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ASSESSING TRACE-METAL EXPOSURE TO AMERICAN DIPPERS IN MOUNTAIN STREAMS OF SOUTHWESTERN BRITISH COLUMBIA, CANADA

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Abstract—To develop a suitable biomonitor of metal pollution in watersheds, we examined trends in exposure to nine trace elements in the diet (benthic invertebrates and fish), feathers (n = 104), and feces (n = 14) of an aquatic passerine, the American dipper (*Cinclus mexicanus*), from the Chilliwack watershed in British Columbia, Canada. We hypothesized that key differences may exist in exposure to metals for resident dippers that occupy the main river year-round and altitudinal migrants that breed on higher elevation tributaries because of differences in prey metal levels between locations or possible differences in diet composition. Metals most commonly detected in dipper feather samples in decreasing order were Zn > Cu > Hg > Se > Pb > Mn > Cd > Al

Trace metals are present in aquatic systems worldwide, largely from underlying substrates, natural erosion, volcanism, and hydrological cycles. However, mining processes [1], urban and agricultural runoff [2], industrial emissions [3], and deforestation [4] also can cause increased metal loads to watersheds. Although mountain streams appear remote from industrialization and urbanization, many still contain significant concentrations of heavy metals from natural and anthropogenic sources [5]. With concerns over environmental impacts of metals to freshwater ecosystems, it is important to be able to monitor the degree of metal exposure to organisms occupying mountain streams.

The American dipper (*Cinclus mexicanus*) is a potentially useful biomonitor of stream pollution because it is a year-round resident of freshwater streams and has an exclusively aquatic diet comprised of benthic macroinvertebrates, small fish, and fish eggs. Many invertebrate taxa have the ability to bioaccumulate metals to high concentrations without inherent toxicity to the host species [6]. Freshwater fish also can bioaccumulate organometallic compounds, particularly methylmercury (MeHg) due to the high assimilation efficiency and the slow elimination rates of this compound [7]. Therefore, predators feeding on metal- contaminated biota, including the dipper, are at risk for elevated exposure from its aquatic diet. Strom et al. [8] confirmed adult and nestling American dippers were exposed to lead through their invertebrate prey in a mine-impacted river system. Therefore, dippers can be an effective model for monitoring metal pollution in mountain streams because they integrate contaminant sources from their aquatic diet over time and space.

Previous studies in the Chilliwack watershed of British Columbia revealed that American dippers have distinct altitudinal patterns of migration, which include seasonal movement upstream and downstream within a watershed [9]. Resident and altitudinal migrants shared common wintering grounds on the river, but most migrants moved upstream onto higher elevation creeks in the spring while residents remained on the river to breed. Eggs of resident dippers had higher levels of mercury and chlorinated hydrocarbons relative to creek migrants as a result of higher cumulative downstream loadings and differences in the proportion of fish and invertebrates in the diet [10]. Dipper diets consisted of 0 to 71% fish, with river residents consuming significantly more fish (42%) compared to creek migrants (22%) [10]. Therefore, we hypothesized that trace-metal concentrations in feathers and feces of resident and migrant American dippers also may reflect the birds' migratory status or specific diet.

Our main objective was to determine if any differences exist in metal exposure for resident dippers occupying the main river and migrant dippers breeding on watershed tributaries using feathers and feces as bioindicators. We further attempted to identify the major sources of metal contamination to resident and migrant dippers via their fish and invertebrate diet. This permitted us to quantify the magnitude of exposure from the major prey groups and potentially relate it to levels observed in feathers. Although some elements biologically are essential, all are toxic at high enough concentrations, with some having a very narrow window of essentiality and toxicity [11]. Thus,

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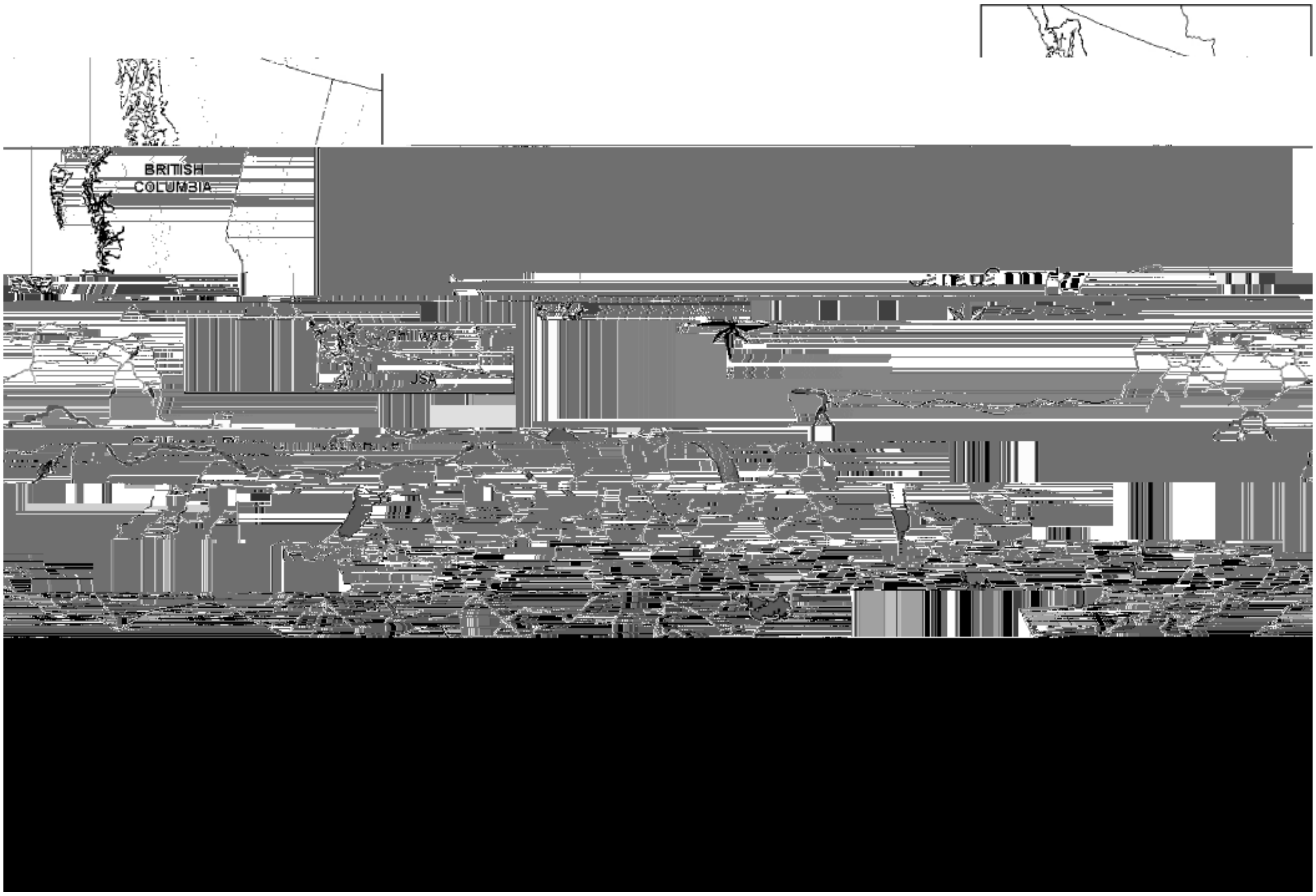

Fig. 1. Map of the study area: The Chilliwack River watershed located near the Canadian–U.S. border in southwestern British Columbia, Canada.

in the interest of using the dipper as a biomonitor, we also modeled the potential toxicological risks of metal exposure to a dipper population with different migratory strategies and diets.

MATERIALS AND METHODS

Collection of samples

Samples were collected from the Chilliwack River watershed (49°10′N, 121°04″W), located in the Cascade Mountains in southwestern British Columbia, Canada (Fig. 1). The watershed drains an area of 1,274 km^2 with elevation ranges from near sea level to over 2,000 m at several mountain peaks. Composite samples of benthic invertebrates and salmon fry were collected at eight different sites spaced at 4- to 5-km intervals along the main stem of the Chilliwack River. Additional composite samples of invertebrates were collected from seven different tributaries in the watershed. Aquatic larval invertebrates (~1-g dry wt) were collected either by kick sampling in the stream (disturbing the rocks directly upstream of a Surber sampler) or by turning over rocks by hand. The sample represented a mixture of insect taxa that dippers naturally would prey upon, including approximately equal proportions of ephemeropteran, plecopteran, and tricopteran larvae in addition to a much smaller fraction by mass of coleopteran and dipteran larvae. Up to 10 individual salmon fry (*Oncorhynchus* spp.) (age 0+) that each weighed 100 to 200 mg fresh weight, were captured live from the Chilliwack River using a dip net and represented a composite sample of predominantly coho and chum salmon fry (~80%), but pink and chinook salmon (~20%) also were included. All samples were collected during the dipper breeding season before the spring freshet over a one-week period in late April 2000 and repeated

Sample preparation and metal analysis

Methods for sample preparation and digestion were adapted and modified from Canadian Wildlife Service method MET-CHEM-AA-02 [14] and U.S. Environmental Protection Agency method 200.3 [15]. Feathers were washed with pure acetone, 1% Triton-X solution alternated with several rinses of distilled deionized water to remove any external surface contamination. Samples were then air-dried for 48 h and finally oven-dried for 12 h. Invertebrate, fish, and fecal samples were freeze-dried for 24 to 48 h until constant weight was achieved. Samples were then weighed accurately into acid-washed glass flasks to the nearest 1 mg. The digestion procedure involved adding 5 ml of 70% ultrapure nitric acid (HNO_3), slow heating to reduce volume, adding an additional 2 ml HNO_3 while heating, and finally adding 1 ml of 30% ultrapure hydrogen peroxide (H_2O_2). All samples were reduced by heat to <1 ml, diluted to 10 ml with distilled, deionized water, and then stored refrigerated in polypropylene vials until metal analysis. A minimum of two certified reference materials (Dolt-2 and Tort-2; National Research Council Canada, Ottawa, ON) and two procedural blanks were digested simultaneously with every batch of samples and analyzed for quality assurance. In addition, a standard calibration curve, analytical blanks, and spiked samples were run with each analysis.

Metal analysis was performed using an inductively coupled plasma mass spectrophotometer (Levelton Engineering, Richmond, BC, Canada) for feathers and fecal samples or an inductively coupled plasma atomic emission spectrophotometer (Cavendish Analytical Laboratories, Vancouver, BC, Canada) for invertebrates and fish. More than 25 different elements were obtained from these analyses, but we report only the data for Hg, Cd, Pb, Se, Mn, Cu, Zn, Al, and As, hereinafter referred to as metals. All metal concentrations are expressed in μg/g dry weight (ppm). Recoveries of reference materials were within 10% of the certified values or were recovery corrected if outside this range (invertebrates and fish from 2000 only).

Data analysis

Both the arithmetic (±standard error) and geometric mean concentrations of metals detected in the diet, feathers, and feces were calculated and reported to facilitate comparison with other studies. In addition, we report the proportion of samples detected for each metal as a measure of prevalence. In the case of prey Hg concentrations, where detection frequency was low, we used a value of one-half the detection limit (invertebrates = 0.005 μg/g and fish = 0.01 μg/g) to permit statistical analysis and to provide a conservative value to use in the exposure models. Metal concentrations generally exhibited a nonnormal distribution (Shapiro-Wilk *W* test) and, therefore, were log-transformed to improve normality before performing statistical comparisons. We used a two-way analysis of variance followed by a Tukey multiple comparison procedure to compare the metal concentrations among river invertebrates, creek invertebrates, and fish by year. A three-way analysis of variance (generalized linear model) was used to analyze feathers for effects of migratory status: River resident ($n = 42$) and creek migrant ($n = 40$), collection year (1999, 2000, 2001), sex (male or female), and interaction terms. Nonsignificant interaction terms were removed sequentially from the analysis and nondetectable samples were not used. Given the limited number of fecal samples ($n = 14$), the power for statistical comparisons was weak and, therefore, is only reported as means of all samples. Pearson product moment correlation coefficients (r) were used to test for correlations among metal concentrations in both feathers and feces. Statistical tests were performed using JMP IN® Version 4.0 (SAS Institute, Cary NC, USA) and the significance level was set at $\alpha = 0.05$.

Exposure models

A mass balance approach was used to calculate daily metal exposure to American dippers depending on migratory status (river resident or creek migrant) and the relative contributions of fish and invertebrates to the diet. Models incorporated geometric mean metal concentrations detected in invertebrates and fish collected from the main river and tributaries of the Chilliwack watershed in addition to estimated daily intake of each prey item using published annual average energy requirements for dippers [16]. Given the importance of body mass in comparing daily exposure among species [17], we further corrected the daily exposure for average dipper body mass (55 g). We assumed that the primary route of exposure would be through oral ingestion. Some metals may be taken up through the water directly by drinking, but this was not accounted for. Therefore, a conservative metal-exposure model for American dippers in the Chilliwack River watershed was calculated as follows:

$$E_{\text{metal}} = \frac{(W_{\text{f}} \times C_{\text{f}}) + (W_{\text{i}} \times C_{\text{i}})}{BW} \quad (1)$$

where E_{metal} = exposure to metal x (μg/g body wt/d), W_{f} = weight of fish eaten per day (g/d), C_{f} = geometric mean concentration of metal in fish (μg/g), W_{i} = weight of invertebrates eaten per day (g/d), C_{i} = geometric mean concentration of metal in invertebrates (μg/g), BW = body weight of dipper (mean = 55 g). Weight of fish (W_{f}) and invertebrates (W_{i}) consumed on a daily basis were calculated using the following equations:

$$W_{\text{f}} = [(P_{\text{f}} + DEE) \times AE] \times ED_{\text{f}} \quad (2)$$

$$W_{\text{i}} = [(P_{\text{i}} + DEE) \times AE] \times ED_{\text{i}} \quad (3)$$

where P = proportion of fish or invertebrates in the diet, DEE = average daily energy required by dippers (estimated ~48.04 kcal/d) [16], AE = assimilation efficiency correction factor for fish diet (85% or 1.15) or invertebrate diet (70% or 1.3), and ED = energy density of juvenile salmon (5.7 kcal/g dry wt) [18] or aquatic invertebrates (4.8 kcal/g dry wt) [19]. These estimates for daily food ingestion averaged 11.8 g/d dry weight, which closely matched the allometric equation of daily food ingestion rate for passerines (12.0 g/d dry wt) given by Nagy [20].

Each metal-exposure model was compared to a tolerable daily intake (TDI) calculated using the Canadian Tissue Residue Guidelines for the Protection of Wildlife Consumers of Aquatic Biota protocol [21]. The TDI is calculated from the results of avian chronic toxicity tests in which the substance was administered orally and sensitive endpoints were measured (Appendix 1). Tolerable daily intake is calculated using the geometric mean of the no-observable-adverse-effect level and the lowest-observable-adverse-effect level and dividing by an uncertainty factor (typically 10–100) to account for differences in sensitivity between species.

$$\text{TDI} = \frac{(\text{LOAEL} \times \text{NOAEL})^{0.5}}{\text{UF}} \quad (4)$$

Table 1. Summary of trace-metal concentrations and frequency of metal detection for aquatic invertebrates (n = 30, except Hg: n = 15) and salmon fry (fish; n = 9, except Hg: n = 17) from the Chilliwack River watershed (BC, Canada). Shown are arithmetic means (μg/g dry wt) ± standard error with geometric means in parentheses. Data for 2000 and 2001 are combined. Geometric means with the same capital letters are not significantly different using one-way analysis of variance and Tukey multiple comparison procedure (α = 0.05)

Metal	River invertebrates	Creek invertebrates	Fish	Significance (p)	Invertebrate % detected	Fish % detected
Hg	ND[ab] (0.005)A	0.018 ± 0.004[b] (0.011)A,B	0.035 ± 0.01[b] (0.022)B	0.002	20	47
Cd	4.58 ± 0.40 (4.31)A	3.66 ± 0.40 (3.35)A	1.37 ± 0.19 (1.27)B	<0.0001	100	100
Pb	0.67 ± 0.12 (0.58)A	0.55 ± 0.15 (0.41)A	0.42 ± 0.10 (0.33)A	NS[c]	70	100
Se	5.83 ± 0.74 (5.55)A	6.08 ± 0.79 (5.14)A	2.68 ± 0.27 (2.58)B	0.006	100	100
Cu	33.29 ± 1.96 (32.48)A	26.43 ± 2.10 (25.17)B	9.05 ± 1.23 (8.39)C	<0.0001	100	100
Mn	129.5 ± 29.7 (99.8)A	107.9 ± 17.4 (96.1)A	8.56 ± 2.13 (7.07)B	<0.0001	100	100
Zn	228.6 ± 17.1 (217.6)A	203.3 ± 18.9 (190.5)A	87.76 ± 7.8 (84.9)B	<0.0001	100	100
Al	1,296.4 ± 216.0 (1,040.3)A	1,586.0 ± 303.7 (1,275.8)A	165.5 ± 49.4 (119.9)B	<0.0001	100	100
As	3.73 ± 0.50 (3.09)A	3.77 ± 0.65 (3.01)A	0.63 ± 0.10 (0.56)B	<0.0001	100	100

[a] ND = no samples with detectable concentrations.
[b] For Hg, a value of half the detection limit was used to permit statistical analyses (detection limit = 0.01 μg/g for invertebrates and 0.02 μg/g for fish).
[c] NS = not significant ($p > 0.05$).

where TDI = tolerable daily intake, LOAEL = lowest-observed-adverse-effect level, NOAEL = no-observed-adverse-effect level, and UF = uncertainty factor. The no-observable-adverse-effect-level and lowest-observable-adverse-effect level for suitable avian toxicity tests were taken from the literature and summarized by Sample et al. [17]. Our TDI estimates use the most-conservative uncertainty factor of 10 for all metals. The TDI value is in units of μg/g body weight/d for direct comparison with the values in the exposure model for American dippers.

RESULTS

Metals in diet items: Invertebrates and fish

Invertebrate samples from the river and the tributaries generally did not differ significantly in metal concentrations (Table 1). Copper was the only metal found to be significantly higher in the river invertebrates relative to those collected from creeks ($t_{28} = -2.45$, $p = 0.02$), although Cd, Pb, Mn, and Zn also showed similar patterns to Cu. In all cases except for Hg and Pb, fish had lower concentrations of metals than both the river

Table 2. Summary of mean trace-metal concentrations (only in detectable samples) and frequency of metal detection in adult feathers of resident and migrant American dippers from the Chilliwack River watershed (BC, Canada), 1999 to 2001 (Hg: n = 104, other metals: n = 82). Shown are arithmetic means (μg/g dry wt) ± standard error with geometric means in parentheses

Metal	All birds	% Detected	River resident	Creek migrant	Significance (p)
Hg	0.69 ± 0.05 (0.56)	97	0.79 ± 0.06 (0.64)	0.58 ± 0.06 (0.50)	0.05
Cd	0.18 ± 0.03 (0.15)	49	0.25 ± 0.04 (0.19)	0.13 ± 0.03 (0.12)	0.01
Pb	0.97 ± 0.15 (0.58)	88	1.06 ± 0.23 (0.57)		
Se	6.03 ± 0.25 (5.68)	92			

dippers belong to the unique family *Cinclidae*, the world's only truly aquatic passerines, direct comparisons of toxicity tests from other passerine species may be inappropriate. For these reasons, we selected the approach of determining a tolerable daily intake value, which included a marginal uncertainty factor.

The only metals to which dippers on any diet clearly exceeded the TDI guidelines were Zn, Se, and Al. Those elements are either homeostatically controlled or are essential elements where the range of essentiality and toxicity is not well understood. Evidence of Zn toxicity to wild birds is limited primarily because Zn is regulated internally even when birds are exposed to high levels of contamination [12,34]. However, mortality and reproductive effects from Se (particularly in the form selenomethionine) have been documented, especially for aquatic birds in areas receiving agricultural drainage [35,36]. Food-chain organisms, such as benthic invertebrates and fish, can accumulate high concentrations of Se without toxicity to the host; however, a dietary toxicity threshold for fish and wildlife is recommended at 3 μg/g dry weight [37]. Although we do not have any information about the concentrations of the more toxic organic form of Se (selenomethionine) in dipper prey, all the invertebrate samples and many fish samples collected from the Chilliwack watershed exceeded the 3-μg/g guideline. Harding and Paton [38] recorded no reproductive impairment with invertebrate Se concentrations of 4.2 μg/g wet weight at a coal mine site and feather Se concentrations almost identical to our study at exposed (pooled sample: 6.5 μg/g dry wt) and reference streams (pooled sample: 6.3 μg/g dry wt). In our study, feather concentrations were not different among migratory groups, but daily Se exposure was six times higher than the TDI levels for birds on exclusively invertebrate diets, indicating migrant dippers may be at a higher risk to potential toxic effects from selenium.

Aluminum also has been reported to influence reproduction of insectivorous passerines breeding in acid-sensitive environments, particularly if Ca and P are limiting [39–41]. Several orders of aquatic invertebrates, including chironomids, caddisflies, stoneflies, and mayflies, have exhibited high Al concentrations of 0.1 to 0.3% body weight (dry wt) [42]. Invertebrates sampled in 2001 from the Chilliwack watershed had elevated Al levels in the range of 0.05 to 0.43% (mean = 0.12% dry wt). Aluminum is of particular concern in acid-sensitive regions, especially in ecosystems with exposed granite or other calcium-poor substrates, which are most severely affected by acidification [43]. The Chilliwack watershed, in addition to many similar river basins in the region, largely is composed of granite bedrock, making this system vulnerable to solubilization of metals that more readily are bioavailable to aquatic biota. Swain [44] listed the Chilliwack Lake, a source at the headwaters of the Chilliwack River, as one of 20% of Briti(abouiguide6d-senludingspecasenludl]TJssenlud)-3-lud t acid-

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APPENDIX

Summary of toxicity data used to calculate tolerable daily intake (TDI) for American dippers. Selected toxicity tests on suitable avian species to ob