

Linking contaminant profiles to the diet and breeding location of American dippers using stable isotopes

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Summary

1. Individual variation in contaminant levels is a common occurrence in many toxicology studies, but the exact cause is often unclear. We investigated the influence of diet and breeding location on individual bird contaminant profiles using a colour-marked population of American dippers *Cinclus mexicanus*. The population comprised two distinct groups within a single river system: resident dippers breeding on the main river and altitudinal migrants breeding on tributaries.

2. Residue analysis revealed that total organochlorines (OC), polychlorinated biphenyls (PCB) and mercury (Hg) were all significantly higher in eggs from river residents compared with tributary migrants. This trend was also apparent for the three most prevalent organochlorine compounds: *p,p'*-dichlorodiphenyl-dichloroethylene (DDE), hexachlorobenzene and *trans*-nonachlor.

3. We hypothesized that the observed differences in contaminant concentrations were partially related to the proportion of salmon fry *Oncorhynchus* spp. in the diet relative to aquatic invertebrates. Stable isotope analyses using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were conducted on blood and feathers of dippers in addition to aquatic invertebrates and salmon fry prey. Linear mixing models using the ^{15}N isotope in the dippers' diet and blood revealed considerable variability in the proportion of fish consumed (0–71%). Resident dippers on the main river ate significantly higher proportions of fish (42%) than tributary migrants (22%) ($P = 0.01$).

4. The difference in diet between migratory groups explained some of the observed variation in egg contaminant profiles, as total OC ($P = 0.002$) in dipper eggs was positively correlated with blood $\delta^{15}\text{N}$ values, indicating fish may be the primary source of contamination.

5. *Synthesis and applications.* We conclude that dipper eggs represent local conditions at the breeding site, making them useful tools for biomonitoring aquatic contaminants in watersheds. However, given the distinct difference in contaminant profiles between resident and migrant dippers and the link with diet, the results emphasize the importance of understanding individual species' ecology for assessing toxicological effects at the population level.

Key-words: biomonitor, mercury, ^{15}N Nitrogen, organochlorines, PCB, rivers, salmon fry, selenium

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Introduction

Water quality in rivers has a large influence on biodiversity and affects its potential use for domestic and agricultural processes (Giller & Malmqvist 2000).

Suitable biomonitors are therefore required to assess the impacts from local and long-range sources of pollution to watersheds. However, there can be marked variation in the response of some biomonitor species to pollutants. For example, contaminant burdens can vary widely between individuals of the same species occupying the same geographical area, thus preventing an accurate assessment of site contamination (Hebert 1998).

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The aquatic songbird American dipper *Cinclus mexicanus* (American Ornithologists' Union 1998) occupies mountainous watersheds year-round and has proven useful as a biomonitor of metal pollution (Strom, Ramsdell & Archuleta 2002). The Eurasian dipper *Cinclus cinclus* L. has also been shown to be a useful indicator of organochlorine (OC), polychlorinated biphenyl (PCB) and mercury (Hg) pollution in upland river catchments (Ormerod & Tyler 1990, 1992; O'Halloran *et al.* 1993, 2003; Ormerod, Tyler & Jüttner 2000). Previous studies have suggested that some populations of American dippers have distinct altitudinal patterns of migration, including seasonal movement upstream and downstream within a watershed (Bakus 1959a,b; Price & Bock 1983; Morrissey 2003). Resident and altitudinal migrant dippers can share common wintering grounds on the main river, but most migrants move upstream onto tributaries in the spring to breed while residents remain on the river year-round (Morrissey 2003). Even such small-scale migration could influence contaminant exposure, producing large variation in observed contaminant concentrations between individuals. Furthermore, dietary composition may vary within the breeding range of individual species, which further contributes to variability in contaminant levels (Hebert, Shutt & Norstrom 1997; Bearhop *et al.* 2000; Bustnes *et al.* 2000).

American dippers feed on benthic invertebrates and small fish during the breeding season. However, the availability of salmon fry to migrants on smaller tributaries is often lower than for residents along the main river because of differences in the distribution of spawning salmon (Crisp 2000). Any resulting difference in diet may be important in understanding American dipper exposure to persistent compounds, which fish tend to bioaccumulate and biomagnify to higher concentrations than invertebrates (Suedel *et al.* 1994; Kidd *et al.* 1995; Kiriluk *et al.* 1995).

Stable isotopes in ecological studies have provided an increasingly powerful means of distinguishing and tracing dietary sources. Applications have focused on determining trophic levels in ecosystems, separating terrestrial and aquatic food webs, and examining local differences in the diets and movements of individuals (Peterson & Fry 1987; Lajtha & Michener 1994; Hobson 1999; Kelly 2000). The use of stable carbon and nitrogen isotopes in this way can provide valuable insight in contaminant studies (Kidd *et al.* 1995; Jarman *et al.* 1996; Hebert, Shutt & Norstrom 1997; Bearhop *et al.* 2000; Braune, Donaldson & Hobson 2002).

Stable isotope ratios of stream biota are now well documented. Animals feeding on fish in addition to invertebrates should have an enriched isotopic signature compared with animals feeding almost exclusively on invertebrates (Rounick & Hicks 1985; Fry 1991; Cabana & Rasmussen 1994). Isotopic analysis of avian whole blood provides information on short-term dietary sources of assimilated foods (half-life time is typically 10–16 days for ^{13}C and 9–15 days for ^{15}N), while avian feathers record past dietary information during

feather growth (Hobson & Clark 1992; Bearhop *et al.* 2002; Evans Ogdén 2002). By using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in blood and feathers, we can infer the relative diet composition of the American dipper and assess further trophic differences between individuals breeding at different locations within a watershed.

In this study, we used a population of individually colour-marked dippers to determine exposure to contaminants by analysing egg samples from known residents and migrants separately. Our first objective was to test whether migratory patterns affected levels of OC, PCB, Hg and selenium (Se) in dipper eggs. We further investigated whether the diet differed between residents and migrants using stable isotopes. We hypothesized that each group would have a distinct isotopic signature of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the blood and feathers, that would not only identify the relative contribution of fish and invertebrates to the diet but could also aid in interpreting any differences in contaminant profiles.

Methods

STUDY AREA

The Chilliwack River watershed (49°1'N, 121°4'W) in the Fraser Valley is approximately 100 km east of Vancouver in the Cascade Mountain Range of southwestern British Columbia, Canada. Because of its unique geographical features, prevailing winds and the large human population found in the lower reaches of the valley, the upper valley receives organic and inorganic pollutants from urban, suburban, marine and agricultural sources as wet and dry deposition (Morrissey 2003). Annual precipitation averages 1850.5 mm, with mean daily temperatures of 10.4 °C (data from 1879 to 1990, Canadian Climate Normals and Averages, Environment Canada). The watershed tributaries are first- to third-order streams. The river is dominantly fourth-order, fed by a large glacial lake at its upper end, and is 43.5 km in length where it merges to become the Vedder River. The Chilliwack River supports populations of Pacific chum *Oncorhynchus keta*, coho *Oncorhynchus kisutch*, pink *Oncorhynchus gorbusha* and chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792), as well as cutthroat trout *Oncorhynchus clarki* (Richardson 1836), steelhead trout *Oncorhynchus mykiss* (Walbaum (1792) and Dolly Varden trout *Salvelinus malma* (Walbaum 1792; BC Fisheries data www.fishwizard.com). Anadromous salmon and steelhead spawn from late summer through winter within the watershed, but peak runs occur along the main stem of the river and at the hatchery in autumn. After egg development and emergence, salmon fry are most abundant in early spring, which coincides with the breeding period of the American dipper.

SAMPLING PREY

In April 2001, 15 composite samples of benthic invertebrates were collected at eight different sites spaced at

4–5 km intervals along the main stem of the Chilliwack River and from seven different Chilliwack tributaries. Aquatic larval invertebrates (*c.* 1 g dry weight) were collected either by kick sampling (disturbing the rocks directly upstream of a Surber sampler (Rickly Hydrological Co., Columbus, OH)) or by turning over rocks by hand. Each sample contained macro-invertebrates, primarily ephemeropteran, plecopteran and tricopteran larvae, that dippers prey upon (Mitchell 1968; Ealey 1977). During invertebrate collections, samples of approximately 10 salmon fry (*Oncorhynchus* spp.) (age 0+), were collected live using a dip net from the same eight sites along the main river. This represented a composite sample of predominantly coho and chum fry (*c.* 80%) but pink and chinook salmon (*c.* 20%) were also included. Faster flows and lower fry densities prevented us from capturing fish on tributaries. Invertebrates and fish were held alive in stream water in polyethylene bags for several hours to allow the stomach contents to purge, to obtain accurate isotopic values of the tissues. All samples were subsequently washed three times with deionized water to remove surface contamination or stream water and were then stored frozen in glass vials until preparation for stable isotope analysis.

SAMPLING AMERICAN DIPPERS AND REFERENCE SPECIES

Adult American dippers were caught throughout the breeding season from late March until July in all areas of the watershed on the main river and associated tributaries. Dippers were captured using 6-m passerine mist nets set up over moving water in narrow channels or on edges of the river and tributaries. A few additional birds were trapped by hand net while on the nest during incubation (females) or nest building (males). All dippers were weighed, measured and banded with a numbered US Fish and Wildlife Service (USFWS) aluminium band and three-coloured celluloid bands to represent unique combinations. We drew approximately 0.7–0.8 mL of blood from the jugular vein using a syringe and needle for most individuals (Kerlin 1964; Hoysak & Weatherhead 1991), or 0.1–0.2 mL in a few individuals by puncturing the metatarsal vein and collecting blood in capillary tubes. In addition, 10–15 breast feathers were removed and stored in polyethylene bags. We randomly selected 31 individual blood samples (16 residents, 15 migrants) and 20 individual feather samples (10 residents, 10 migrants) for analysis of stable isotope ratios. Blood and feathers used for the stable isotope analysis were not always from the same individuals because we did not obtain blood from all birds and used many feather samples for analyses unrelated to the present study.

In addition to American dippers, we collected blood and feathers from two reference groups in the Chilliwack River watershed. Common mergansers *Mergus merganser* (American Ornithologists' Union 1998) were considered a model for an obligate piscivore and three species of swallows, *Tachycineta thalassina*, *Tachyci-*

neta bicolor and *Stelgidopteryx serripennis* (American Ornithologists' Union 1998), were considered a model for an obligate insectivore. Common mergansers ($n = 12$) were captured using mist nets set up across sections of the river, and swallows ($n = 9$) were captured in the nest cavity using a hand net. We opportunistically captured a single belted kingfisher *Ceryle alcyon* (American Ornithologists' Union 1998), an obligate piscivore, from which we also sampled blood and feathers. All individuals were banded with a USFWS metal band. A 1.0-mL blood sample was collected from the brachial vein of mergansers with a syringe and needle and 0.1 mL was collected from the swallows and kingfisher by puncturing the brachial vein and drawing off blood with capillary tubes. In addition, several breast feathers were taken from each individual and stored in sealed polyethylene bags. All sample collections were done in accordance with Animal Care Committee approval and with a valid Environment Canada scientific permit. We observed no negative effects from blood or feather sampling of individuals. Furthermore, egg collections and handling of breeding birds caused no known nest abandonment or hatching failure.

EGG COLLECTION AND CONTAMINANT RESIDUE ANALYSIS

A single egg from a clutch of four or five eggs was collected from dipper nests throughout the watershed in 1999, 2000 and 2001. Viable and non-viable eggs were selected at random from each of 33 different clutches from resident dippers on the main river ($n = 17$) and from migrants on the tributaries ($n = 16$). Considerable variation in contaminant levels occurs within and between clutches allowing for equal treatment of eggs regardless of their position in the clutch or in repeat clutches (C.A. Morrissey, unpublished data). Whole eggs were refrigerated for up to 4 weeks. Egg contents were then transferred into an acetone : hexane-rinsed glass jar to be frozen at -25°C until analysis.

Chemical analyses of eggs were carried out at the National Wildlife Research Centre (NWRC), Hull, Quebec, Canada. OC analyses included determination of chlorobenzenes (tetrachlorobenzene, pentachlorobenzene, hexachlorobenzenes), hexachlorocyclohexanes (α -, β - and γ -HCH), chlordane-related compounds (oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide), DDT and metabolites (*p,p'*-DDE and *p,p'*-DDD), mirex and photomirex, and dieldrin. Total PCB were calculated by summing the peaks of 62 individual congeners identified. Samples were analysed quantitatively by capillary gas chromatography coupled with a mass selective detector, operated in selected ion monitoring mode according to CWS method no. MET-CHEM-OC-04B (Won, Mulvihill & Wakeford 2000). Briefly, samples underwent neutral extraction with 1 : 1 dichloromethane (DCM) : hexane after dehydration with anhydrous sodium sulphate, removal of lipids and biogenic materials

by gel permeation chromatography, and further cleanup by Florisil (Floridin Co., Quincy, FL, USA) column chromatography. All samples were spiked with internally labelled ^{13}C standards prior to extraction. Each sample extract was injected twice, once for determination of OC and once for PCB. As part of the quality control, blanks and CWS reference material (1989 Lake Ontario Herring Gull QA) were run concurrently. The nominal detection limit for all compounds was $0.0001 \mu\text{g g wet weight}^{-1}$. Internal standard recoveries were typically between 80% and 110% and residues were not recovery corrected. Egg concentrations are reported on a wet weight basis (Peakall & Gilman 1979) with arithmetic corrections of desiccated samples that deviated by more than 5% from the mean moisture content (78.6%) of freshly collected, undeveloped eggs.

Total Hg and Se were analysed according to CWS method no. MET-CHEM-AA-02E (Neugebauer, Sans Cartier & Wakeford 2000). The sample homogenates were freeze-dried to determine moisture content and analysed for Hg without prior acid digestion on the AMA-254, Advanced Mercury Analyser, which employs direct combustion of the sample in an oxygen-rich atmosphere. Samples for which there was sufficient material remaining were digested in nitric acid according to standard techniques for Se analysis. Se was analysed by graphite furnace atomic absorption spectrometry (GFAAS) using a Perkin Elmer 3030b equipped with a Deuterium background corrector and HGA-300 Graphite furnace. Accuracy of analysis was determined using certified reference materials Dolt-2 and Dorm-2 (National Research Council of Canada, Ottawa, ON, Canada) and blank samples. Recoveries of reference materials were within the certified range (95–121%). Additionally, random egg samples were analysed in duplicate to check precision. All values for Hg and Se are reported on a dry weight basis and detection limits under these conditions were $0.18 \mu\text{g g dry weight}^{-1}$ for Hg and $0.10 \mu\text{g g dry weight}^{-1}$ for Se.

STABLE ISOTOPE ANALYSIS

Invertebrate and fish samples were stored frozen and then freeze-dried for 24–48 h until completely dry before being ground into a fine powder. Three separate subsamples of each of the composites of invertebrates and fish were analysed to ensure homogeneity of the mixture, particularly the invertebrates. Whole blood samples were also stored frozen until preparation for determination of stable isotope ratios. Samples were then freeze-dried for 24 h and homogenized. Feather samples were washed with a 2:1 chloroform and methane solution and thoroughly rinsed with distilled deionized water to remove any surface lipids or external contamination. They were subsequently oven dried and cut-up into $< 1 \text{ mm}$ pieces. A subsample of each tissue ($c. 1 \text{ mg}$) was weighed into miniature tin capsules ($5 \times 9 \text{ mm}$; Costech Analytical Technologies Inc., Valencia, CA, USA) for combustion at over $1000 \text{ }^\circ\text{C}$ using an on-line elemental analyser.

Stable isotope ratios of carbon and nitrogen were analysed at the Stable Isotope Facility, University of Davis, California, USA, using a Europa Hydra 20/20 continuous flow isotope ratio mass spectrometer (CFIRMS) (PDZ Europa, Cheshire, UK). Sample isotope ratios were compared with the standard gases Pee Dee Belemnite for ^{13}C and atmospheric nitrogen (AIR) for ^{15}N , which were injected directly into the CFIRMS before and after the sample peaks. Values for ^{13}C and ^{15}N were calculated and reported using the standard delta (δ) notation in parts per thousand (‰) as follows:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

where X is ^{15}N or ^{13}C and R is the corresponding ratio $^{15}\text{N} : ^{14}\text{N}$ or $^{13}\text{C} : ^{12}\text{C}$. Replicate laboratory standards (ammonium sulphate $^{15}\text{N} = 1.33\text{‰}$ and sucrose $^{13}\text{C} = -23.83$) were analysed before and after every 12 samples to determine the accuracy of ^{13}C and ^{15}N values. Measurement errors averaged $\pm 0.1\text{‰}$ for nitrogen and $\pm 0.04\text{‰}$ for carbon.

DATA ANALYSIS

A two-sample t -test was used to compare mean total OC (ΣOC), total PCB (ΣPCB), Hg and Se residues in resident and migrant American dipper eggs. OC and ΣPCB residues were not normally distributed (Shapiro–Wilk W -test); therefore values were log-transformed to approximate normal distributions prior to parametric analysis. OC and PCB values are reported as arithmetic means \pm SE and geometric means. Where concentrations were below the detection limit for OC compounds in eggs, a value equal to the minimum detection limit ($0.0001 \mu\text{g g wet weight}^{-1}$) was applied. For Hg, we used a value of one-half the detection limit ($0.09 \mu\text{g g}^{-1}$) to permit statistical analyses. Sample size was 32 eggs for OC/PCB analysis, 33 for Hg and 27 for Se based on available sample mass.

We determined differences in stable isotopic signatures ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) among prey types (tributary invertebrates, river invertebrates and salmon fry) using a K -nearest neighbour randomization test (Rosing, Ben-David & Barry 1998), which treats the isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as spatial data. The same analysis was then used to determine differences in blood and feather isotope values among resident and migrant dippers and between reference species (mergansers and swallows). Where appropriate, ANOVAs were used to assess variation in mean $\delta^{15}\text{N}$ values among groups, followed by a multiple comparison Tukey HSD test. Statistical analyses were performed using JMP v.4.0 (SAS Institute Inc. 2002).

A relative index of the invertebrate and fish component of the diet was calculated for each dipper as well as a mean for each group, using only the $\delta^{15}\text{N}$ values in a two-source linear-mixing model (Phillips & Gregg 2001). As larger changes in $\delta^{15}\text{N}$ occur with trophic level, this isotope is more sensitive to variation in diet. In addition,

we found low variability among prey samples for $\delta^{15}\text{N}$, allowing for increased precision in estimating diet composition. River dippers were analysed separately from the tributary dippers because invertebrate $\delta^{15}\text{N}$ signatures were significantly higher on the river, probably as a result of enriched marine-derived nutrients from spawning salmon in the main river (Bilby, Franston & Bisson 1996; Johnston *et al.* 1997). Isotope ratios of salmon fry collected from the river were assumed to be representative of both river and tributary locations. We used fractionation values of 2.9‰ for $\delta^{15}\text{N}$ in invertebrate samples and 1‰ for $\delta^{15}\text{N}$ in fish samples, which were calculated from the reference species (swallows and mergansers/kingfisher). These were comparable to values obtained in captive-feeding experiments of birds on high protein diets (Bearhop *et al.* 2002; Evans Ogden 2002). Proportions of fish in the diet were arcsine transformed to permit parametric analyses. Male and female $\delta^{15}\text{N}$ values and relative proportions of fish in the diet were compared using a two-sample *t*-test to determine if sex affected diet composition. Combined sex isotope data were then used to represent overall diet composition for comparison with contaminant profiles in dipper eggs.

Results

CONTAMINANT RESIDUES IN AMERICAN DIPPER EGGS

Mean concentrations of ΣOC ($t_{29} = -4.9$, $P < 0.0001$) and ΣPCB ($t_{28} = -3.6$, $P = 0.001$) were higher in eggs from river residents compared with the tributary migrants (Fig. 1). Residues of DDT metabolites, chlordane compounds and chlorobenzenes were all significantly higher in resident eggs compared with migrants (Table 1). Detection of HCH was low and, therefore, differences between residents and migrants were not formally significant. Mirex, photomirex and dieldrin were not detected in any of the eggs. Mercury was also significantly higher in eggs of river residents than tributary migrants ($t_{31} = -4.7$, $P < 0.0001$; Table 2). However, there was no difference in Se concentrations between groups ($t_{25} = -1.15$, $P = 0.26$).

STABLE ISOTOPE ANALYSIS

Prey samples indicated significant differences existed in isotopic signatures between river invertebrates, tributary invertebrates and fish (*K*-nearest neighbour randomization test, $P < 0.0003$; Table 3). The $\delta^{15}\text{N}$ values in tributary invertebrates were significantly different from river invertebrates ($F = 239.2$, $P < 0.0001$); therefore we tested for trophic level differences separately for resident dippers on the main river and for migrants on the tributaries.

Isotopic signatures differed between whole blood samples from the reference species (swallows and mergansers) and American dippers (river resident and tributary migrants; Fig. 2). The *K*-nearest neighbour randomization test indicated that all four groups were significantly spatially separated ($P < 0.0001$). $\delta^{15}\text{N}$ values were also different among groups ($F_{3,48} = 79.8$, $P < 0.0001$), with no overlap of confidence intervals. River resident dippers had a $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signature intermediate between the two reference species, but tributary dippers could not be compared because of a

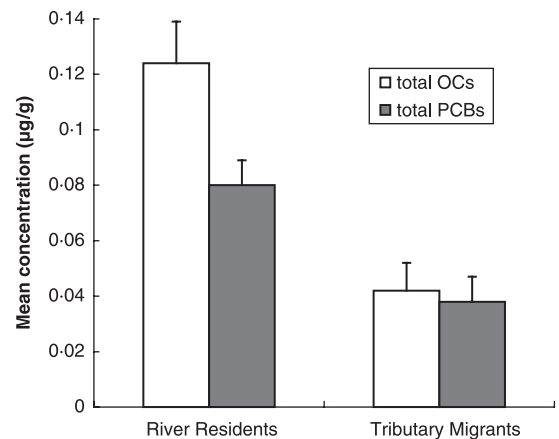


Fig. 1. Arithmetic mean concentrations (\pm SE) of total organochlorines (OC) and total polychlorinated biphenyls (PCB) in $\mu\text{g g wet weight}^{-1}$ detected in American dipper eggs from river and tributary nests within the Chilliwack River watershed, British Columbia, Canada. Statistical tests were performed on log-transformed values (geometric means).

Table 1. Mean concentrations \pm SE (geometric mean) of organochlorine residues detected in American dipper eggs collected from river residents and altitudinal migrants in the Chilliwack River watershed, British Columbia, Canada

Group	% lipid	Concentration of OC ($\text{ng g wet weight}^{-1}$)*					
		ΣDDT	ΣClBz	ΣCHLOR	ΣHCH	ΣMirex	Dieldrin
River resident	6.6 ± 0.4	118.0 ± 15.2 (102.2)	4.3 ± 0.5 (4.1)	2.4 ± 0.6 (1.5)	4.7 ± 1.9 (0.83)	ND†	ND
Tributary migrant	6.3 ± 0.3	42.6 ± 8.7 (29.9)	2.7 ± 0.5 (2.5)	0.7 ± 0.1 (0.42)	1.1 ± 0.6 (0.21)	ND	ND
Significance (<i>P</i>)	NS	0.0002	0.006	0.009	0.06	–	–

* ΣDDT = DDE, DDT, DDD; ΣClBz = chlorobenzenes (tetrachlorobenzene, pentachlorobenzene, hexachlorobenzene); ΣCHLOR = chlordane compounds (oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, *cis*-nonachlor and heptachlor epoxide); ΣHCH = hexachlorocyclohexanes (α -, β -, γ -hexachlorocyclohexanes); ΣMirex = mirex and photomirex. †ND, all samples non-detectable; detection limit for all compounds = 0.1 ng g^{-1} .

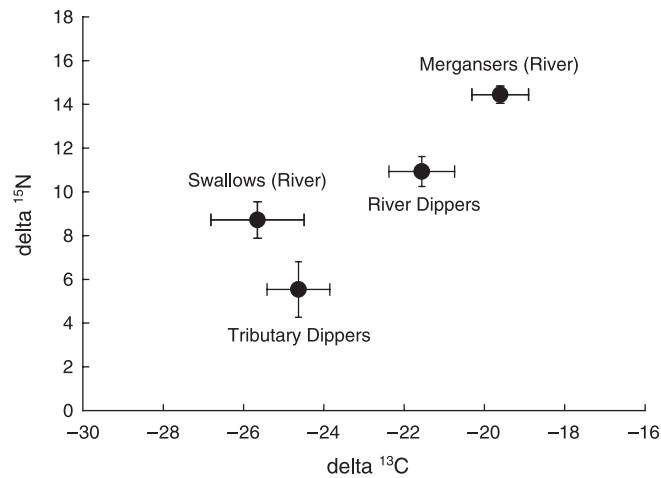


Fig. 2. Stable isotope signatures ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in blood samples of American dippers and two reference species, mergansers and swallows, with 95% confidence intervals (CI) on both axes. Samples were collected from the Chilliwack River watershed, British Columbia, Canada.

Table 2. Mean concentrations (\pm SE) of mercury and selenium residues in resident and migrant American dipper eggs collected in the Chilliwack River watershed, British Columbia, Canada

Group	% moisture	Total mercury ($\mu\text{g g dry weight}^{-1}$)*	Selenium ($\mu\text{g g dry weight}^{-1}$)
River resident	75.7 \pm 1.9	0.19 \pm 0.01	2.96 \pm 0.16
Tributary migrant	76.8 \pm 1.6	0.09 \pm 0.02	2.67 \pm 0.19
Significance (<i>P</i>)	NS	< 0.0001	NS

*For samples below detection, a value of one-half the detection limit was used. Hg detection limit = 0.18 $\mu\text{g g}^{-1}$.

Table 3. Summary of mean isotopic values (\pm SE) for prey samples collected at eight sites on the main stem of the Chilliwack River watershed and seven different tributaries, British Columbia, Canada

Prey type	<i>n</i>	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Salmon fry	8	13.6 \pm 0.5	-20.1 \pm 0.6
River invertebrates	8	5.4 \pm 0.3	-25.4 \pm 1.1
Tributary invertebrates	7	0.1 \pm 0.4	-27.7 \pm 0.9

Table 4. Summary of mean blood isotopic values (\pm SE) and relative diet composition (% fish obtained from linear mixing model for $\delta^{15}\text{N}$ isotope) of male and female American dippers in the Chilliwack watershed, British Columbia, Canada

	Males (<i>n</i> = 20)	Females (<i>n</i> = 11)	Significance (<i>P</i>)
$\delta^{15}\text{N}$ (‰)	8.23 \pm 0.75	8.48 \pm 1.01	0.8 (NS)
$\delta^{13}\text{C}$ (‰)	-23.35 \pm 0.47	-22.48 \pm 0.64	0.3 (NS)
% fish in diet	30.0 \pm 4.9%	37.4 \pm 6.5%	0.4 (NS)

lack of a suitable insectivore reference (i.e. swallows breeding on tributaries). However, river and tributary dippers had significantly different blood isotopic signatures, with river residents more enriched in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (*K*-nearest neighbour randomization test, $P < 0.0001$; Fig. 3).

According to the mixing model using $\delta^{15}\text{N}$, the amount of fish in the diets of American dippers ranged widely from 0% to 71% (mean = 33%; Fig. 4). Male and female dippers did not differ in blood $\delta^{15}\text{N}$ values ($t_{29} = 0.20$, $P = 0.8$) or diet composition ($t_{29} = 0.96$, $P = 0.4$; Table 4). However, resident dippers occupying the river ate a higher percentage of fish (42% \pm 7%) than migrants on tributaries (22% \pm 6%) ($t_{29} = -2.7$, $P = 0.01$).

Isotopic signatures in feather samples from the reference species and the dippers were not consistent with blood samples. Feathers of the mergansers and swallows were significantly spatially separated (*K*-nearest

neighbour randomization test, $P = 0.003$) but with considerable variation among individuals. Spatial separation of isotopic signatures between resident and migrant dipper feathers was marginally insignificant (*K*-nearest neighbour randomization test, $P = 0.06$; Fig. 5). Given the lack of any clear distinction between resident and migrant dippers, or suitable end points from the reference species, we could not assess the relative contributions of fish and aquatic invertebrates to the diets of dippers during feather growth in the previous year. Therefore, we could not definitively infer moulting location by feather isotope signatures.

We had a small sample ($n = 8$) of data where both egg contaminants and blood isotope ratios were collected from a bird in the same territory in the same season (four river residents and four tributary migrants). ΣOC (log-transformed) in dipper egg samples was positively

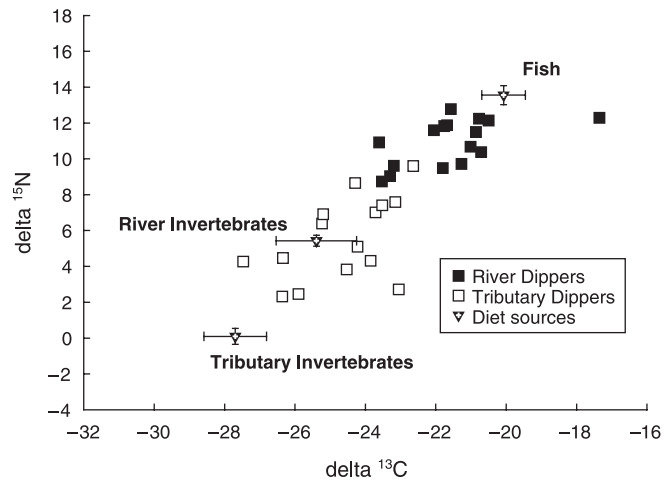


Fig. 3. Stable isotope signatures ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) of river resident and tributary migrant American dippers in the Chilliwack River watershed, British Columbia, Canada, relative to the mean isotopic signature (\pm SE) of benthic invertebrate and salmon fry diet sources.

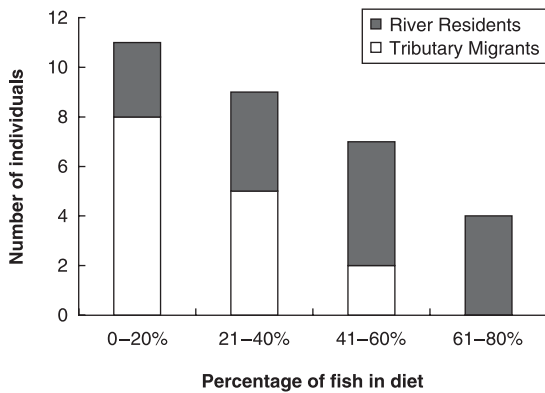


Fig. 4. Relative contribution of salmon fry to the diets of river resident and tributary migrant dippers, as determined by a two-source linear-mixing model (Phillips & Gregg 2001) using the ^{15}N isotope.

correlated with $\delta^{15}\text{N}$ values in blood from birds sampled from the same territory and year ($r = 0.91$, $P = 0.002$). Given that DDT metabolites comprised more than 90% of the OC pesticide residues in dipper eggs,

the log sum of DDT metabolites was also significantly correlated with $\delta^{15}\text{N}$ values in blood ($r = 0.91$, $P < 0.002$; Fig. 6a). Correlation between ΣPCB and $\delta^{15}\text{N}$ values in dipper blood was marginally insignificant ($r = 0.68$, $P = 0.06$; Fig. 6b). Data points in the correlations tended to cluster by location, with river residents comprising the upper end with higher $\delta^{15}\text{N}$ and $\Sigma\text{DDT}/\text{PCB}$ concentrations than tributary migrants. ΣOC was not intercorrelated with ΣPCB ($P = 0.3$). There was no relationship between egg Hg levels and $\delta^{15}\text{N}$ values in blood, primarily because many samples from tributaries were below detection limits for Hg.

Discussion

SOURCES AND SIGNIFICANCE OF CONTAMINANT RESIDUES

Although few studies have noted the importance of small-scale migratory movements on the interpretation of contaminant concentrations in focal species, they are a significant factor in explaining individual variability

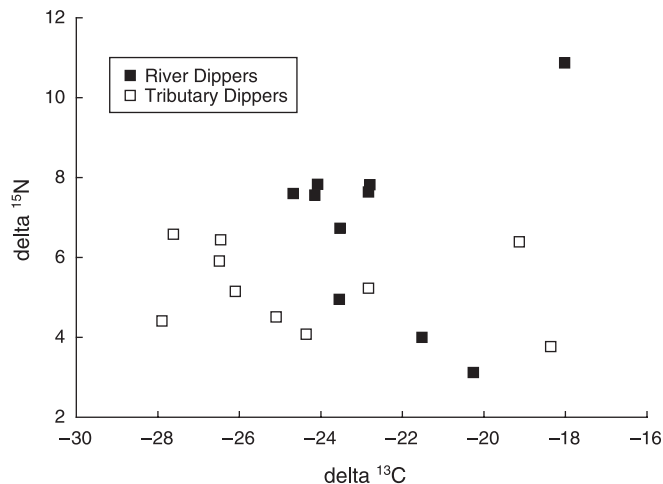


Fig. 5. Stable isotope signatures ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in breast feather samples of river resident and tributary migrant American dippers in the Chilliwack River watershed, British Columbia, Canada.

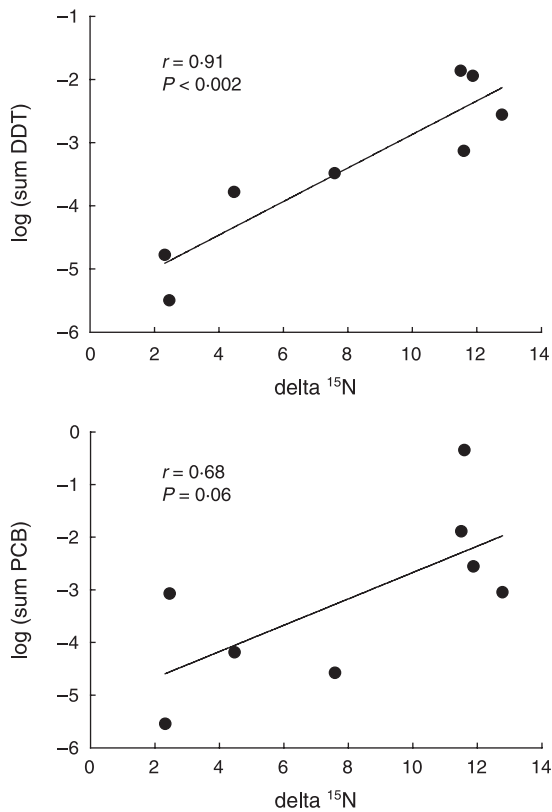


Fig. 6. Correlation between $\delta^{15}\text{N}$ values in American dipper blood and contaminant concentrations, (a) log ΣDDT and (b) log ΣPCB , in egg samples. Blood and eggs were from individuals from the same territory in the same year (2001).

in contaminant concentrations. Hebert (1998) noted that winter weather influenced migratory movements of herring gulls *Larus argentatus* (American Ornithologists' Union 1998) in the Great Lakes, which ultimately affected the birds' exposure to local OC contaminants. Our data on the American dipper further demonstrate the importance of spatial trends in species that are often classified as non-migratory.

Variability in contaminant concentrations in American dipper eggs was largely explained by the bird's status as a resident on the main river or an altitudinal migrant on one of the watershed tributaries. Each group had significantly different levels of OC, PCB and Hg in their eggs, with residents showing higher concentrations of all contaminants measured, except Se. This trend was apparent despite the fact that the system is a single continuous watershed where distances separating migrant and resident dippers are relatively minor (< 1–15 km). Residents and migrants in the Chilliwack watershed are known to winter on the main river together, but the migrants move upstream onto the tributaries to breed (Morrissey 2003). Our data imply that contamination in eggs is largely determined by exposure on the breeding site, and that nutrients are deposited in the eggs primarily from recent dietary uptake. This finding is consistent with Ormerod, Tyler & Jüttner (2000), who suggested that eggs of the Eurasian dipper also reflected local sources of contamination. Most nutrients required

for egg formation in passerines are not stored prior to laying but are gathered on a daily basis during the laying period (Perrins 1996). This is not unexpected given that egg formation in passerines is costly in terms of energy and nutrient requirements as the mass of the clutch can often equal or outweigh the female's own body weight (Perrins 1996). Therefore, recent dietary intake in dippers will largely influence contaminant levels in eggs, making them a useful biomonitor of local contamination.

Given that persistent lipophilic organic compounds bioaccumulate and biomagnify with increasing trophic levels (Connel 1990), dippers with a larger proportion of fish in their diet were expected to have greater exposure to chlorinated hydrocarbons than those primarily on an invertebrate diet. The results from the stable isotope data support this hypothesis, demonstrating that river residents were consuming a larger proportion of fish during the breeding season. In addition, log ΣDDT and log ΣOC in eggs were significantly correlated with the $\delta^{15}\text{N}$ signature in blood of banded birds despite the limitations of sample size. These results are consistent with other studies where an increased $\delta^{15}\text{N}$ signature was associated with a higher trophic level and subsequently higher contaminant burdens (Broman *et al.* 1992; Kidd *et al.* 1995; Jarman *et al.* 1996; Bearhop *et al.* 2000).

Differences in location between river residents and tributary migrants within the watershed may have further contributed to the observed contaminant trends. Patterns exist in watersheds with respect to stream ecosystem structure and function (e.g. the river continuum concept) that suggest a predictable transition from the headwaters to higher order reaches (Vannote *et al.* 1980). Changes occur in discharge, chemistry, allochthonous and autochthonous energy sources, species richness and biomass, along with increasing contaminant loads through atmospheric deposition from upstream to downstream (Giller & Malmqvist 2000). Atmospheric deposition can explain some of the variability in contaminant profiles between resident and migrant dippers. However, some contaminants, such as Hg and DDE, did not differ significantly in benthic invertebrate prey collected from river and tributary locations (Morrissey 2003). Instead, Hg and DDE were detected at a higher frequency and at higher concentrations in fish tissues compared with invertebrates. Additionally, PCB were not detected in any invertebrate samples regardless of location, but were detected in salmon fry, indicating fish are the primary source of exposure (Morrissey 2003). Therefore, variations in diet and available prey provide a mechanism by which breeding locations can affect contaminant exposure and uptake.

With the exception of Se, which was at or near the threshold level of $3 \mu\text{g g dry weight}^{-1}$ for reproductive toxicity (Lemly 1993), the levels of OC, PCB and Hg in dipper eggs were all relatively low and generally below levels known to cause toxicity (Beyer, Heinz & Redmon-Norwood 1996). Despite the low concentrations, many

contaminants were detectable, with a highly significant trend existing among residents and migrants. This further emphasizes the utility of American dipper eggs in monitoring pollutants in watersheds. American dipper blood samples have also been used successfully to indicate lead pollution in a mine-impacted stream (Strom, Ramsdell & Archuleta 2002). Future studies using dippers as indicators of water quality are expected to emerge for monitoring anthropogenic impacts to watersheds and following stream restoration projects. However, consideration as to the differences in breeding location and feeding ecology of residents and migrants will be important in interpreting the sources of contaminant burdens.

BLOOD AND FEATHER ISOTOPE ANALYSIS

Based on controlled laboratory studies of captive birds, whole blood is a useful tissue for stable isotope analysis because it incorporates both ingested and assimilated diet items over a window of 20–30 days (Hobson & Clark 1992; Bearhop *et al.* 2002). In passerines, this information can be used directly to identify the diet and nutrient sources at the time of egg formation, but it will depend on the timing of blood sampling and the diet consistency.

The difference in blood isotopic signature between residents and migrants was related to the proportion of fish in the diet but also to the relative isotopic signature from the aquatic invertebrates. As marine-derived nutrients are enriched in ^{15}N , spawning salmon contribute their marine signature to the stream benthos through carcass decay (Bilby, Franson & Bisson 1996, Bilby *et al.* 1998; Johnston *et al.* 1997). Salmon abundance is considerably higher on the Chilliwack River relative to the tributaries (BC Fisheries data 1985–2000). Salmon spawn in high densities along rivers but fewer fish migrate onto the tributaries because of steep gradients, faster flows and the presence of barriers (Crisp 2000). Therefore, ^{15}N signatures in benthic invertebrates from the tributaries were considerably lower than river invertebrates. We had to assume isotope ratios of salmon fry collected from the river were representative of both river and tributary locations because we were unable to capture fish on tributaries. If salmon fry are significantly depleted in ^{15}N on the tributaries as a result of lower salmon densities, we may have underestimated the proportion of fish in the diet of tributary birds. However, differences in stable isotope values of salmon fry caused by lower salmon densities on the tributaries are probably less than 10–20% for $\delta^{15}\text{N}$ and only 2% for $\delta^{13}\text{C}$ (Johnston *et al.* 1997; Bilby *et al.* 1998). This equates to a relative increase of 3–6% in the mean proportion of fish in tributary dippers' diet (maximum 28% fish instead of 22%). Linear-mixing models have considerably greater error than this (Ben-David & Schell 2001); therefore our estimates for diet proportions should be taken as an index rather than an absolute value of diet proportions. Ultimately, isotopic signatures were generally higher in river dippers compared with tributary

birds because of the higher consumption of salmon fry by river residents during the breeding season and the higher densities of spawning salmon, which contribute enriched marine-derived nitrogen to the river biota.

The isotopic signature in feathers reflects the diet during the period of regrowth, and isotopic compositions are essentially locked into the feather structure post-moult (Hobson & Clark 1992; Mizutani, Michio & Kabaya 1992). It was apparent that the reference species did not have an isotopic signature in the feathers that resembled their blood. We have no information on the moult location of the mergansers and swallows; therefore, we could not use them to determine isotopic values for obligate fish and insect feeders to compare with American dippers.

As dippers tend to moult when the breeding season is complete (usually from July to September; Kingery 1996), stable isotope analysis can give information on the previous year's breeding and moulting location based on known isotopic signatures of residents and migrants. Although resident and migrant dippers showed some similarities between feathers and blood with respect to diet, clearly not all the birds had the same pattern. Mizutani, Michio & Kabaya (1992) showed that both ^{13}C and ^{15}N were enriched in feathers relative to the diet of nine species of aquatic birds. Therefore, if resident and migrant American dippers moulted in the same location as they bred in the preceding year, we expected their feather isotopic signatures to show a similar pattern to the blood. Notably, at least two individual residents and two migrants had an alternate isotopic signature that did not reflect the majority of birds. A change in diet during the period of feather growth may have caused this result, or a movement away from the breeding site during moult. A study by Smith & Ormerod (1986) found no evidence of a change in diet during the moult period of the Eurasian dipper. Although we assumed that dippers were breeding and moulting in the same location, dippers are highly secretive and often not seen on their territories during the moult (Price & Bock 1983). Therefore, some individuals may seek out refuge or accessible food in new locations, ultimately altering their isotopic signature. Further research is needed to determine if feather isotope analysis is a useful technique for identifying breeding and moulting locations in this species.

CONCLUSIONS AND APPLICATIONS

Given that contaminant levels in dipper eggs are largely determined by exposure on the breeding site, and that nutrients are deposited in the eggs primarily from recent dietary uptake, dipper eggs can be used as an effective tool for monitoring local contaminant conditions. Although there appeared to be no additional effect of migration on egg contaminant profiles, spatial segregation as a result of differential migration strategies proved an important factor in exposure to OC, PCB and Hg. As such, contaminant levels in dipper eggs reflected

prey selection of the individual. Therefore, if trends in contaminant concentrations are to be correctly inferred by indicator species such as the dipper, a sound understanding of the population's structure, migration strategy and diet is crucial. We recommend that results of future contaminant studies on indicator species be interpreted with a more integrative approach that accounts for the spatial variation in breeding sites and relative prey availability.

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