniques (biopsy tips measured 0.6 × 3.5 cm) (Barrett-Lennard, 2000; Hoelzel et al., 1998; Ylitalo et al., 2001). Biopsy samples were stored on ice in the field and then stored at −80 °C until analyzed. Each biopsy sample was first split in half lengthwise and then one-half the sample was cut horizontally to a standardized depth of 2 cm as reported previously (Herman et al., 2005). The remainder (about 3/4 of the sample) was archived at −80 °C. Because lipid-adjusted POP concentrations are not highly stratified by blubber depth in killer whales (Krahn et al., 2004b), biopsy samples are a good representation of lipid-normalized concentrations in the entire blubber layer.

2.2. Analyses for persistent organic pollutants and stable isotopes

Killer whale blubber was analyzed for POP concentrations using the procedure of Sloan et al. (2005). Total lipids were measured by a TLC/FID method (Ylitalo et al., 2005). All POP concentrations in

Table 1
Life history data, concentrations of persistent organic pollutants (ng/g lipid) in blubber, percent lipid of blubber, and stable isotope values (mean ± 1 SD) in epidermis from biopsy samples of Southern Resident killer whales.

Whale ID[a]	Sampling date	Sex[a]	Age (years)[a]	Birth order[a,b]	Calves born/ surviving[a,c]	lipid (%)	∑PCBs[d]	∑DDTs[e]	∑PBDEs[f]	∑CHLDs[g]	∑HCHs[h]	HCB	$\delta^{13}C$[i]	$\delta^{15}N$[i]
J-pod														
J22	6/10/07	F	22	3	2/2	28.4	4600	1500	880	290	62	76	−15.8 ± 0.07	16.6±0.08
J38	6/8/07	M	4	2	NA	20.9	41,000	24,000	14,000	5100	1000	1200	−15.7 ± 0.05	16.7±0.20
K-pod														
K7	12/14/07	F	est 97	U	3[c]/1	28.5	120,000	44,000	6700	16,000	1100	650	−16.8 ± 0.04	16.0±0.18
K13	12/14/07	F	35	1[b]	4[c]/4	22.0	8900	11,000	1200	1400	300	270	−16.2 ± 0.05	15.9 ± 0.14
K21	12/14/07	M	21	4[b]	NA	26.6	38,000	73,000	2900	6400	410	360	−16.2 ± 0.05	16.1 ± 0.24
K34	12/14/07	M	6	4	NA	22.3	39,000	61,000	10,000	7900	1200	1200	−16.5 ± 0.04	15.7 ± 0.29
K36	12/14/07	F	4	4[b]	NA	18.3	62,000	95,000	15,000	12,000	1700	2000	−16.4 ± 0.05	16.0 ± 0.14
L-pod														
L21	6/12/07	F	est 57	U	2[c]/1	18.7	55,000	99,000	4200	9500	750	450	−16.0 ± 0.10	16.3 ± 0.18
L26	9/6/07	F	est 51	U	4[c]/1	22.1	17,000	27,000	4400	3700	580	500	−15.9 ± 0.08	15.8 ± 0.21
L67	9/11/07	F	22	2[b]	2/2	29.2	5600	4300	680	730	150	150	−15.9 ± 0.03	15.8 ± 0.12
L73	9/6/07	M	21	2	NA	23.8	32,000	55,000	3400	5500	450	370	−16.3 ± 0.12	16.1 ± 0.19
L87	12/14/07	M	15	5[b]	NA	25.6	24,000	44,000	2600	4500	410	350	−16.1 ± 0.10	16.4 ± 0.04

[a] Information from Center for Whale Research (2008). F = female; M = male; U = unknown; est = estimated age; NA = not applicable.
[b] The birth order is an estimate; previous siblings may have been born before record keeping began or may have died before being observed.
[c] The number of calves is an estimated minimum; other calves may have been born before record keeping began. The number of calves "surviving" is from 2008.
[d] Sum of all 45 congeners analyzed (Sloan et al., 2005).
[e] Sum of o,p′-DDD, p,p′-DDD, p,p′-DDE, o,p′-DDE, o,p′-DDT and p,p′-DDT.
[f] Sum of congeners 28, 47, 49, 66, 85, 99, 100, 153, 154 and 183.
[g] Sum of oxychlordane, γ-chlordane, nona-III-chlordane, α-chlordane, trans-nonachlor, and cis-nonachlor.
[h] Sum of α-, β-, and γ-HCH isomers.
[i] Mean (±1 SD) of three analyses of each epidermis sample.

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this paper were calculated using a surrogate (internal) standard (Sloan et al., 2005) and were lipid-normalized. Quality control samples (i.e., method blank, replicate and SRM®1945) 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 SRM®1945. Other quality control samples met established laboratory criteria (Sloan et al., 2006).

Stable isotope analyses were conducted on lipid-extracted epidermis (skin) from killer whale biopsy samples as described previously (Herman et al., 2005). SRM®1946 (National Institute of Standards and Technology) was used as a control material and was analyzed with every 20 analyses to monitor stable isotope analytical accuracy and precision (Sloan et al., 2006). All quality control samples met established laboratory criteria (Sloan et al., 2006).

2.3. Statistics

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 ($\alpha = 0.05$).

Field observers have reported that L87 was associated with K-pod for most of the year prior to biopsy sampling, so its diet may not have been typical of those of other L-pod members (NWFSC, Unpublished data). Therefore, L87 was excluded from the statistical analyses of stable isotope results. However, because contaminants are acquired over a lifetime, L87's year-long association with K-pod would not greatly affect contaminant concentrations, patterns and ratios.

3. Results and discussion

3.1. Concentrations of POPs

All biopsy samples in this study were analyzed for lipid, POPs and stable isotopes (Table 1). POP concentrations are usually compared among whale groups using adult males, because reproductive female whales transfer a substantial portion of their contaminant burden to their calves (Ross et al., 2000). Concentrations of POPs in females are generally lower than in males and are also partially dependent of the number of times they have given birth (Borga et al., 2004; Ross et al., 2000; Ylitalo et al., 2001). For example, this study showed that three recent mothers (J22; K13; L67) had POP levels lower than those for males of similar ages (Table 1). Thus, biases may result when POP data from reproductive female whales are used for comparisons. Levels of POPs in the three male whales from this study (Table 1 and Fig. 1) were in the low to middle portion of the range found for adult males in the earlier study (Krahn et al., 2007b), except that levels of HCHs and HCB were slightly below the ranges observed in that study (Table 1). However, this may be an age-related effect, because the mean age of adult males presented here was 19 years ($n = 3$; approximate ages 15, 21, 21) compared to 26 years previously ($n = 7$; 15, 15, 15, 18, 18, 29, 55) and contaminant concentrations have been reported to increase with age in adult male killer whales (Krahn et al., 2007b; Ross et al., 2000).

In the previous study, J39, a male 3-year-old (at the time of biopsy sampling) was found to have the highest concentrations of ∑PBDEs, ∑HCHs and HCB (Krahn et al., 2007b). Biopsies from three additional juvenile whales (J38, K34, K36) were obtained for this study. POP levels from the new biopsies were compared to those from J39 (Table 1 and Fig. 2). For example, J38 had concentrations (Table 1) comparable to those found for J39 [Fig. 2 and Krahn et al. (2007b)] for all POP groups. Furthermore, the K-pod

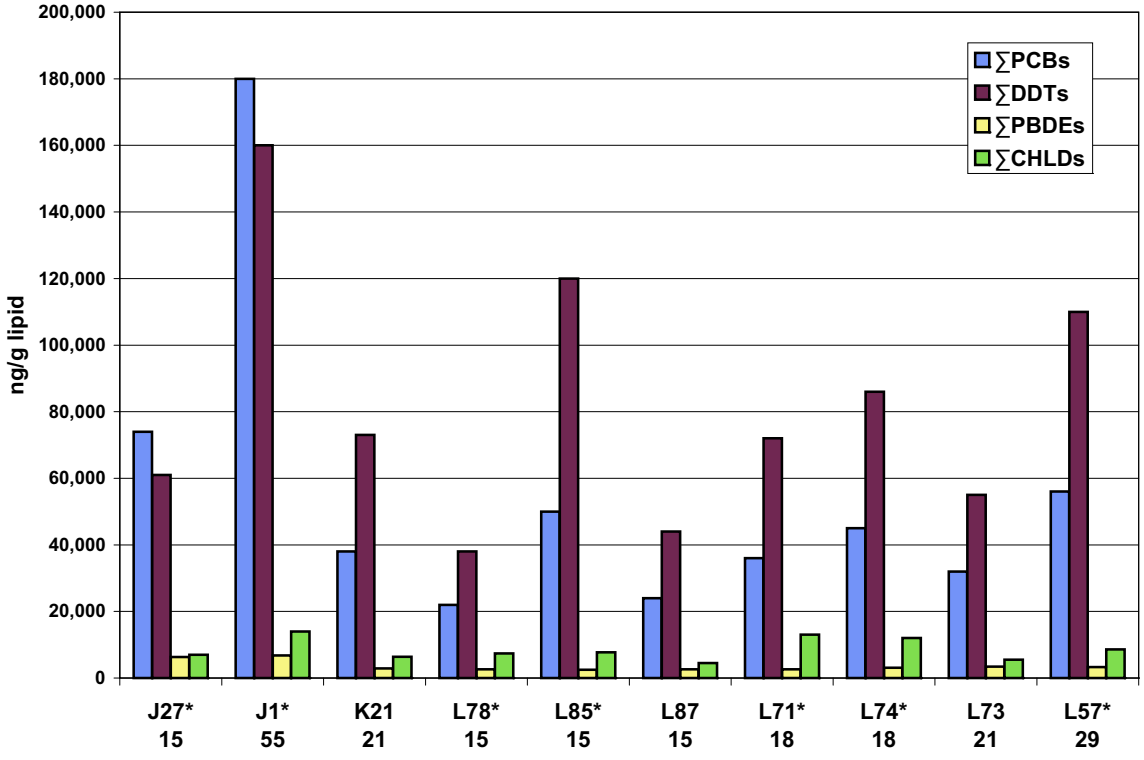

Fig. 1. Concentrations of ∑PCBs, ∑DDTs ∑PBDEs, ∑CHLDs in blubber samples from male Southern Resident killer whales (J-, K- and L-pods). Estimated ages of the whales are designated below their ID number. POP concentrations for whales identified with an asterisk (∗) were reported in Krahn et al. (2007b).

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juvenile whales (K34 and K36) had blubber concentrations of ∑DDTs and ∑CHLDs that were substantially higher (2–3 times) than those of the J-pod juveniles (Fig. 2). Among the K-pod whales, K36 had the highest levels for all POPs (Table 1 and Fig. 1) compared to the other juvenile (K34) and to all the adult whales, except for the long-lived female (K7, est 97 years). Finally, the young whales (J38, J39, K34 and K36) had statistically higher concentrations of certain contaminants (i.e., ∑PBDEs, ∑HCHs and HCB; $p < 0.0001$) than were found for adult males from both studies. These new results indicate that the high levels of POPs previously reported for J39 were not anomalous and are found – not only in J-pod juveniles – but also in young whales from K-pod.

3.2. Transfer of POPs from mother to offspring

Two mother–offspring pairs of Southern Residents were biopsied in the current study. In the first pair, J38 was the second calf of J22, born 5 years after its first-born sibling (Center for Whale Research, 2008). In the other pair, K34 was the fourth calf of K13, born 7 years after the birth of a previous calf (Center for Whale Research, 2008). Birth order has proven to be a major influence on contaminant levels transferred to killer whale calves from their mothers, with first-borns having the highest levels (Ylitalo et al., 2001). Females can also accumulate POPs between pregnancies (see next paragraph) and then the mother transfers those contaminants to her new calf (Fig. 2). POP concentrations in the juveniles were much higher than in the mothers (Table 1 and Fig. 2). Namely, J38 (4 years) had 8.9–17.6-fold higher concentrations of POPs compared to those measured in its mother J22. Similarly, POP levels in juvenile K34 (6 years) were 4.0–8.3 times higher than those found in its mother K13. A large portion of the mother's POP burden, transferred to the calf during gestation and lactation, is assimilated into its blubber and other tissues. Because the size of the calf is much less than that of the mother, POP concentrations are higher in the calf.

When the transfer of POPs to a calf ceases following lactation, the mother's contaminant levels begin to increase again (Table 2). Although senescent females have been reported to show increases in POP levels with increasing age (Ross et al., 2000), no data have been available to determine whether younger females show the same sort of increases in POP concentrations during the non-reproductive years following the birth of a calf. Consequently, simple linear regression analyses were conducted on POP concentrations in female killer whales vs. "years to accumulate POPs" – defined as the number of years since a female had her last calf minus 2 years for lactation (Haenel, 1986). Even though only a limited number of animals ($n = 6$) were available for these analyses, statistically significant correlations resulted. For example, the correlations of concentrations for ∑PCBs and ∑CHLDs in reproductive females, as well as those thought to have reached senescence, vs. "years to accumulate" were very strong

Table 2
The number of years Southern Resident killer whale females had to accumulate POPs after weaning their last calf, assuming weaning occurs 2-years post-partum.

Animal ID	J22 F-22y	K7 F-est 97y	K13 F-35y	L21 F-57y	L26 F-51y	L67 F-22y
Last calf weaned[a]	2004	1956	2002	1978	1994	2003
Years to accumulate[b]	2	50	4	28	12	3

[a] Arbitrarily, the date the last calf was weaned was chosen to be 2 years after its birth (Haenel, 1986).
[b] Years between the date the last surviving or observed calf was weaned and the collection of the biopsy sample from the mother.

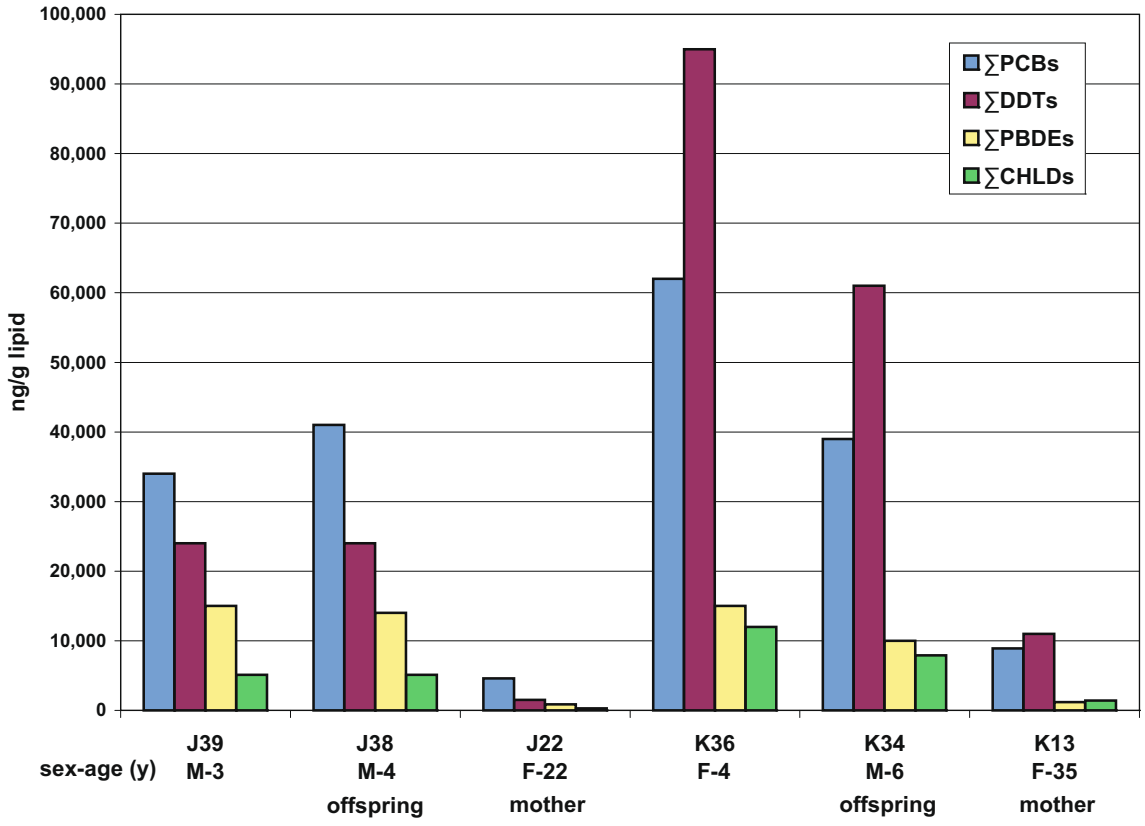

Fig. 2. Concentrations of ∑PCBs, ∑DDTs ∑PBDEs, ∑CHLDs in blubber samples from juvenile Southern Resident killer whales and the mothers of two of the whales. J22 is J38's mother and K13 is K34's mother. For the sex-age designations: M = male; F = female.

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(r^2 = 0.983 and 0.998, p = 0.0001 and <0.0001, respectively). The correlations for ∑HCHs and ∑PDBEs were also strong (r^2 = 0.912 and 0.847, p = 0.003 and 0.009, respectively). Although the correlations for HCB and ∑DDTs with "years to accumulate" were not as strong (r^2 = 0.744 and 0.646, p = 0.03 and 0.166, respectively), this relationship was significant for HCB. The significant correlations indicated that reproductive females also accumulate certain POPs over the time period between pregnancies at about the same rate as found for senescent females. Because accumulation of ∑DDTs is strongly dependent on the primary foraging sites of the whales' prey (e.g., California Chinook salmon have high ∑DDTs; see Section 3.4), it is not surprising that this contaminant did not correlate with "years to accumulate."

3.3. Effects of POPs

Based on captive-feeding studies of harbor seals (*Phoca vitulina*), Kannan et al. (2000) derived a threshold for PCB-related health effects in marine mammals (17,000 ng/g lipid in blubber) using immune system and endocrine endpoints. All Southern Resident killer whales in this study and the previous one (Krahn et al., 2007b) – with the exception of three recent mothers (J22, K13 and L67; see Tables 1 and 2) – had ∑PCBs that exceeded the health-effects threshold reported by Kannan et al. (2000). Most notably, the four juvenile whales exceeded the threshold by factors of 2–3.6 (Table 1 and Fig. 2). Recently, this threshold has been incorporated into risk assessments for cetaceans. For example, Jepson et al. (2005) found that, for harbor porpoises (*Phocoena phocoena*) having total PCB concentrations in blubber above the marine mammal threshold (17,000 ng/g lipid), total PCBs were significantly higher in porpoises that died of infectious disease compared to those that died from acute physical trauma. However, this association was not significant with porpoises having PCB concentrations below that threshold. In addition, Hickie et al. (2007) have demonstrated the importance of age and sex in determining health risks from PCBs and found the killer whale population segments most at risk were adult males and young juveniles and least at risk were neonates and reproductive females. Their results showed that nursing killer whales can go from being the least to the most contaminated members of the population in about 1 year because of the rapid transfer of POPs from the mother during lactation (Cockcroft et al., 1989) before a significant amount of growth dilution occurs (Hickie et al., 1999, 2007). Thus, maternal transfer of POPs to juvenile Southern Residents has resulted in high levels during a period when their biological systems are undergoing rapid development (Eriksson et al., 2006, 2002; Viberg et al., 2003), so these juveniles may be at greater risk than adults for POP-related health effects. Furthermore, a contaminant risk model developed by Schwacke et al. (2002) is consistent with low survivorship in calves exposed to high POPs levels from maternal transfer. Consequently, it is apparent that a large proportion of the Southern Resident killer whale population, and particularly the young whales, is at risk for serious health effects.

During the approximately 20% decline of the Southern Resident killer whale population experienced in the mid-1990s, survivorship was lowest for calves, adult males and post-reproductive females (Krahn et al., 2004a, 2002). Furthermore, it has been demonstrated in the current and other recent studies of Southern Residents (Krahn et al., 2007b; Ross et al., 2000) that the highest levels of POPs occurred in juveniles, adult males and post-reproductive females. Although direct evidence for effects of POPs on survival of this population is lacking, certain lines of evidence support the role of contaminants in the 1990s population decline. For example, a strong correlation was reported by Ford et al. (2005) between the increased mortality of Southern Residents in the mid-1990s and decreased abundance of Chinook salmon, a preferred prey item (Ford and Ellis, 2006). Because the Southern Residents likely experienced poorer nutrition due to decreased availability of prey, lipid stores would have been metabolized to meet energetic needs and the associated lipophilic POPs would have been released from blubber to circulate in blood. In male bottlenose dolphins, a decline in immune function occurred as contaminant concentrations increased in the blood (Lahvis et al., 1995). Thus, during the 1990s when Southern Resident prey was limited and lipid stores were likely needed to provide energy, these killer whales may have experienced immune suppression as a result of high levels of circulating POPs, thus increasing their risk of disease (Hall et al., 2006; Jepson et al., 2005) or other pollutant-related health effects.

3.4. POP ratios

The ratios of certain POPs in marine mammals can provide insight into whales' forging locations (Calambokidis and Barlow, 1991; Krahn et al., 2007a; Muir et al., 1990). For example, mean ∑DDTs/∑PCBs ratios of whales from K-pod and L-pod were not significantly different from each other (1.68 ± 0.19 and 1.93 ± 0.08, respectively), but were significantly higher than the ratio of J-pod (0.72 ± 0.24). The similarity of K- and L-pod ratios suggests that K-pod, in addition to L-pod (Krahn et al., 2007b), travels to forage on prey that reside in California, where high levels of DDTs are found in the marine environment (the "California signature"). These results have been substantiated by multiple sightings of both K- and L-pods in waters off the coast of California (Krahn et al., 2007b; NWFSC, Unpublished data). Furthermore, both pods have been observed feeding on salmon in Monterey Bay (Black et al., 2001). As reported previously, support for the "California signature" in K- and L-pods can be found by examining ∑DDTs/∑PCBs ratios in prey (Krahn et al., 2007b). For example, the ∑DDTs/∑PCBs ratios in Chinook salmon from California (n = 10; 2.60 ± 0.50) and the Columbia River (n = 10; 1.17 ± 0.23) indicated higher ∑DDT compared to ∑PCBs, whereas those of Chinook from the Fraser River (n = 13; 0.73 ± 0.21) or Puget Sound (resident "blackmouth;" n = 26; 0.24 ± 0.29) reflected a lower ratio (O'Neill et al., 2006). Given the whales' preference for Chinook salmon (Ford and Ellis, 2006) and because Chinook from the Central Valley of California are thought to remain in California waters (Myers et al., 1998), these salmon likely contributed to the relatively high DDT levels observed in K- and L-pods. In contrast, J-pod has not been observed in California waters and is more frequently observed than the other pods (Hauser et al., 2007) in Puget Sound/Georgia Basin waters where prey that have low ∑DDTs relative to ∑PCBs (O'Neill et al., 2006).

3.5. Stable isotopes

Decreased quantity and quality of prey are likely risk factors in the population decline of Southern Resident killer whales (Baird, 2001; Ford and Ellis, 2005; Krahn et al., 2004a, 2002). Southern Residents are reported to prefer Chinook salmon (*Oncorhynchus tshawytscha)* (Ford and Ellis, 2006) over other potential prey species (e.g., groundfish, herring and other salmon species) that are generally of lower trophic levels (O'Neill et al., 2006). Nitrogen stable isotope values are often used to assess the trophic level at which marine mammals feed (δ^{15}N values increase as trophic level increases) and carbon values are indicative of offshore vs. nearshore feeding (δ^{13}C values decrease as distance from shore increases) (Kelly, 2000). Thus, δ^{15}N and δ^{13}C values were determined in the epidermis of Southern Resident killer whales to gain information about their diet (Table 1). Hicks et al. (1985) reported that a complete isotope turnover of skin cells in bottlenose dolphins was estimated to take 73 days, but changes in stable

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isotope values were apparent within a month of the diet change. Therefore, stable isotope values in Southern Residents – assuming epidermis turnover is similar to that of bottlenose dolphins – reflect their diet in the previous month or two.

Stable isotope values of nitrogen and carbon measured in the whales did not differ significantly between J-pod ($n = 6$) and L-pod ($n = 6$) (Table 3). In contrast, K-pod had significantly lower $\delta^{13}C$ values compared to the other two pods and had significantly lower $\delta^{15}N$ values than those of J-pod, but not L-pod (Table 3). However, all K-pod samples were collected in December 2007 ($n = 5$) and J-pod samples were collected in May and June, 2006/2007 ($n = 6$). L-pod had the widest range of sampling dates (L87 was excluded; see Methods) – May/June 2006 ($n = 2$), June 2007 ($n = 1$) and September 2007 ($n = 3$).

The carbon and nitrogen stable isotope results for J- and L-pods were comparable to previous results reported for these pods by Krahn et al. (2007b), so the pods appear to be consuming prey from the same trophic level and region. However, only K-pod samples were collected in December, so the differences in K-pod carbon and nitrogen stable isotope values compared to the other pods might reflect seasonal variations, as well as differences in trophic levels and foraging regions. For example, the $\delta^{13}C$ differences between K-pod and the other pods could result from seasonal changes in stable isotope ratios at the base of the food chain. Alternatively, K-pod's lower $\delta^{13}C$ values might result from these whales consuming a larger proportion of prey that feed in "offshore" areas compared to the other whales. Furthermore, K-pod's $\delta^{15}N$ value was significantly lower than that of J-pod, so these results could point to seasonal changes in diet. Field data support stable isotope results showing diet differences among the killer whale pods. Salmon species available in Puget Sound vary on a seasonal basis (Washington Department of Fish and Wildlife, 1993), the whales have been observed to consume salmon from different species and populations during different seasons (NWFSC, Unpublished data) and field observations indicate that K- and L-pods spend more time outside of Puget Sound/Georgia Basin waters than J-pod does (Osborne, 1999).

Previously, it was demonstrated that stable isotope values of carbon and nitrogen measured in epidermis samples from three L-pod whales sampled in 1996 did not differ greatly from those of L-pod whales sampled in 2006, but the sample size was small ($n = 3$ in 1996 and $n = 2$ in 2006) (Krahn et al., 2007b). In 2007, additional biopsies were acquired from L-pod whales and the combined results from 2006/2007 ($n = 6$ excluding L87; Table 2) showed that the nitrogen ($p = 0.05$), but not the carbon, stable isotope values were significantly different (lower) than those of L-pod sampled in 1996. However, three of six biopsies from 2006/2007 were taken in early September, whereas the other three were collected in May and June. Therefore, to minimize seasonal variation, the 1996 samples collected in late September and early October (17.0 ± 0.4,) were compared to the September 2007 biopsies (15.9 ± 0.2) and a larger significant decrease in $\delta^{15}N$ values was observed ($p = 0.009$). Field data (Baird et al., 2005) have suggested there may have been a shift in diet from 1993 through 2002 based on differences in dive depths of Southern Resident killer whales instrumented with time-depth recorders. Thus, these results indicate a possible change in the diet of L-pod Southern Residents over the last decade, suggesting that Southern Residents consume fish species other than Chinook salmon, particularly in the fall months.

Table 3
Stable isotope values (mean ± 1 SD) in 2006/2007 epidermis samples from Southern Resident killer whales compared to those from 1996.

Mean ± SD	$\delta^{13}C$	$\delta^{15}N$
J-pod (2006/2007; $n = 6$)[a]	−15.7 ± 0.27	16.4 ± 0.23
K-pod (2007; $n = 5$)	−16.4 ± 0.25	15.9 ± 0.15
L-pod (2006/2007; $n = 6$)[b]	−16.0 ± 0.20	16.2 ± 0.46
L-pod (1996; $n = 3$)[c]	−15.9 ± 0.20	17.0 ± 0.37

[a] Mean (±1 SD) for biopsy samples from this study and from Krahn et al. (2007b).
[b] Mean (±1 SD); L87 has been omitted from the mean because this whale had been foraging with the K-pod for approximately 1 year.
[c] Mean (±1 SD) from biopsy samples collected in 1996 and reported by Krahn et al. (2007b).

4. Conclusions

In conclusion, juvenile Southern Resident killer whales had statistically higher concentrations of certain contaminants (i.e., ∑PBDEs, ∑HCHs and HCB) than were found for adult males and these results confirmed the high levels of POPs previously reported for a single juvenile whale. Furthermore, all Southern Resident killer whales – with the exception of three recent mothers – exceeded the health-effects threshold for total PCBs in blubber and, most notably, the four juvenile whales exceeded the threshold by factors of 2–3.6. Thus, maternal transfer of POPs to the juvenile Southern Residents has resulted in high POP levels during a period when their biological systems are undergoing rapid development, so these juveniles may be at greater risk than adults for POP-related health effects (e.g., immune and endocrine system dysfunction).

The similarity of the ∑DDTs/∑PCBs ratios for K- and L-pods, in contrast to the lower ratio for J-pod, suggests that both K-pod and L-pod travel to California to forage, where high levels of DDTs are found in prey. In contrast, J-pod generally remains near Puget Sound/Georgia Basin waters where prey has lower ∑DDTs/∑PCBs ratios. These results have been substantiated by multiple sightings of both K- and L-pods, but not J-pod, in waters off the coast of central California. Nitrogen stable isotope values, as well as field observations, indicated a possible change in the diet of L-pod Southern Residents over the last decade, suggesting that Southern Residents consume fish species other than the preferred Chinook salmon, particularly in the fall months.

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