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PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles

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ABSTRACT

Concentrations of persistent contaminants often vary widely among individuals within a population. We hypothesized that such variation was caused mainly by differences in diet (biomagnification) and in coastal systems by the tendency of marine systems to act as contaminant sinks. We examined the relationship between contaminant concentrations and stable isotope ratios in nestling plasma from an apex predator with a particularly broad diet. Our study included freshwater, estuarine, inshore and pelagic breeding sites. Bald eagles (*Haliaeetus leucocephalus*) at the pelagic marine sites showed high trophic level and marine input, eagles at the freshwater sites showed low trophic level and marine input, and eagles at the estuarine and inshore marine sites had intermediate values. The relationship between trophic level and marine input may reflect longer food chains in pelagic compared to terrestrial ecosystems. $\sum$PCBs and DDE concentrations generally increased with trophic level and marine input, with the exception of the freshwater sites, while $\sum$PBDEs, hydroxylated-PBDEs and hydroxylated-PCBs increased with marine input, but were independent of trophic level. The relationships for $\sum$PCBs and DDE were often slightly stronger with marine input than trophic level, suggesting that oceanographic processes may be more important than trophic level. At freshwater locations, spatial variation may be more important than trophic level due to the heterogeneity of contaminant profiles between feeding locations (lakes, rivers, agricultural fields). Adults had similar isotopic composition to their chicks but higher contamination. Based on nests where prey composition was determined independently, isotopic enrichment values for nestling plasma were 1.6 ± 0.1 (δ^{15}N) and -0.4 ± 0.2 (δ^{13}C). We conclude that trophic level and marine influence are significant factors influencing PCB and DDE concentrations in eagles. However, trophic level in particular did not influence PBDEs, possibly due to their being metabolized by eagles.

1. Introduction

Within a given biotic community, concentrations of persistent pollutants tend to increase with trophic level across taxa (Bowles et al., 2001; Elliott, 2005a,b; Weech et al., 2006). Relationships and processes have been explored and modeled for many of these compounds (Fisk et al., 1998; Mackay and Fraser, 2000; Veltman et al., 2005; Borgå et al., 2004), although not for hydroxylated metabolites (McKinney et al., 2006; Verreault et al., 2008; Gebbink et al., in press). There is less information about variation within species or populations (but see Bearhop et al., 2000; Bustnes et al., 2000; Morrissey et al., 2004), where differences in trophic level and contaminant exposure would be expected to be less broad. Nonetheless, body burdens of persistent organic pollutants often are highly variable among individuals within a given avian population (Hebert et al., 1997; Elliott and Norstrom, 1998; Morrissey et al., 2004). The reasons are usually complex, and include individual variation in diet, particularly trophic level, foraging area, age, gender, pregnancy, sexual development and ability to metabolize xenobiotics (Hobson et al., 1997; Braune et al., 2002; Das et al., 2003). Variation in carbon source (freshwater vs. marine vs. terrestrial) is also an important variable and often a better predictor of contaminant burden than trophic level in aquatic ecosystems because differences in mixing rates between aquatic zones result in differences in contaminant concentrations (Kainz et al., 2002; Ethier et al., 2008).

Sea eagles (genus *Haliaeetus*; hereafter "eagles") are opportunistic predators feeding at the top of the aquatic food chains. They have often been used successfully as indicators of ecosystem health (Bowerman et al., 1998; Dykstra et al., 1998, 2005; Elliott and Harris, 2001), and some populations continue to be stressed by contaminants (Bowerman et al., 1995; Clark et al., 1998; Helander et al., 2002). Thus,

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understanding the causes of variation in exposure continues to be of conservation interest. Within a single region (e.g. Great Lakes, Pacific Coast), individual contaminant levels often vary by several orders of magnitude (Elliott and Norstrom, 1998; Dykstra et al., 1998; Donaldson et al., 1999; Helander et al., 2002). Although such variability often has been attributed to spatial variation in contaminant concentrations in the environment, it may also reflect differences in trophic level as eagles are highly opportunistic and individuals will consume widely different food items resulting in widely different exposures (Dykstra et al., 1998; Gill and Elliott, 2003). For example, extremely high DDE levels in some eagles from coastal California appear to result from consumption of marine mammal carcasses (Garcelon et al., 1989; Garcelon and Thomas, 1997).

Stable isotope values provide an independent measure of trophic level and carbon source. The ratio of ^{15}N to ^{14}N increases by about 2–5‰ per trophic level due to preferential removal of light amine groups during deamination and transamination (Kiriluk et al., 1995; Ruus et al., 2002; Hoekstra et al., 2003). The ratio of ^{13}C to ^{12}C varies depending on the carbon source for the local community, with terrestrial communities usually being depleted in ^{13}C compared to marine communities and benthic/littoral communities being enriched compared to pelagic communities (Kainz et al., 2002; Ethier et al., 2008).

Here, we examine concentrations of chlorinated and brominated hydrocarbon concentrations relative to stable isotope ($\delta^{15}N$ and $\delta^{13}C$) signatures for plasma of nestling eagles collected along the Pacific Coast. Eagle contaminant concentrations and diet in coastal regions vary widely (Elliott et al., 1996a,b,c, 1998, 2005; Gill and Elliott, 2003; Cesh et al., 2008), and we hypothesized that contaminant concentrations would be closely related to trophic level. We also hypothesized that eagles foraging in freshwater environments, where contaminants are unlikely to be readily dispersed leading to high spatial heterogeneity in environmental contaminant levels, would show a weaker relationship with trophic level than eagles foraging in marine environments, where contaminants more readily disperse (Ruus et al., 2002); even in high flow riverine systems, eagles generally feed in back-eddies, sloughs and nearby ponds where contaminant dispersion is likely low. In addition, we examine what stable isotopes can tell us about the foraging ecology of adult eagle diet in different environments, as most previous studies during the breeding season have focused on direct observations of nestling diet which necessitate small sample sizes and are biased towards easily-observed prey items (Dykstra et al., 1998; Elliott et al., 2005; Thompson et al., 2005) or prey remains which are biased towards prey items that do not decompose rapidly (Mersmann et al., 1992; Elliott et al., 2005).

2. Methods

2.1. Study areas

Nestling bald eagle (*Haliaeetus leucocephalus*) blood samples were collected from five sites in British Columbia during 1993, 1994, 2001 and 2003 and one site in California in 2003. They included freshwater lakes (North Interior, $N=21$), coastal freshwater (Fraser Valley, $N=14$), estuarine (Fraser Delta, $N=14$), inshore marine (Strait of Georgia, $N=16$), Californian offshore marine (Santa Catalina Island, $N=3$), marine (western Vancouver Island, $N=20$) and northern offshore marine (Johnstone Strait and Haida Gwaii, $N=8$) eagle breeding sites (Fig. 1). Adult blood samples were also collected from freshwater lakes ($N=5$) and marine (Vancouver Island and Fraser Delta; $N=13$) sites by trapping breeding adults on the water. The offshore marine sites are unlikely to be contaminated by local sources, but may be influenced by atmospheric transport, especially trans-Pacific transport from Asia (Elliott et al., 1996a,b,c; Cesh et al., 2008). The coastal freshwater, estuarine and inshore marine sites were historically influenced by input of PCBs from urban and industrial areas such as the cities of Vancouver and Victoria, and of agricultural DDT input from farmlands particularly in the Fraser

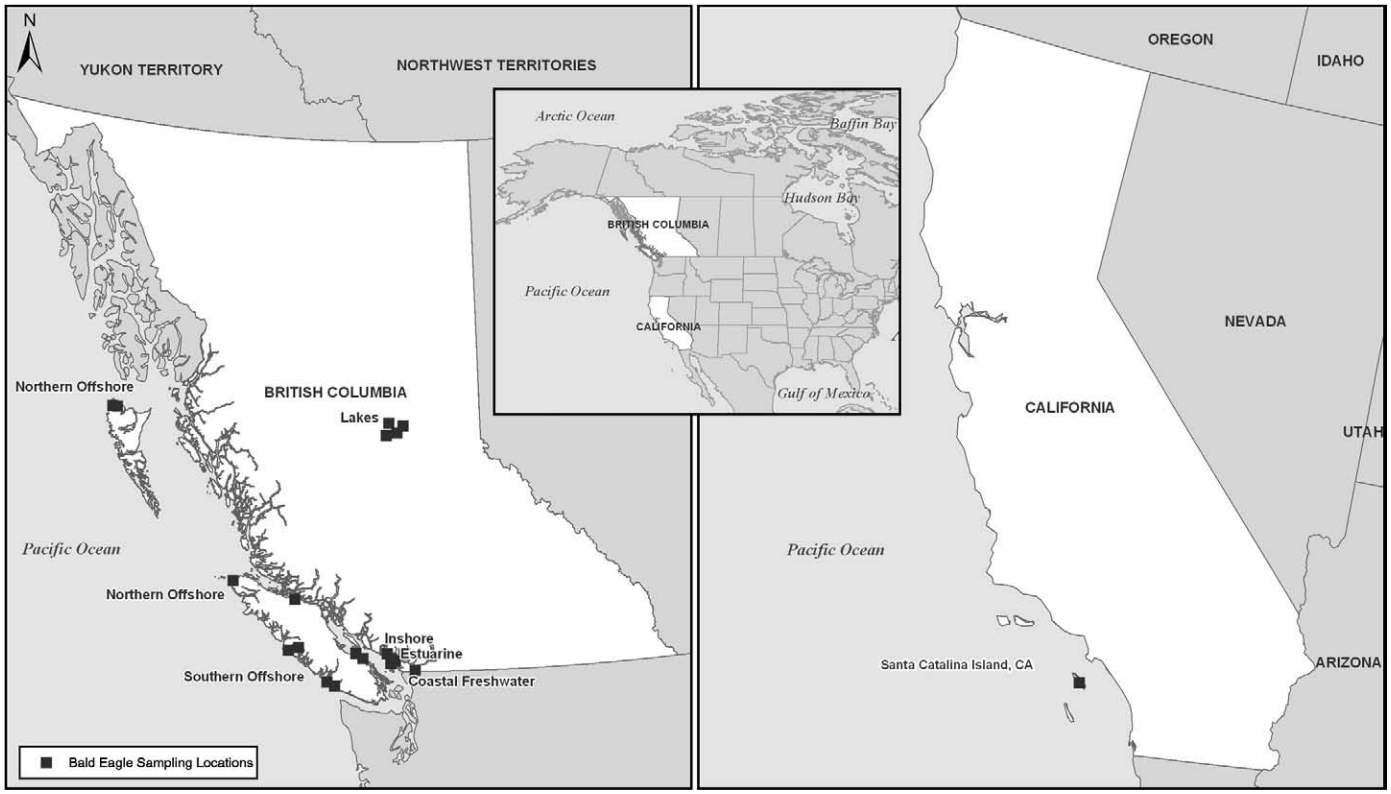

Fig. 1. Study locations for bald eagles in coastal British Columbia.

River Valley; residual inputs for both compounds are now likely to be minimal (Cesh et al., 2008). Highest levels of PCBs were found at inshore marine sites in British Columbia and highest levels of DDE were found at offshore marine sites in California influenced by DDT discharge from industry in the Los Angeles area; lowest levels for both contaminants were at montane sites in British Columbia (Cesh et al., 2008). The freshwater lake sites are unlikely to be contaminated by local sources, and contaminant levels there likely represent atmospheric transport (Cesh et al., 2008).

PBDEs, primarily as flame retardants, are present in a vast array of consumer and industrial products; uses of penta- and octa-, but not deca-formulations, have been regulated and are no longer in primary use. However, inputs to the environment of all formulations are expected to continue for some time, as products containing various PBDEs are disposed of, mainly in landfills. Concentrations of ΣPBDEs and most major congeners were found to have increased exponentially from the 1970s until 2002 in eggs of other fish-eating top avian predators from sites in the Strait of Georgia (Elliott et al., 2005). The relative contribution of different PBDEs varied by site, with southern California having relatively higher levels of higher brominated compounds (McKinney et al., 2006) although both southern California and inshore marine sites in British Columbia had the highest levels of ΣPBDEs and most individual PBDEs (McKinney et al., 2006).

For all chicks, blood samples were collected (up to 24 mL) from the brachial vein using a needle and syringe and immediately transferred to vacutainers containing sodium heparin. After up to 6 h on ice, plasma was extracted from whole blood by centrifugation and stored at −20 °C. Further details on sample collection are available elsewhere (Elliott and Norstrom, 1998; McKinney et al., 2006; Weech et al., 2006; Cesh et al., 2008) and all data are compiled in Appendix A.

2.2. Contaminant analysis

Determination of organochlorine (OC) pesticides and polychlorinated biphenyls (PCBs) were performed at the Canadian Wildlife Service National Wildlife Research Centre (NWRC; Ottawa, ON, Canada), as described previously (Elliott and Norstrom, 1998; Cesh et al., 2008). The suite of organochlorines analyzed included: chlorobenzenes (tetra, penta, and hexa), hexachlorocyclohexanes, chlordane-related compounds (oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor, cis-nonachlor, and heptachlor epoxide), p,p-DDT and metabolites (p,p-DDE and p,p-DDD), mirex, photomirex, and dieldrin. Sixty-seven major PCB congeners were analyzed and summed to present the level of total PCBs. The plasma samples were denatured with formic acid (1:1 v/v) after the addition of internal standards. OCs/PCBs were extracted with activated C18 cartridges, deluted with DCM/hexane (1:1), and cleaned up by Florisil column chromatography. Samples were quantitatively analyzed by capillary gas chromatography coupled with a mass selective detector operated in the selected ion monitoring mode. The instruments used were the HP 5890 GC with HP Mass Selective Detector HP 5971A for PCB analysis, and Agilent 6890 GC with HP Mass Selective Detector Agilent 5973 for OC analysis. As part of the quality control, blanks and CWS reference material (2003 Herring Gull QA) were run concurrently with the samples. The nominal detection limit for all compounds was 0.1 ng/g wet weight (ww). Residues were not corrected for internal standard recoveries, which were typically between 70% and 90%. Plasma lipids were determined colorimetrically.

Samples from 2003 only were analyzed for a suite of polybrominated diphenyl ethers (PBDEs; seven congeners; BDE-47, -100, -99, -153, -138, -183, -209), hydroxylated, OH-PBDEs (14 congeners) and hydroxylated, OH-PCBs (30 congeners) at the former Letcher labs at the University of Windsor (Windsor, ON); details of analytical methods and data have been reported elsewhere (McKinney et al., 2006).

2.3. Stable isotope analysis

The same nestling plasma samples used for contaminant analysis were freeze-dried, homogenized, sealed into 1 mg tin samples at NWRC and sent to the University of California Davis Stable Isotope Laboratory. Analysis of dual isotopes ($^{15/14}$N and $^{13/12}$C) for samples and standards was conducted on a Europa Scientific Hydra 20/20 continuous flow isotope dilution mass spectrometer (CD-IRMS). Adult samples from 1997 were sent to the G.G. Hatch Laboratory at the University of Ottawa where they were loaded with internal standards into a Vario EL III (manufactured by Elemetar) elemental analyzer interfaced (Conflo II manufactured by Thermo) to an isotope ratio mass spectrometer (Delta XP Plus Advantage manufactured by Thermo). Samples were flash combusted with oxygen using Dumas combustion and the resulting gas products carried by helium through columns of oxidizing and reducing chemicals optimized for CO_2 and N_2. The gases were then separated by a purge and trap adsorption column and sent to the mass spectrometer. Values are reported as parts per thousand (‰) relative to standards (atmospheric nitrogen for ^{15}N and Pee Dee belemnite for ^{13}C). Internal standards were C-51 nicotiamide, C-52 ammonium sulphate and sucrose mixture, C-54 caffeine and C-55 glutamic acid. Blind standards had standard deviations of 0.03‰ (−0.01‰ from expected value) for ^{13}C and 0.06‰ (−0.21‰ from expected value) for ^{15}N. Further details are outlined by Weech et al. (2006). Within-sample repeatability averaged 0.12‰ for ^{13}C and 0.07‰ for ^{15}N, which is much smaller than the variability between nestlings at the same site.

To estimate isotopic enrichment values, we estimated nestling prey composition (taxon and weight) from direct observations at the offshore and inshore marine sites (Elliott et al., 1998, 2003, 2006; Gill and Elliott, 2003). At the montane freshwater site we estimated nestling prey composition by collecting prey remains. We obtained taxon stable isotope values from the literature and multiplied these by the percent mass contribution to the diet assuming no enrichment (Hobson and Welch, 1992; Hobson et al., 1994; Weech et al., 2006). Actual enrichment was the observed stable isotope value minus the estimated stable isotope value assuming no enrichment (Hobson and Welch, 1992).

Although carbon isotopes are usually considered measures of marine input and nitrogen isotopes measures of trophic level, carbon isotopes were also enriched with trophic level and nitrogen isotopes were also enriched with marine input (Cabana and Rasmussen, 1996; Bannon and Roman, 2008). To obtain a closer estimate of trophic level, we subtracted 2‰ from nitrogen isotopic values at freshwater sites and 1‰ from nitrogen values at the estuarine site; baseline isotopic values for particulate organic matter are roughly 2‰ lower in freshwater compared to marine ecosystems in the Pacific Northwest (Hobson et al., 1994; France 1994).

2.4. Statistical analyses

All statistical analyses were completed with the statistical package R 3.2.1. Prior to using the parametric statistics, we tested for normality (Shapiro–Wilk test). All contaminant values were ln-transformed to meet the normality requirements prior to analyses. All values reported are means ± SE. For multiple comparisons, we used the sequential Bonferroni correction (Rice, 1989). Note that due to the small sample sizes at each location and resulting low power, and thus high probability of rejecting a true result, we also quote values significant at the $\alpha = 0.05$ level (Nakagawa, 2004). DDE values did not change over our study period (Cesh et al., 2008) and PBDEs were only sampled in one year (2003). Consequently, temporal changes in contaminant levels are unlikely to affect our results for those contaminants. However, total PCB concentrations did decrease over our study period at some sites (Cesh et al., 2008). Thus, some of the

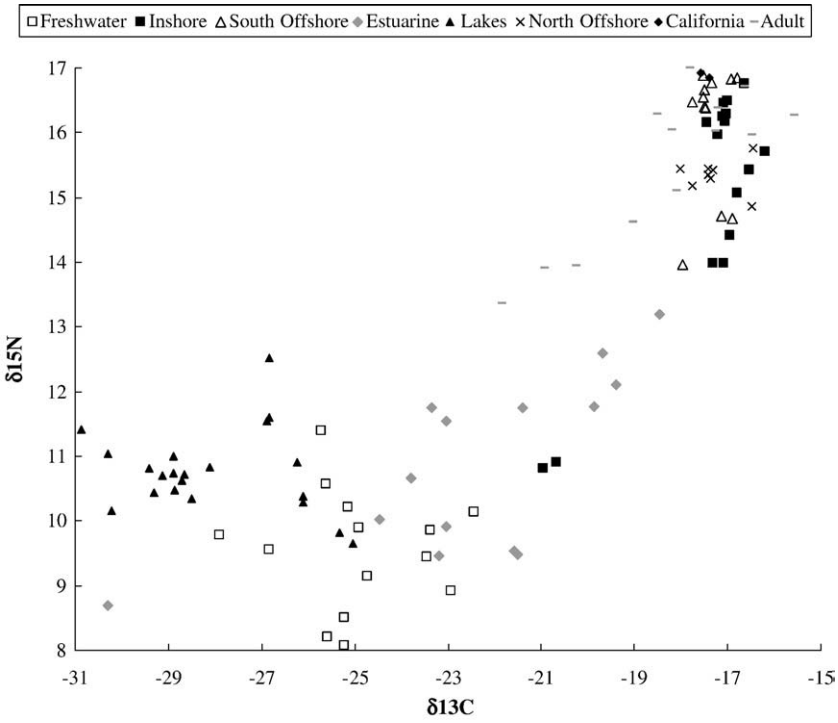

Fig. 2. δ^{15}N and δ^{13}C values for bald eagle plasma from the North American Pacific coast 1993–2003.

variability in our relationships for PCBs may be due to temporal differences in PCB concentrations.

3. Results

Values for δ^{13}C and δ^{15}N were correlated at all locations except the freshwater (coastal and lakes) sites (Fig. 2, Table 1). Generally, δ^{15}N and δ^{13}C values were greatest on the offshore, followed by the inshore, estuarine and freshwater sites. There was almost 9‰ difference in δ^{15}N between eagles feeding in the freshwater sites and those feeding at the offshore sites (Fig. 2).

Table 1
Correlation coefficients (r^2) for relationships within and between stable isotopes and contaminants for nestling bald eagle plasma from the Pacific Coast.

Location	*N*	δ^{13}C vs. δ^{15}N	DDE vs. PCB	δ^{13}C vs. DDE	δ^{15}N vs. DDE	δ^{13}C vs. PCB	δ^{15}N. vs. PCB
Inshore	16	***0.80***[a]	***0.71***[b]	0.16	0.04	**0.38**	**0.35**
Southern offshore	12	**0.33**	***0.76***	**0.57**	**0.51**	**0.66**	**0.30**
Northern offshore	8[c]	**0.70**	**0.86**	0.01	**0.32**	0.07	**0.30**
Estuarine	14	**0.57**	**0.54**	**0.31**	**0.38**	**0.44**	**0.35**
Coastal freshwater	14[d]	0.00	0.04	0.00	0.07	0.00	0.08
Freshwater lakes	21[e]	0.04	***0.93***	0.20	0.00	0.18	0.02
Adults (pooled)	13	***0.74***	***0.84***	**0.45**	0.04	**0.55**	0.06
All sites	90[f]	***0.69***	***0.47 (0.76)***[g]	**0.18**	0.04 (0.01)[h]	***0.51***	***0.35 (0.30)***[h]

[a] Values in bold are significant at the $P=0.05$ level, values in italics are significant at the Bonferroni-corrected $P=0.001$ level using the sequential method. Note that the Bonferroni-corrected values applied to small sample sizes lead to a high probability of rejecting a true result (Nakagawa, 2004)
[b] Contaminant values were ln-transformed prior to all analyses in Table 1.
[c] Contaminant values unknown for two samples, excludes adults.
[d] Contaminant values unknown for four samples, excludes adults.
[e] Contaminant values unknown for 16 samples, excludes adults.
[f] Contaminant values unknown for 22 samples, excludes adults.
[g] Value in brackets includes adults.
[h] Values in brackets are corrected for different baseline levels of ^{15}N in particulate organic matter.

DDE and PCB were correlated at all locations except the coastal freshwater site (Fig. 3, Table 1). PCB values were generally correlated with both δ^{15}N and δ^{13}C, with the relationships being slightly better for δ^{13}C compared with δ^{15}N (Fig. 4, Table 1). The exceptions were the freshwater and Northern Offshore sites (Table 1). DDE generally increased with δ^{13}C, but was generally independent of δ^{15}N, except at the estuarine and offshore sites (Fig. 4, Table 1). Concentrations of PBDE, OH-PBDEs and OH-PCBs increased with δ^{13}C, but did not increase with δ^{15}N or baseline-corrected δ^{15}N once δ^{13}C was accounted for (Fig. 5).

At the freshwater lake sites, adult plasma δ^{15}N values were 11.2 ± 0.6‰ while chick values were 11.0 ± 0.7‰ ($t_6=1.74$, $P=0.06$). Adult plasma δ^{13}C values ($n=5$) were −26.7 ± 1.2‰ while nestling values were −28.2 ± 2.0‰ ($t_{10}=1.97$, $P=0.03$). At the inshore site, adult plasma δ^{15}N values ($n=7$) were 15.8 ± 0.3‰ while chick values ($n=16$) were 15.1 ± 0.5‰ ($t_{21}=1.36$, $P=0.19$). Adult plasma δ^{13}C values were −17.4 ± 0.4‰ while nesting values were −17.4 ± 0.4‰ ($t_{21}=0.05$, $P=0.96$). Estimated isotopic enrichment values for nestling plasma were 1.62 ± 0.14‰ (δ^{15}N) and −0.39 ± 0.17‰ (δ^{13}C; Table 2). There was no relationship between nestling age and δ^{15}N at the estuarine ($t_8=0.76$, $P=0.47$, $r^2=0.01$), inshore ($t_6=-1.29$, $P=0.25$, $r^2=0.25$) or coastal freshwater ($t_5=-1.40$, $P=0.23$, $r^2=0.33$) sites. There was also no relationship between nestling age and δ^{13}C at the estuarine ($t_8=0.95$, $P=0.37$, $r^2=0.12$), inshore ($t_6=-0.81$, $P=0.46$, $r^2=0.12$) or coastal freshwater ($t_5=0.25$, $P=0.81$, $r^2=0.02$) sites.

4. Discussion

Bald eagles from coastal British Columbia showed a high degree of variation in trophic level and marine input, as shown from stable isotope values (Fig. 2). The variation explained a significant portion of the variation in PCB and *p,p'*-DDE concentrations within most marine sites (Fig. 4, Table 1). The absence of a significant relationship with *p,p'*-DDE and trophic level across all sites presumably reflects the greater relative importance of spatial processes (atmospheric and oceanographic transport) in determining small- and large-scale variation in *p,p'*-DDE contamination. It is unlikely to reflect high individual variation in the ability to breakdown or sequester *p,p'*-DDE (Clark et al., 1987).

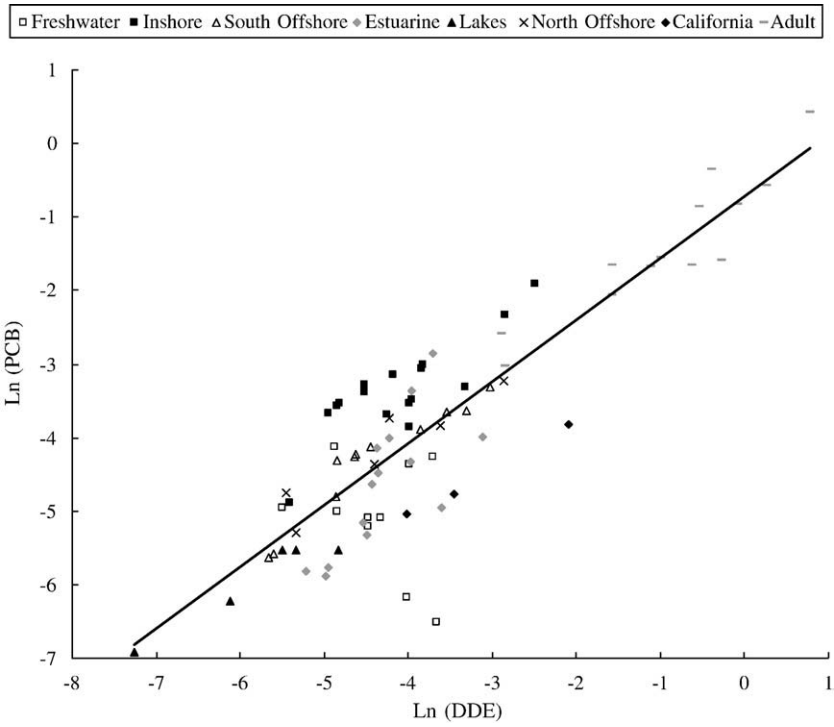

Fig. 3. DDE and PCB concentrations are correlated in bald eagle nestling plasma from the Pacific Coast 1993–2003.

We hypothesized that PBDEs would increase with trophic level given that those compounds appear to bioaccumulate in birds (e.g. Norstrom et al., 2002; Law et al., 2003; Lindberg et al., 2004; Elliott et al., 2005; Veltman et al., 2005; Gauthier et al., 2008), and their overall similarity in toxicokinetic behaviour to PCBs in a laboratory study with another bird of prey, the American kestrel

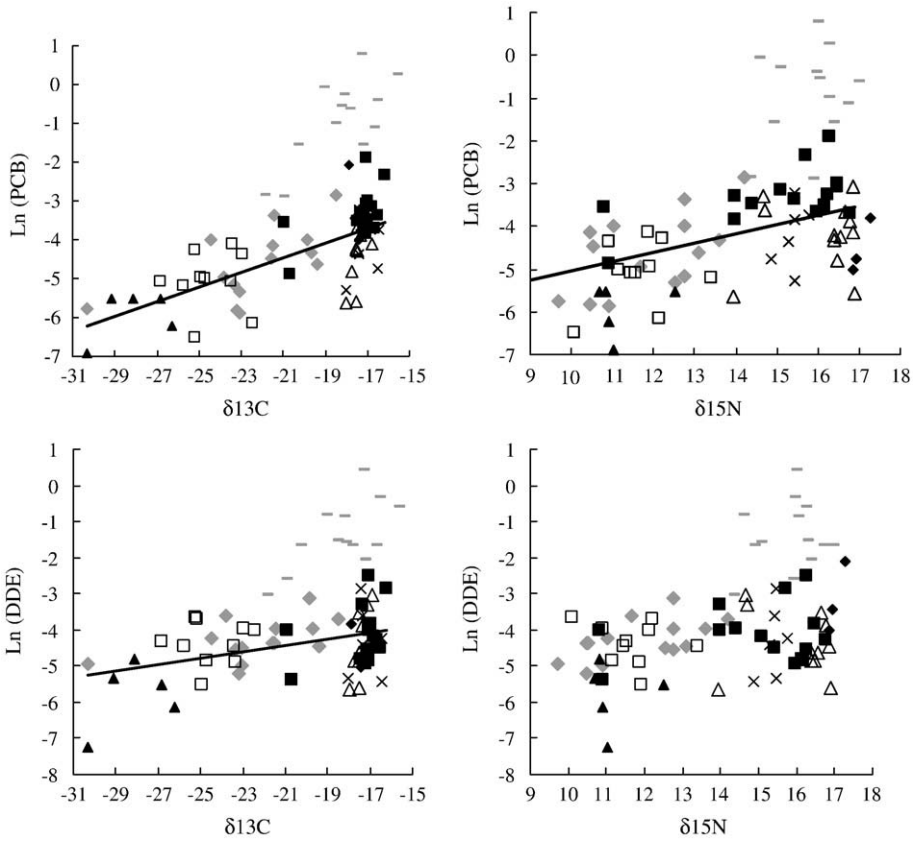

Fig. 4. Contaminant values relative to $\delta^{13}C$ and baseline-corrected $\delta^{15}N$ values for bald eagle plasma from the Pacific coast 1993–2003. Regression excludes adult values. Legend is the same as Fig. 3.

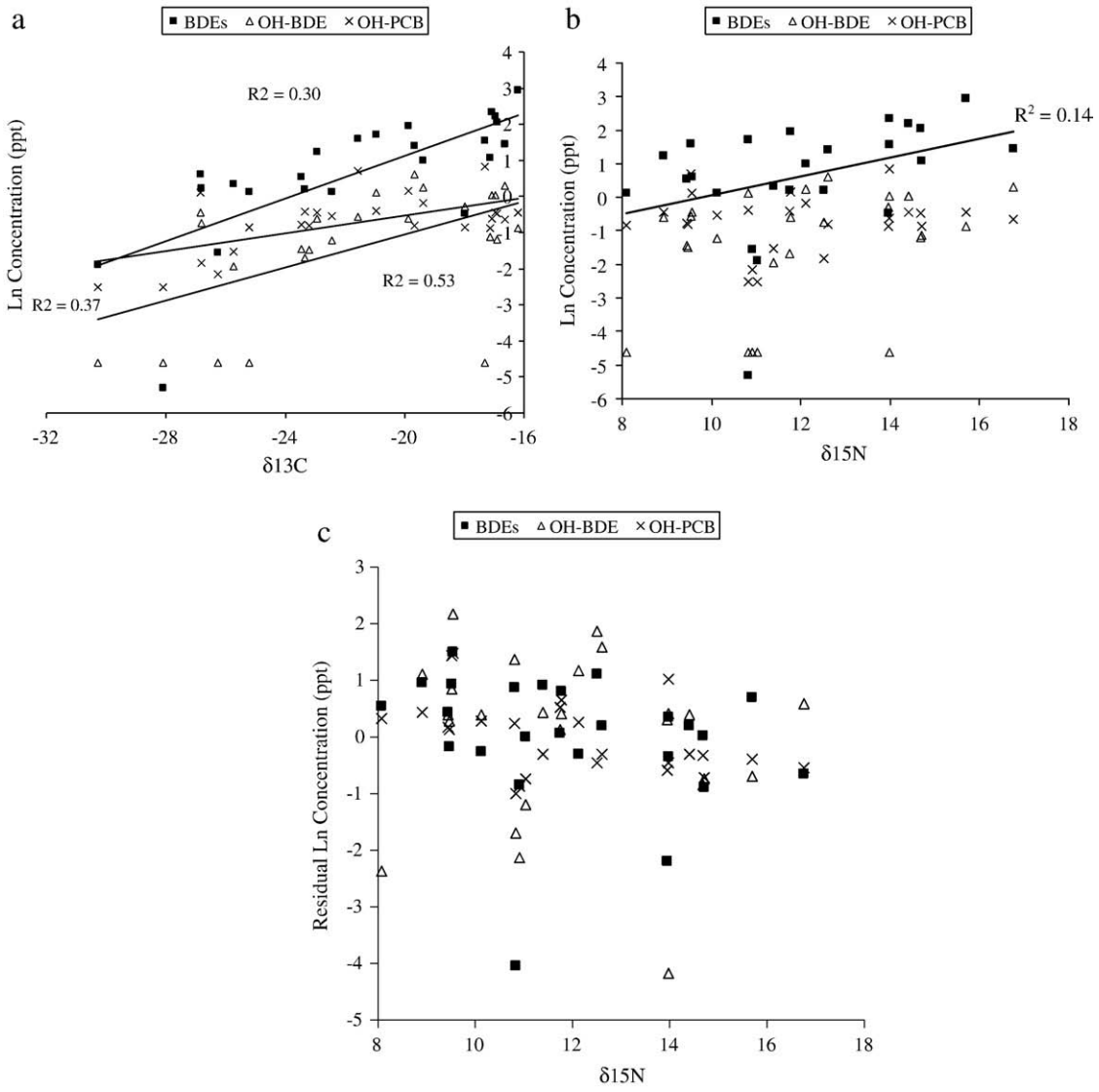

Fig. 5. BDEs and associated compounds (a) increase with marine input, (b) slightly increase (BDEs) or have no effect (OH-BDE, OH-PCB) with trophic level and (c) do not increase with residual trophic level once marine input is accounted for in bald eagle nestling plasma from the Pacific coast of North America. Legend is same as Fig. 3.

(*Falco sparverius*; Drouillard et al., 2007). However, a recent investigation of biomagnification of persistent organic pollutants in an arctic marine food web found that, similar to our results, PBDEs generally did not increase with trophic level, especially in homeotherms, including birds (Kelly et al., 2008). A greater rate of biotransformation of PBDEs compared to PCBs was hypothesized to explain that finding, supported by reports of OH-PBDE formation. Evidence of the ability of eagles to metabolize PBDEs is supported by our previous finding of OH-PBDEs in some of these same eagle samples (McKinney et al., 2006).

Table 2
Estimated isotopic enrichment values for bald eagle nestling plasma.

Location	$\delta^{15}N$			$\delta^{13}C$		
	Estimated value	Actual value	Enrichment	Estimated value	Actual value	Enrichment
Inshore	14.25	15.79	1.54	−16.61	−17.33	−0.72
Southern offshore	13.63	15.05	1.42	−17.31	−17.44	−0.13
Lakes	9.06	10.95	1.89	−27.84	−28.16	−0.32
All sites			1.62 ± 0.14			−0.39 ± 0.17

The cytochrome P-450 system of the bald eagle may be more responsive to exposure at least to some known xenobiotic inducers than the kestrel, given that a significant increase in EROD activity occurred at 0.4 ng/g TEQs in bald eagle hatchlings (Elliott et al., 1996a; Elliott and Harris, 2001) compared to 23.3 ng/g TEQs (233 ng/g PCB-126) in the kestrel (Hoffman et al., 1998). The hepatic and renal P450 systems of adult kestrels were also relatively insensitive to induction by PCBs (Elliott et al., 1997), while in adult eagles exposure to ambient concentrations of 2,3,7,8-TCDD appeared to induce metabolism of 2,3,7,8-TCDF (Elliott et al., 1996c). Perhaps that difference stems from adaptation to a primarily aquatic, including marine, diet of the bald eagle, compared to the terrestrial diet of the kestrel? Previous studies found differences among avian taxa in cytochrome P450 activity, generally that marine birds had less developed capability to metabolize xenobiotic compounds, at least compared to omnivores and even raptors (Ronis and Walker, 1989; Fossi et al., 1995). However, marine environments contain compounds such as halogenated dimethyl bipyrroles (HDBPs) thought to be naturally produced by marine bacteria or other organisms (Tittlemeier et al., 1999). Concentrations up to140 ng/g of a HDBP were found in eggs of a Pacific population of Leach's storm petrels (*Oceanodroma leucorhoa*), a known prey species of the bald eagle at their colonies. It is possible,

therefore, that birds feeding in marine food chains may have developed an enhanced ability to metabolize similar compounds.

The negative relationship between residual δ^{15}N and OH-PCBs found here may indicate a depletion of PCB metabolites in prey and predators at higher trophic levels, probably via conjugation and excretion, but requires further study. The increase in BDEs, OH-PBDEs and OH-PCBs with marine input rather than trophic level also suggests that spatial variation, rather than trophic level, is more important for determining levels of those compounds.

Except along the northern offshore sites, where sample size was low, marine input (δ^{13}C) was generally a better predictor of contaminant concentrations than trophic level while at the freshwater sites there were no relationships between contaminants and stable isotopes (Table 1). Furthermore, those sites showing the strongest correlations between δ^{15}N and δ^{13}C, generally had the closest connections between stable isotopes and contaminants (Table 1). We suggest that was due to atmospheric and oceanographic processes tending to make marine sites more uniformly contaminated while also driving the processes that correlate δ^{15}N and δ^{13}C (Elliott et al., 1996a,b,c; Atwell et al., 1998; Elliott, 2005a,b). That uniformity results in a strong correlation between *p*,*p*'-DDE and PCBs and reduces local spatial variability as "noise" in the relationship between trophic level and contaminants. At freshwater sites, admixture is lower and the influence of local sources of contaminants greater, obscuring the relationships between trophic level and contaminants. For example, birds feeding on freshwater food chains along the Fraser River could be influenced by local industrial point sources, and thus have greater exposure to PCBs despite feeding at a low trophic level. In contrast, birds feeding on freshwater or terrestrial communities influenced by local agricultural sources in the Fraser Valley could have higher burdens of *p*,*p*'-DDE despite feeding at a low trophic level, while birds feeding on scavengers of human waste (rats, gulls) would have low exposure to both PCBs and *p*,*p*'-DDE despite feeding at a high trophic level. Similarly, contaminant concentrations may increase and trophic levels decrease with altitude (Campbell et al., 2000; Blais et al., 2001), leading to a strong correlation between *p*,*p*'-DDE and PCBs and leading to birds feeding at a high trophic level (large salmonids at low altitude lakes) having a lower contamination than those feeding at a low trophic level (smaller fish at high altitude lakes) (Blais et al., 2001). Those are two examples of how different dispersal patterns in freshwater may lead to greater spatial heterogeneity in contaminant levels at freshwater compared to marine sites, where contaminants are more easily dispersed and, thus, more uniform across large spatial scales (Atwell et al., 1998).

At the marine locations, trophic level and carbon input tended to explain a large portion of the variation in PCB and *p*,*p*'-DDE concentrations (Table 1). Those sites are among the most remote in subarctic western North America and upwind from any North American industry. Thus, with a high degree of admixture and limited local inputs, atmospheric sources appear to dominate across most of offshore and inshore coastal regions of Pacific coastal North America, resulting in a lack of variability among sites for mean concentrations of legacy persistent organic pollutants (POPs) such as PCBs or organochlorine insecticides (Elliott et al., 1996a,b,c, 1998; Elliott and Norstrom, 1998; Cesh et al., 2008). Therefore, the high r^2 values at marine sites are consistent with the idea that trophic level explains much of the individual variation in the absence of significant small-scale heterogeneity in contamination. California eagles were not at a significantly higher trophic level than birds from other offshore marine sites, so it is unlikely that they obtained high contaminant levels from marine mammals; in fact, PCB levels were lower than would be expected from trophic level.

Eagles have a broad diet in coastal regions, including mammals, birds and fish, both from natural and anthropogenic sources (Gill and Elliott, 2003; Elliott et al., 2005, 2006). Our data (Fig. 2) show that eagles on the outer coast rely more exclusively on marine organisms at a high trophic level, presumably pelagic fish such as herring and salmon (Gill and Elliott, 2003; Elliott et al., 2005). Eagles in the freshwater aquatic system rely more on freshwater or terrestrial organisms at a low trophic level. That may include small aquatic fish and human waste (chicken farm byproducts, household refuse, Elliott et al., 2006). At intermediate locations, stable isotope values suggested a stronger reliance on estuarine or benthic fish (Elliott et al., 2003, 2005). Our results support the conclusion that coastal eagles at most locations largely do not feed on other piscivorous birds, as was previously reported based on prey remains in nests (Vermeer and Morgan, 1989), as even the highest δ^{15}N values were similar to those of other piscivorous birds (Hobson and Welch, 1992; Hobson et al., 1994). Our δ^{13}C values were all more depleted than the value of −15.0‰ quoted as a terrestrial endpoint for gulls feeding at a landfill in our study site (Hobson, 1987), even for those known from direct observations to be feeding exclusively on marine fish. Thus, we suggest that terrestrial endpoint is not a useful value for eagles (a value of −17.0‰ would be more appropriate; Table 2), and that eagles are not using landfills as a prey source for nestlings (cf. Elliott et al., 2006).

Although we were able to estimate isotopic enrichment factors, and the ^{15}N enrichment factor of 1.62‰ was similar to the value of 3–5‰ quoted elsewhere (Hobson and Welch, 1992; but similar to that reported for other piscivorous birds in Becker et al., 2007), the large amount of potential prey items and their variability in isotopic signatures, prevented us from obtaining a robust estimate of diet from stable isotopes. For example, eaglets from western Vancouver Island fed at a higher trophic level than eaglets from Johnstone Strait and Haida Gwaii, even though some of the latter eaglets were fed regularly on seabirds. This is presumably because planktivorous seabirds feed at a lower trophic level than carnivorous fish, such as salmon, midshipman and herring. Nonetheless, stable isotopes may be a useful tool for assessing the role of extremely high trophic levels, such as marine mammals in diet.

Adults fed at similar trophic levels as their chicks, but had higher contaminant levels. The similar isotopic signature suggests that adults largely did not discriminate between the prey they fed themselves and those they fed their chicks, but accumulated higher levels of contaminants through their life. Based on central-place foraging theory, it is expected that adults would bring back larger prey items for their chicks than they eat themselves (Davoren and Burger, 1999; Ropert-Coudert et al., 2004), leading to a higher trophic signature for chicks than for adults, as has been observed previously for seabirds (Hobson and Welch, 1992; Cherel et al., 2005). Presumably, the absence of such a relationship in eagles reflects the small foraging radius of eagles compared with colonial seabirds, and consequently low foraging costs. Perhaps, adults may have selected prey items with specific nutritional requirements for their offspring, or they may have supplemented their own diet with large prey items (carrion, large fish) that they were unable to carry back to their offspring. Nestlings at our study site were fed primarily with small fish (Gill and Elliott, 2003; Elliott et al., 1998, 2005).

We conclude that trophic level is an important factor influencing concentrations of the legacy compounds PCBs and *p*,*p*'-DDE, explaining 30–40% of the variation at most marine sites. Those compounds have been heavily restricted in North America for over three decades, and are therefore likely near equilibrium particularly in marine systems not impacted by major local point sources (Elliott et al., 1996a,b,c; Elliott and Norstrom, 1998), However, trophic level *per se* may be less important at locations where there are ongoing point or major regional sources such as proximate to waste dumps or highly contaminated sediment deposition zones (Jenkins et al., 1994; Anthony et al., 1999). Trophic level was less important at freshwater sites, possibly due to greater spatial heterogeneity in contaminant sources. Trophic level also did not significantly influence concentrations of PBDEs in these eagles as compared to PCBs or *p*,*p*'-DDE, possibly due to the greater biotransformation via debromination, suggested

previously for highly brominated PBDE congeners (such as BDE-209) in Great Lakes herring gulls (Gauthier et al., 2008).

Furthermore, stable isotopes were useful for general information about eagle diet, such as assessing eagle trophic level, with an enrichment factor of about 1.62‰, and marine input, with marine prey items dominating below about −17‰. Thus, the technique may be useful for studying eagle diet where direct observation is difficult (adults on migration) or for augmenting sample sizes where direct observation is expensive.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2009.02.027.

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