Limnol. Oceanogr., 50(5), 2005, 1511–1519

Trophic transfer of metals along freshwater food webs: Evidence of cadmium biomagnification in nature

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Abstract

We conducted a study with cadmium (Cd) and copper (Cu) in the delta of San Francisco Bay, using nitrogen and carbon stable isotopes to identify trophic position and food web structure. Cadmium is progressively enriched among trophic levels in discrete epiphyte-based food webs composed of macrophyte-dwelling invertebrates (the first link being epiphytic algae) and fishes (the first link being gobies). Cadmium concentrations were biomagnified 15 times within the scope of two trophic links in both food webs. Trophic enrichment in invertebrates was twice that of fishes. No tendency toward trophic-level enrichment was observed for Cu, regardless of whether organisms were sorted by food web or treated on a taxonomic basis within discrete food webs. The greatest toxic effects of Cd are likely to occur with increasing trophic positions, where animals are ingesting Cd-rich prey (or food). In Franks Tract this occurs within discrete food chains composed of macrophyte-dwelling invertebrates or fishes inhabiting submerged aquatic vegetation. Unraveling ecosystem complexity is necessary before species most exposed and at risk can be identified.

Ecosystems are threatened by a steadily increasing number of pollutants that cause adverse effects. Yet the link between metal exposure and effects in aquatic organisms remains poorly known, likely because biological responses differ among species, metals, physicochemical conditions, and exposure routes. For example, marine crustaceans appear more sensitive to metals accumulated from food rather than from their aqueous environment (Hook and Fisher 2001*a*,*b*). This implies that ingested metals are likely to cause toxicity not only at the base of food webs but also in top consumers if the assimilated metals build up through the food web (e.g., Cabana and Rasmussen 1994). However, controversy surrounds the question of metal biomagnification, defined here as the progressive accumulation of chemicals with increasing trophic levels (Leblanc 1995). For instance, Gray (2002) concluded that metal biomagnification is an exception rather than a rule among metals and metalloids. Reinfelder et al. (1998) suggested that trophic transfer potential (TTP) could be described from the biodynamic parameters weight-specific ingestion rate, assimilation efficiency (AE) and rate constant of loss. Among metals, organic mercury is the most likely to biomagnify because organisms efficiently assimilate methylmercury and very slowly eliminate it in proportion to biomass (Mason et al. 1996; Reinfelder et al. 1998). There are theoretical reasons to suspect that selenium (Se), Cd, and perhaps even silver could biomagnify under some circumstances, but only Se has been carefully evaluated in the field (Stewart et al. 2004). Unambiguous evaluations of metal biomagnification in nature are rare because metal concentrations in whole-body prey are often compared with those in predator's specific tissues without knowledge of bioaccumulation processes, feeding relationships, and trophic status (Reinfelder et al. 1998; Gray 2002).

Here we study two metals that can be both toxic (i.e., they commonly appear on government priority-substances lists, e.g., the U.S. Environmental Protection Agency), but that contrast in their biological functions and perhaps their potential for biomagnification. Copper (Cu) can act as essential micronutrient (e.g., Sunda and Huntsman 1995). However, little is known about its trophic transfer potential mainly because the lack of a suitable radioisotope prevented quantification of AE and loss-rate constants (until recently; Croteau et al. 2004). In contrast, Cd has no known biological use in animals (although it may substitute for zinc in certain enzymes in phytoplankton; Lane and Morel 2000). Cadmium might biomagnify if consumers efficiently assimilate and slowly lose it (Reinfelder et al. 1998; Wang 2002); however, this has not been demonstrated directly in the laboratory or in nature. First, we address the question as to whether Cd and Cu concentrations differ, and to what degree, among species collected from the same habitat and at the same time. We then ask whether feeding relationships could be used to explain at least some of those differences; if different types of food webs transfer metals differently; and lastly, whether Cd and Cu differ in food web transfer.

The study of trophic transfer is also limited by the difficulty of discriminating food webs and accurately ascribing trophic position to organisms. Stable isotope ratios of carbon ($^{13}C:^{12}C$; $\delta^{13}C$) and nitrogen ($^{15}N:^{14}N$; $\delta^{15}N$) are now recognized as powerful tools to provide time-integrated evalu-

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Acknowledgments

The technical assistance of F. Parchaso, G. E. Moon, M. K. Shouse, and K. Higgins with sample collections is acknowledged. We thank L. Grimaldo and E. Santos (DWR) for their assistance with fish sampling. Guidance with ICP-MS analysis by B. R. Topping is recognized. We thank C. B. Lopez (USGS) and L. Hare (INRS-ETE) for their assistance in identifying algae and chironomids, respectively. Critical comments from B. R. Topping and J. K. Thompson are appreciated. Funding was provided by the U.S. Geological Survey Toxic Substances Research Program; with some support to S. N. Luoma from a WJ Fulbright Distinguished Scholar Award. M.-N. Croteau was supported by postdoctoral fellowships from Fonds de Recherche sur la Nature et les Technologies (Québec) and NSERC Canada.

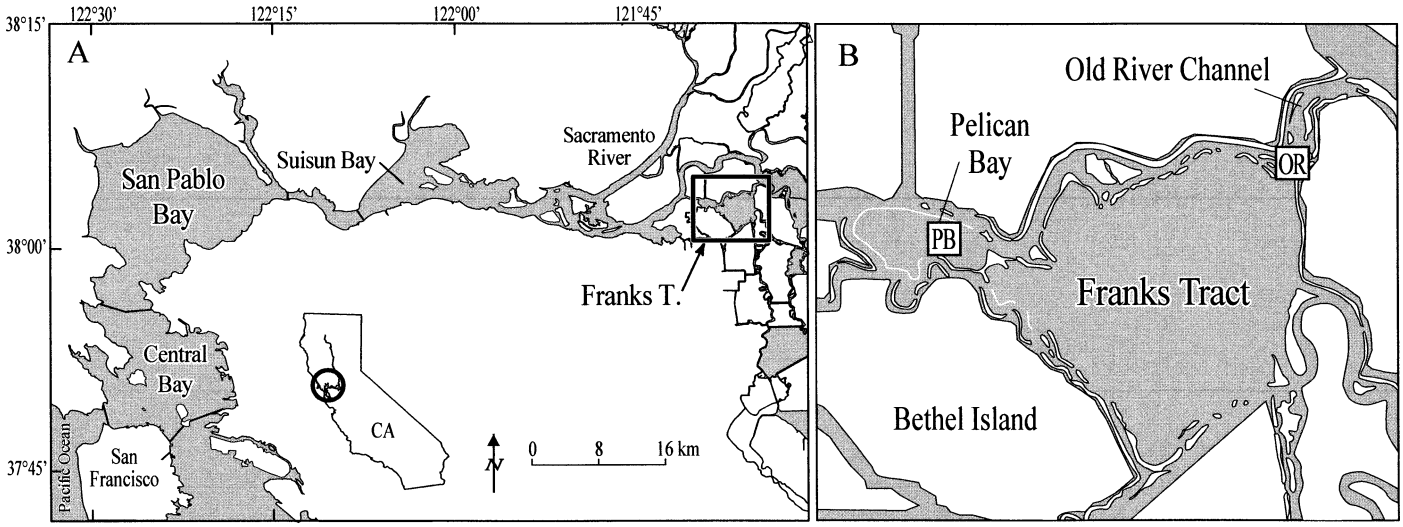

Fig. 1. (A) Map of northern San Francisco Bay and Sacramento–San Joaquin River delta, California, USA. (B) Enlarged map of Franks Tract showing our two sampling stations, OR and PB.

ations of energy flow and food web structures in ecological communities (Peterson and Fry 1987). As a result of differential fractionation during food assimilation, $\delta^{13}C$ can be used to identify food sources (if food items have distinct isotopic signatures; France 1995), whereas $\delta^{15}N$ can be used for inferring the relative trophic position of an individual within a food web (Minagawa and Wada 1984). Comparing data on pollutant concentrations with trophic levels inferred from stable isotope methodologies can enhance our understanding of trophic and contaminant interrelationships in aquatic biota. For example, $\delta^{15}N$ has been used to successfully predict mercury levels in lake trout (Cabana and Rasmussen 1994). Stewart et al. (2004) used $\delta^{13}C$ and $\delta^{15}N$ to show that estuarine food webs in San Francisco Bay biomagnify Se. But due to differences in biodynamics of Se at the base of food webs, predatory fishes belonging to food webs for which the first link is bivalves accumulate much more Se than those from food webs for which the first link is zooplankton. Here we use $\delta^{13}C$ and $\delta^{15}N$ to separate freshwater food webs and describe trophic position in food webs of the San Francisco Bay delta and thereby evaluate trophic enrichment of Cd and Cu.

Methods

Collection of field samples—Organisms were collected in fall 2002 from a flooded farm tract in the Sacramento–San Joaquin River system (Fig. 1A). Franks Tract (hereafter referred to as FT) is a shallow (mean depth of ~3 m), eutrophic (chlorophyll *a* from 0.4 to 6 $\mu g\ L^{-1}$), open freshwater habitat (surface area of 12.9 km^2) influenced by tides and bounded by levees that allow connections with surrounding river channels (Lucas et al. 2002). We constrained our sampling to a season and a specific geographical area (i.e., at the confluence of Old River [OR] channel; OR in Fig. 1B) to minimize the confounding influences of time and space.

Submerged aquatic vegetation (SAV) (mainly the nonindigenous water hyacinths *Eichhornia crassipes* and macrophyte *Egeria densa*) dominates the littoral areas in FT and at OR, forming dense mats of vegetation. The littoral site was contrasted to an open-water site located 200 m offshore of the OR littoral zone (i.e., at FT's deepest zone: $Z_{max} \sim 8$ m). No macrophytes were present at the open-water site, presumably because of its depth. Invertebrates were collected on two sample days (7 October and 8 November 2002) from the water column and sediment compartments at both littoral and open-water sites. Fishes were mainly collected from the OR littoral site (25 October 2002), although we also sampled them from an additional station in Pelican Bay (PB in Fig. 1B) to ensure sufficient numbers within some species and to expand the number of species collected. At the littoral station, water-column invertebrates were handpicked from the leaf surfaces of macrophytes (mainly *E. densa*). Those included amphipods (*Hyalella azteca, Gammarus daiberi,* and *Americorophium* spp.), gastropods (*Physa gyrina* and *Gyraulus* sp.), insect larvae (Zygoptera, Orthocladiinae, and Chironomidae midges), flatworms (*Dugesia tigrina*), and water mites. Bottom-dwelling invertebrates (mainly the oligochaete *Sparganophilus eiseni* and the leeches *Mooreobdella microstoma* and *Helobdella stagnalis*) were collected using an Ekman grab and isolated from sediment by sieving through a 0.5-mm–mesh net. Fish were sampled using a seine net drawn throughout SAV areas. Species captured were redear sunfish (*Lepomis microlophus*), bluegill sunfish (*Lepomis macrochirus*), black crappie (*Pomoxis nigromaculatus*), largemouth bass (*Micropterus salmoides*), inland silverside (*Menidia beryllina*), rainwater killifish (*Lucania parva*), and shimofori goby (*Tridentiger bifasciatus*). At the open-water station, we collected benthic invertebrates (i.e., the clam *Corbicula fluminea,* the polychaete worm *Neanthes limnicola,* and chironomid larvae) by Ekman grab. Zooplankton were sampled by hauling a 75-μm plankton net horizontally through the water column. The net's path ensured that plankton were collected from all depths. Organisms as well as specimens of *E. densa* collected from littoral (OR) were held in plastic bags (filled

with FT water for the invertebrate samples) and transported to the laboratory in coolers.

Laboratory methods—To minimize inadvertent metal contamination, labware, vials, and Teflon sheeting used for metal analysis were soaked for 24 h in 15% nitric acid, rinsed several times in ultrapure water (Milli-Q system water, >18 Mohm cm^{-1}), and allowed to dry under a laminar-flow hood prior to use.

Under a microscope, *E. densa*'s surfaces were scraped to collect epiphytic biofilm, which included attached-algae (i.e., pennate diatoms), protozoans, bacteria, fish eggs, and detritus. Invertebrates were sorted according to taxon and placed either individually (soft tissue for mollusks) or pooled (Table 1) on a piece of acid-washed Teflon sheeting that was frozen until analysis. When numbers permitted, 3–15 replicates of pooled samples or 2–15 replicates of single individuals were prepared for each invertebrate taxon (Table 1). Thawed fishes were identified, measured, weighed, and dissected to remove skinless muscle tissue and liver. Fish livers were individually placed on a piece of acid-washed Teflon sheeting and frozen. Muscle tissue from each fish was placed in 2.5-ml polystyrene vials and frozen. For fish species that were abundant (i.e., redear sunfish and inland silverside), size classes were defined. One to 23 replicate samples were prepared for each fish species and size class (Table 2).

Analysis—Previously frozen invertebrates and fish tissues were freeze-dried (Virtis 12ES). Large-sized invertebrates (i.e., clams and oligochaetes) and invertebrate taxa that were less abundant (i.e., midge and leech) were transferred to acid-washed 2.5-ml polystyrene vials, ground to a fine powder using a mixer mill (5100 SPEX CertiPrep), and subsampled (0.2–8.0 mg dry weight [d.w.]). Samples for metal analysis were weighed (Sartorius M2P electronic microbalance) and digested at room temperature in Teflon vials with concentrated nitric acid (Baker Ultrex II grade, 100 μl mg^{-1} d.w. sample) for 7 d (Croteau et al. 2001). Hydrogen peroxide (Baker Ultrex II grade, 40 μl mg^{-1} d.w. sample) was added prior to final dilution with ultrapure water (760 μl mg^{-1} d.w. sample). Samples of similar weight from the certified reference material TORT-2 (lobster hepatopancreas from National Research Council of Canada [NRCC]) were submitted to the same digestion procedures during each analytical run. Metal concentrations measured in TORT-2 were within the certified range.

Additional freeze-dried samples collected for stable isotope analysis were ground to a fine powder using a mixer mill. These samples as well as those of the previously ground large-size and rare taxa were subsampled (1–2 mg d.w.), packed in 4 × 6-mm tin capsules, and analyzed for ^{13}C, ^{12}C, ^{15}N, and ^{14}N by continuous-flow isotope-ratio mass spectrometer (IRMS Europa Hydra 20/20) at the Stable Isotope Facility of University of California, Davis. All samples were standardized against atmospheric nitrogen or CO_2 in PeeDee limestone as follows:

$$\delta^{13}C \text{ or } \delta^{15}N\ (‰) = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (1)$$

where R is $^{15}N:^{14}N$ or $^{13}C:^{12}C$.

Metal concentrations in organisms were analyzed by either graphite furnace atomic-absorption spectrophotometry or by inductively coupled plasma–mass spectroscopy (ICP-MS). Two or three replicates were measured for each sample. A replicate consisted of 32 individual measurements that were averaged. Certified reference riverine water samples (NRCC; SLRS-4) were analyzed for Cd and Cu during each analytical run, and measured metal concentrations were within the certified range. To check for the instrument drift and change in sensitivity, we reanalyzed one of our standards after every five samples.

Statistical analysis—Comparisons of metal concentrations and stable isotope ratios among invertebrates, fish species, and between locations were made by *t*-tests. We used linear regressions to relate metal concentrations to sizes (or weights) as well as to trophic positions.

Results

Differences in metal concentrations among invertebrates and fishes—Metal concentrations varied greatly among invertebrate taxa, i.e., 175- and 44-fold metal concentration differences were found for Cd and Cu, respectively (Table 1). Leeches were the least contaminated organisms (i.e., Cd and Cu concentrations of 0.02 μg g^{-1} and 5 μg g^{-1}, respectively). In contrast, Cd concentrations reached 3.4 μg g^{-1} in the clam *Corbicula fluminea* (hereafter referred as *Corbicula*), whereas Cu levels as high as 205 μg g^{-1} and 159 μg g^{-1} were found in gastropods (*P. gyrina*) and bivalves (*Corbicula*), respectively. Taxonomically similar species differed markedly in metal concentrations. For instance, Cu concentrations in the snail *P. gyrina* (Physidae) were 5–9 times higher than those found in another snail, *Gyraulus* sp. (Planorbidae). Cadmium concentrations ranged from as low as 0.2 μg g^{-1} in *Chironomus* larvae collected from offshore sediment to as high as 1.8 μg g^{-1} in orthocladiinae midges sampled within the SAV's water column (Table 1). For most invertebrate taxa, metal concentrations were similar between sample days ($p > 0.05$; Table 1), although not all taxa were present at both sampling times.

Metal concentrations in fish livers were generally lower than in invertebrates and were highly variable among species (i.e., a 30-fold concentration gradient was found for both metals; Table 2). The benthivorous feeder shimofori goby was the least contaminated species, having liver Cd and Cu concentrations of 0.02 μg g^{-1} and 3 μg g^{-1}, respectively. In contrast, liver Cd concentrations in largemouth bass reached 0.32 μg g^{-1}, whereas livers from rainwater killifish collected at PB showed Cu levels as high as 98 μg g^{-1} (Table 2). Metal concentrations were not different between fish collected at PB and OR ($p > 0.05$), except in rainwater killifish (i.e., Cd and Cu concentrations of 0.12 μg g^{-1} and 98 μg g^{-1} compared to 0.05 μg g^{-1} and 35 μg g^{-1} were found in killifish collected at PB and OR, respectively). For the subsequent food web analysis we used metal and isotope data from both sites for sunfishes (Centrarchidae) and gobies (Gobiidae), but we only used data from OR for rainwater killifish.

Allometric parameters (e.g., reproductive cycle, growth) did not influence metal concentrations for most invertebrate

Table 1. Stable isotope ratios (‰) and metal concentrations (μg g^{-1}) in organisms collected in FT in fall 2002. Also given are number of individual composite for a sample: S for single; P for pooled individuals (number used in parentheses); and whether metal analysis was performed on a subsample of homogenized tissues (H). Organisms are sorted according to the habitat where collected; nd, not determined.

Habitat	Organism	Sampling month	Stable isotope ratios ± 95% C.I.			Metal concentration ± 95% C.I.			Type of sample
			δ^{13}C	δ^{15}N	*n*	Cadmium	Copper	*n*	
Littoral, water column	Insecta								
	Zygoptera	Oct	−20.35	15.54	1	0.48±0.13	13±1.7	7	S
		Nov	nd	nd	nd	0.35±0.28	12±4.4	3	S
	Midge Chironominae, Orthocladiinae	Oct	nd	nd	nd	1.8±0.30	20±2.6	3	P (7–30)
	Amphipoda*								
	Hyalella azteca	Oct	−20.37±0.01	14.67±0.07	2	0.43±0.05	66±4.8	11	P (3)
		Nov	−18.92±0.49	15.09±0.23	4	0.37±0.05	77±5.5	15	S
	Gammarus daiberi	Oct	−21.37±1.09	14.19±0.70	2	0.59±0.19	71±9.8	9	S
		Nov	−20.79±0.44	15.13±0.34	15	0.54±0.11	71±4.5	15	S
	Americorophium spp.	Oct	nd	nd		0.51±0.11	63±9.6	6	S
		Nov	−28.44±0.25	11.15±0.09	4	0.43±0.05	72±8.9	15	P (2)
	Chelicerata	Oct	nd	nd		2.2±0.48	28±3.4	3	P (3–5)
	Gastropoda*								
	Physa gyrina	Oct	nd	nd		0.50±0.15	145±52	3	S
		Nov	−18.61±1.77	15.33±0.29	5	0.45±0.20	205±54	15	S
	Gyraulus sp.	Oct	−14.75	14.1	1	0.62±0.57	31±9.5	2	S
		Nov	nd	nd	nd	0.40±0.11	24±3.5	10	S
	Flatworm (*Dugesia tigrina*)	Oct	−20.50±1.26	15.78±1.18	3	1.0±0.16	10±1.8	10	P (2–3)
		Nov	−21.26±0.47	17.49±0.24	4	1.5±0.14	31±11	16	S
	Epiphytic algae	Nov	−23.76±1.20	11.07±0.89	5	0.07±0.01	12±1.0	15	P
Littoral, sediment	Annelida*								
	leeche (*Mooreobdella microstoma* and *Helobdella stagnalis*)	Oct	−24.38	15.40	1 (H)	0.02	4.7	1	S
		Nov	−24.17±2.91	15.88±0.94	4 (H)	0.03±0.02	8.6±4.6	4	S
	Oligochaete (*Sparganophilus eiseni*)	Nov	−26.69±0.42	13.77±0.27	12 (H)	0.09±0.03	11±2.7	15	S
	Oligochaete (Tubificidae)	Nov	nd	nd		1.6±0.76	25±9.1	2	S
Open-water, water column	Copepoda	Oct	−31.79±0.35	11.87±0.59	4	0.38±0.02	21±1.3	11	P (125–150)
		Nov	−32.69±0.20	11.92±0.04	3	0.26±0.03	18±2.9	5	P (125–150)
Open-water, sediment	Insecta								
	Midge Chironomidae, *Chironomus* sp.	Oct	−38.53	6.71	1 (H)	0.16±0.03	17±3.9	2	S
	Bivalva								
	Corbicula fluminea	Oct	−30.12±0.37	10.35±0.26	6 (H)	3.4±0.60	159±29	15	S
		Nov	−30.33±0.07	10.35±0.07	13 (H)	2.5±0.24	70±20	15	S
	Annelida*								
	Polychaete (*Neanthes limnicola*)	Oct	−31.44±2.99	12.27±0.50	2 (H)	0.26±0.01	22±15	2	S
		Nov	−30.49	12.70	1 (H)	0.18	16	1	S

* Species determined according to the Department of Water Resources benthic data (http://www.iep.water.ca.gov/emp/Data_access.html) and advice of H. A. Peterson (U.S. Geological Survey).

Table 2. Stable isotope ratios (‰), liver metal concentrations (μg g^{-1}), average length (±95% C.I.), and some feeding characteristics of fishes collected in FT in fall 2002 at two different littoral sites.

Species	Feeding characteristics*	Sampling site†	Length (mm)	Stable isotope ratios ± 95% C.I. δ^{13}C	Stable isotope ratios ± 95% C.I. δ^{15}N	Metal concentration ± 95% C.I. Cadmium	Metal concentration ± 95% C.I. Copper	*n*
Sunfishes (Centrarchidae)	Opportunistic feeders of							
Redear sunfish	invertebrates and fishes;	PB	38±7	−19.94±0.78	16.42±0.20	0.12±0.01	8.5±1.3	38
	forage in littoral SAV	OR	57±34	−20.09±2.72	16.08±0.74	0.14±0.05	7.1±0.6	6
Bluegill sunfish	areas	PB	46±52	−21.67±1.51	17.34±0.23	0.21±0.14	7.3±1.2	5
		OR	133±5	−22.30±1.53	17.89±1.35	0.28±0.13	7.4±3.7	2
Black crappie		PB	117	−20.36	16.08	0.12	26	1
Largemouth bass		PB	59±18	−17.62±1.43	17.30±0.58	0.32±0.10	25±11	6
		OR	66±12	−15.75	17.06	0.18	8.5	1
Silversides (Atherinopsidae)	Shoal feeders of							
Inland silverside	zooplankton and planktonic insect larvae	OR	47±3	−25.67±0.55	16.96±0.14	0.09±0.01	8.8±0.5	73
Killifishes (Fundulidae)	Opportunistic feeders of							
Rainwater killifish	most abundant inverte-	PB	21±2	−19.25±1.15	16.49±0.29	0.12±0.03	98±30	8
	brates in their habitat (e.g.,	OR	21±1	−19.66±1.07	15.87±0.37	0.05±0.01	35±4	15
	water surface's biofilm‡)							
Gobies (Gobiidae)	Benthic feeders on							
Shimofori goby	substrate (bottom-living	PB	40±21	−22.70±2.37	14.78±0.48	0.02±0.02	3.3±1.1	2
	invertebrates and detritus)	OR	39	−21.82	−14.43	0.01	2.6	1

* From Moyle (2002).
† OR, Old River; PB, Pelican Bay.
‡ L. Grimaldo (pers. comm.).

and fish taxa (assuming uniformity in metal exposure). Slopes of regression between metal concentrations and weight (for invertebrates) or size (for fish) were not significantly different from 0, except for physid snails ($p < 0.001$; Fig. 2A,B) and clams ($p < 0.001$ for Cu and $p = 0.049$ for Cd; Fig. 2C,D). A somewhat significant ($p = 0.02$) but poorly predictive ($r^2 = 0.07$) relationship was also found between Cu concentrations and size (or size classes) in inland silverside (data not shown).

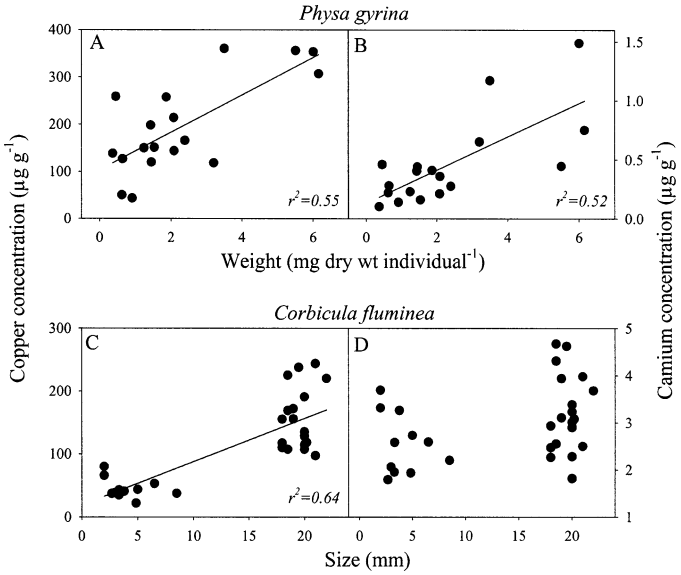

Fig. 2. Influence of weight (A,B) and size (C,D) on metal concentrations in the snail *Physa gyrina* and the clam *Corbicula fluminea.* Each point corresponds to an individual.

Habitat-specific food webs—Stable carbon isotope ratios in primary producers differed markedly between habitats. Epiphytes harvested from littoral macrophytes were enriched in ^{13}C (as depicted by a less negative δ^{13}C value, −23.8‰; Table 1) compared to phytoplankton collected offshore (−28.6‰; Cloern et al. 2002). Conditions of low turbulence experienced by epiphytes within the SAV likely favored high diffusive boundary layer resistance that restrained isotopic discrimination during carbon fixation (Hecky and Hesslein 1995).

Values of δ^{13}C were used to ordinate algae and invertebrates along a littoral to open-water gradient. We ascribed primary producers and most consumers to either an epiphyte-based food web (in the littoral zone) or a phytoplankton-based food web (in the open water). The food web characterizations for each species were consistent with knowledge of their feeding habits as well as from where they were collected. These factors were considered in assigning organisms to the food web ellipses shown in Fig. 3. All littoral invertebrates (except the filter-feeding amphipods *Americorophium* spp.) were significantly enriched in ^{13}C compared to those from open-water ($p < 0.001$). For instance, δ^{13}C values averaged (±95% confidence interval [C.I.]) −21.9 ± 0.9‰ and −30.8 ± 0.6‰ for the former and latter invertebrates, respectively. The isotopic correspondence of primary producers and consumer's δ^{13}C values between habitats is consistent with the concept that δ^{13}C differentiates food webs (signatures are conserved through the food web) but is not enriched with trophic transfer (i.e., 1‰ or less; Peterson and Fry 1987).

Fishes show nearly the same range of δ^{13}C values as those found in littoral invertebrates (Fig. 3), suggesting that most

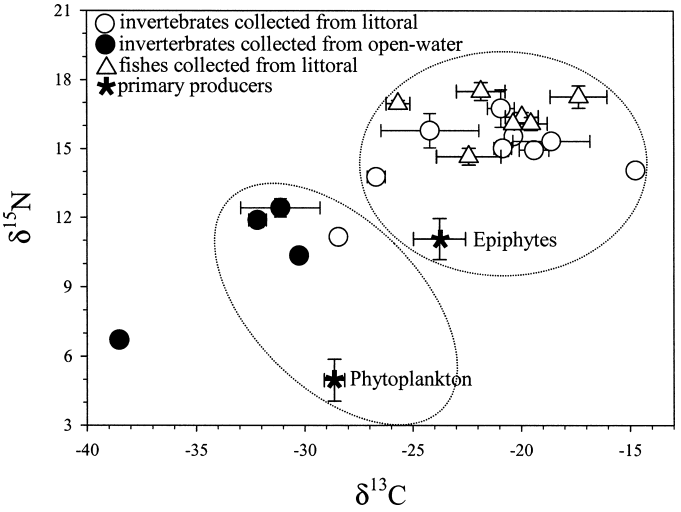

Fig. 3. Relationship between mean (± 95% C.I.) δ^{15}N and δ^{13}C values for invertebrates and fishes collected in fall 2002 in FT. Each point corresponds δ^{15}N and δ^{13}C values averaged either for both sampling months (for invertebrates when data for both months were available) or for both sampling sites (for sunfishes, largemouth bass, and gobies). Only data for rainwater killifishes collected at OR were used (see text). Open circles and triangles represent invertebrates and fishes collected from the littoral site; filled circles represent invertebrates collected from the open-water site. Stars represent primary consumers, namely phytoplankton (data from Cloern et al. 2002) and epiphytic algae. Refer to Tables 1–2 for species names. Ellipses enclosed organisms assumed to belong to a similar food web (i.e., either phytoplankton- or epiphyte-based food web) from knowledge of their feeding habits as well as from their habitat of collection.

species fed on prey items derived from the SAV. Like metal concentrations, fish δ^{13}C signatures were similar between sampling sites ($p < 0.001$). Thus, we ascribed all fish species to the epiphyte-based food web, which indeed appears consistent with their dietary habits (Table 2). For example, δ^{13}C values found for sunfishes (ranged from −18.0‰ to −21.9‰; Table 2) were similar to those of their potential prey (i.e., snails, amphipods, insect larvae, and most fish species; Fig. 3). Inland silversides, in contrast, forage in both open-water and littoral habitats, and they had a depleted δ^{13}C signature that integrated the isotopic δ^{13}C range of their heavier (i.e., amphipods) and lighter (i.e., zooplankton) food items (Table 1; Fig. 3).

Stable carbon isotope ratios also were different depending upon whether invertebrates were collected from the sediment or the water-column. Within littoral habitats, δ^{13}C values for sediment-dwelling taxa were more negative (e.g., −26.7‰ and −24.2‰ for oligochaetes and leeches, respectively) than those for most other water-column invertebrates (δ^{13}C ranged from −16.0‰ to −20.9‰, $p < 0.001$; Table 1), suggesting that the invertebrates collected from these two compartments were assimilating different sources of carbon. Benthic invertebrates that feed deeply in sediment were also more depleted in ^{13}C than those that feed at and above the sediment–water interface (e.g., *Chironomus* larvae versus the polychaete *Neanthes limnicola*). Benthic organisms and especially those from profundal habitats are presumably fixing respired CO_2 (which is depleted in ^{13}C; Rau 1980). The *Chironomus* δ^{13}C value stands out among the phytoplankton-based food web invertebrates, showing the most negative δ^{13}C value (−38.5‰; Table 1). This suggests that *Chironomus* likely belongs to a profundal (rather than an open-water) food web.

Food web structure within habitats—Stable nitrogen isotope ratios in primary producers differed markedly between habitats—i.e., epiphytes had higher δ^{15}N value than phytoplankton (Fig. 3)—despite the fact that isotopic signatures of inorganic nitrogen were similar between habitats (SAV δ^{15}N = 7.38‰ and open-water δ^{15}N = 7.26‰; A. R. Stewart, unpubl. data) This suggests that trophic linkages influenced the δ^{15}N values found for epiphytes. That is, while scraping macrophytes surfaces to harvest epiphytes, we likely collected a mix of materials (i.e., algae, bacteria, fungi, protozoa, and small metazoans; Goldman and Horne 1983) that led to a higher δ^{15}N signature. If consumers feed on the full mix of species found in the epiphyte community, then we might expect their δ^{15}N values to be higher than if algae were solely consumed (Fig. 3). This example highlights the difficulties in comparing trophic positions among environments when δ^{15}N values characterizing organisms at the base of the food web are determined using particulate organic material where algae cannot be effectively separated from the sample.

Trophic fractionation of δ^{15}N between primary producers and herbivorous consumers (i.e., most invertebrate taxa) averaged 5.3‰ and 4.1‰ in the phytoplankton- and epiphyte-based food webs, respectively (Table 1; Fig. 3). Assuming that 2.5–5‰ enriches a consumer's δ^{15}N value over that of its diet (Peterson and Fry 1987), this suggests that all invertebrates shared a similar trophic position, likely forming a single prey–predator link. However, the range of δ^{15}N signatures shown by the invertebrates in the epiphyte-based food was almost twice that found for those in the phytoplankton-based food web (i.e., 4.1 vs. 2.1; Tables 1–2). This might imply that the invertebrates from the epiphyte-based food web more likely encompassed two trophic links. For example, flatworms, which had the most enriched δ^{15}N values (16.8‰), are known to feed on invertebrates such as amphipods, snails, and insect larvae (δ^{15}N ~ 14.8‰), which likely graze and/or browse on epiphytes (δ^{15}N = 11.7‰; Table 1; Smith 2001). Presumed feeding habitats and the isotope values therefore agreed. The highest δ^{15}N values occurred in largemouth bass and bluegill sunfish, consistent with known trophic linkages with invertebrates (e.g., amphipods, snails, and insect larvae) or gobies as intermediate prey. But a single prey–predator link characterized most fish species (Tables 1–2; Fig. 3).

Metal concentrations along food webs—There were no significant relationships between Cd concentrations and stable nitrogen isotope ratios among all data ($p > 0.1$; Tables 1–2). However, significant trends emerged if discrete epiphyte-based food webs were further characterized. Cadmium concentrations progressively increased among trophic levels (as inferred by δ^{15}N values) within epiphyte-based food webs composed of either macrophyte-dwelling invertebrates (for

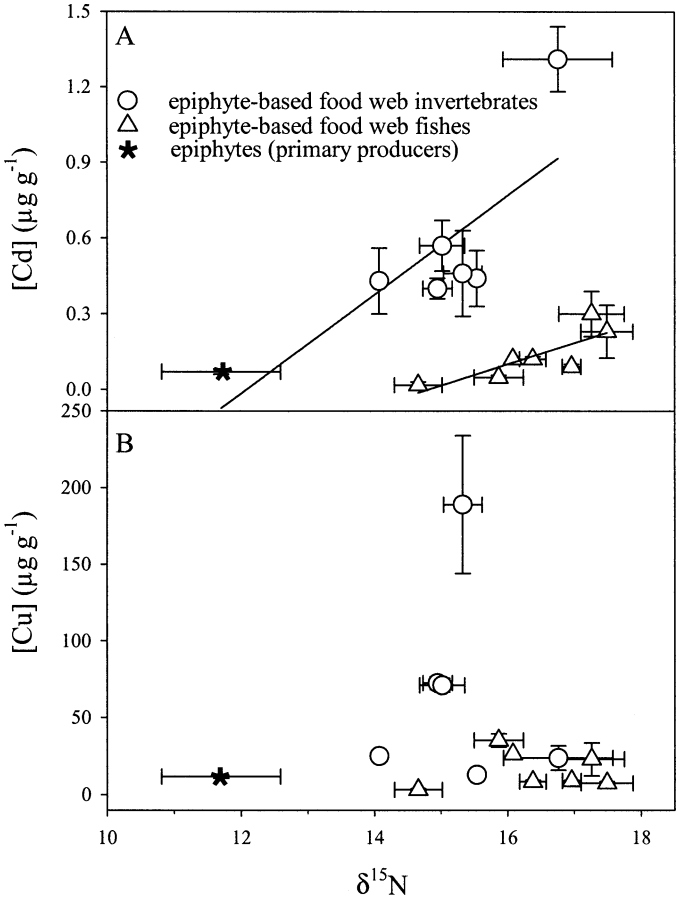

Fig. 4. Metal concentrations (± 95% C.I.) in invertebrates and fish collected from littoral as a function of δ^{15}N values. Each point corresponds metal concentrations and stable isotope ratios averaged either for both sampling months (for most invertebrates when data for both months were available) or for both sampling sites (for sunfishes, largemouth bass, and gobies). Only data for rainwater killifishes collected at OR were used (see text). (A) Cd; (B) Cu. Open circles represent the epiphyte-based food web invertebrates and triangles represent the epiphyte-based food web fishes. Star represents epiphytic algae (included in the regression). Refer to Tables 1–2 for species names.

which the first link is epiphytic algae; $r^2 = 0.66$, $p = 0.026$) or fishes (for which the first link is goby; $r^2 = 0.67$, $p = 0.025$; Fig. 4A). For example, Cd concentrations ranged from 0.07 μg g^{-1} in epiphytes to up to 1.3 μg g^{-1} in flatworms in the invertebrate food web. In the fish food web Cd concentrations ranged from 0.02 μg g^{-1} in shimofori goby to 0.32 μg g^{-1} in largemouth bass. Concentrations of Cd were biomagnified about 16 times within the scope of two trophic links in both food webs. However, Cd trophic enrichment (determined using slope of the regressions shown in Fig. 4A) was 2.3 times higher for invertebrates than for fishes (i.e., slope ± standard error of 0.196 ± 0.063 and 0.084 ± 0.0027, respectively). Cadmium concentrations significantly decreased from fish (e.g., largemouth bass) to their potential invertebrate prey (e.g., amphipods, snails, and insect larvae), that is, those averaged 0.46 μg g^{-1} for the above prey items compared to 0.28 μg g^{-1} in largemouth bass (Fig. 4, Tables 1–2).

Copper concentrations were not significantly enriched along trophic levels ($p > 0.1$), regardless of whether organisms were sorted by food web type or treated on a taxa basis within discrete food webs (e.g., Fig. 4). Concentrations of Cu in invertebrates belonging to the phytoplankton-based food web were negatively related to δ^{15}N values ($p = 0.048$). However, amphipods, polychaetes, and zooplankton are not *Corbicula*'s prey items, so this is probably a spurious relationship. In addition, all these taxa likely shared the same trophic position (Table 1).

Discussion

Differences in metal concentrations among invertebrates and fish—Metal concentrations in invertebrates and fish varied widely among taxa and even within closely related taxa (Tables 1–2). Possible explanations for intertaxon variability in metal concentrations include species-specific differences in bioaccumulation dynamics as well as differences in metal exposures, such as those being induced by habitat characteristics (e.g., temperature, metal speciation, and partitioning) or dictated by dietary preferences, foraging behaviors, food web structure, and trophic position (Reinfelder et al. 1998; Croteau et al. 2002; Stewart et al. 2004). We next examine how each of the latter possibilities might contribute to the differences that we measured in Cd and Cu levels in invertebrate and fish taxa.

First, we found that exposure concentrations were not uniform among habitats. Sediment-dwelling invertebrates and bottom-feeding fish (e.g., gobies) were generally less contaminated than organisms collected in the water column (Tables 1–2). For example, midges collected offshore in the sediment (*Chironomus*) had lower Cd concentrations and a more depleted δ^{13}C signature (−38.5‰) relative to their congeners (Orthocladiinae) collected within the SAV's water column (δ^{13}C of −17.8‰; M.-N. Croteau, unpubl. data; Table 1). This suggests that different organic carbon pools are available to these organisms (i.e., organic coatings on sediment and epiphytes, respectively), thereby implying different dietary metal exposure (if isotopic carbon composition of these consumers resembles their diets; DeNiro and Epstein 1978).

Second, consumer's food choices within habitat can also drive wide differences in metal exposure and accumulation. Foraging and feeding behavior are known to vary greatly among fish species (Table 2). For example, topminnows (killifishes) that showed the highest Cu concentrations are known to feed opportunistically on biofilm at the water surface, whereas gobies that are the least contaminated species feed on benthic prey and bottom detritus. Because biofilms are huge reservoirs of organic biomass that can greatly accumulate contaminants (Barranguet et al. 2002), feeding on water surface might explain why killifishes had the highest Cu concentration among fish. However, metal concentrations in sunfish varied greatly among species (Table 2), despite the fact that sunfish species shared similar feeding characteristics. This suggests that foraging and feeding behavior

could only explain some of the interspecific differences in fish metal concentrations.

We also cannot rule out the possibility that specific differences in factors unrelated to habitat, such as metal biodynamics (e.g., metal AE, loss-rate constant), play a key role in explaining why metal concentrations differed among related species that share similar carbon sources. As recently shown for coexisting species (Croteau et al. 2001, 2002; Stewart et al. 2004), differences in metal accumulation among species could be explained by interspecific variations in metal assimilation efficiency, rate constant of loss, feeding habits as well as by temperature (which influenced prey ingestion rates). For example, rate constant of loss for Cu in mollusks appears more important for bioaccumulation than is food web structure (Croteau et al. 2004). Similar knowledge of metal biodynamics might help to explain why fish accumulate less Cd than invertebrates.

A final explanation for the intertaxon variability in metal concentrations resides in the likelihood of metal trophic transfer within food webs. Cadmium concentrations showed a progressive enrichment among trophic levels within discrete epiphyte-based food webs composed of either macrophyte-dwelling invertebrates (for which the first link is epiphytic algae) or fishes (for which the first link is goby; Fig. 4A). This implies that processes driving Cd trophic transfer appear more complex than those of organics, organometals, or Se (Cabana and Rasmussen 1994; Kidd et al. 1995; Stewart et al. 2004).

Our results suggest that to accurately predict Cd biomagnification in nature, physiological biodynamics, habitat, food web structure, and trophic position have to be considered. These factors seem to provide an initial ''set point'' at lower trophic levels that determines the concentration from which Cd transfers up the food web. ''Traditional approaches'' (ascribing trophic position as the sole predictor of contaminant levels) would have failed in predicting Cd enrichment along the discrete epiphyte-based food webs we described. Rather than corroborating Gray's (2002) conclusion (metal biomagnification rarely occurs in nature), our results suggest that Cd enrichment along food webs might be more common that expected. However, identifying those occurrences mandates a meticulous characterization of food webs and other important ecological processes (e.g., bioaccumulation dynamics).

Our results also reiterated the well-known advantages of using stable isotopes to understand food webs. A good example is *Americorophium* spp., which had a δ^{13}C signature more negative than predicted by its littoral habitat, and than its congeners *Gammarus daiberi* and *Hyalella azteca* (that were clearly macrophyte dwellers). In contrast to *G. daiberi* and *H. azteca* that browse on the film of microscopic plants, animals, and organic debris covering littoral vegetation (Smith 2001), *Americorophium* spp. filter feeds on algae derived from the open-water environment. Inferring carbon sources of consumers based on habitat (e.g., littoral, pelagic, and profundal zones as proposed by Vander Zanden and Rasmussen [1999]) should only be done circumspectly; for in our case a ''categorical variable'' such as lake habitat (rather than δ^{13}C signatures) would have failed in properly ascribing *Americorophium* spp. to the phytoplankton-based food web.

Our study is the first to unequivocally demonstrate that Cd can be magnified along certain food chains in nature. Trophic enrichment of Cd increases the vulnerability of consumers at the highest trophic levels. Other studies show that adverse effects of Cd should be sought among species ingesting Cd-rich food sources (Larison et al. 2000). Organisms from higher trophic levels within food webs like those based upon epiphytes might provide another example, given a Cd-contaminated environment. For some metals, unraveling ecosystem complexity will be necessary before the species most exposed and at risk can be identified.

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Received: 18 October 2004
Accepted: 24 March 2005
Amended: 4 April 2005