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Exchange rates of cadmium between a burrowing mayfly and its surroundings in nature

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Abstract

We conducted a reciprocal transfer of nymphs of the burrowing mayfly *Hexagenia limbata* between two rivers harboring populations of this insect that differed in their cadmium (Cd) concentrations. We measured Cd uptake and loss rates simultaneously in the field at several levels of biological organization: in the gut and body of whole nymphs as well as in three subcellular fractions. The most marked changes in Cd concentrations occurred in the insect's gut, where Cd was largely associated with heat-stable proteins, a fraction that includes the metal-binding protein metallothionein. Because most of the Cd that entered nymphs was detoxified in this manner, we would not expect it to have direct toxic effects; however, it would be readily transferred to higher trophic levels. Our data on subcellular partitioning suggest that there is a fast-exchanging pool of Cd bound to heat-stable proteins such as metallothionein and a slow-exchanging pool bound to either or both of the remaining fractions (heat-denatured proteins; remainder, which includes exoskeleton and granules). Nymphs of *H. limbata* responded rapidly to changes in bioavailable Cd, which suggests that they would be useful for monitoring changes in ambient Cd for weeks or months in the field. A one-compartment model successfully explained the changes we observed in nymph Cd values. Furthermore, values of the model constant for Cd loss were close to those reported for *Hexagenia rigida* in the laboratory, which suggests that accumulation patterns observed in the laboratory can be representative of those measured in nature.

Cadmium (Cd) is a potentially toxic trace metal that commonly appears on government priority-substances lists such as that in the Canadian Environmental Protection Act (Environment Canada 1994). Because this metal is released as a by-product of the mining and smelting of copper, nickel, and zinc ores, it can be an important contaminant of lakes and rivers in mining regions, where it is readily accumulated by organisms (Croteau et al. 2002*b*). As part of risk assessment protocols for Cd in aquatic systems, reliable tools are required to evaluate the exposure of organisms to this metal, their accumulation of Cd, and its toxic effects (Chapman and Wang 2000). Such information is often obtained in the laboratory, where conditions tend to be unrealistic; that is, exposures tend to be of short duration, to a single metal, in dissolved form only, at high concentrations (Luoma 1995). We avoided such constraints by conducting a field experiment in which we transferred the mayfly *Hexagenia limbata* between two rivers that differed in their Cd concentrations. By this means, we were able to measure Cd gain and loss simultaneously without adding Cd to the insect's surroundings.

We selected the burrowing mayfly *Hexagenia* for our reciprocal transplant experiment because it has several qualities that make it a good biomonitor for metals in that it is: of adequate size for contaminant measurements; often present at high densities in suitable sediments (Charbonneau and Hare 1998); widely distributed in temperate lakes and rivers (McCafferty 1975); and tolerant of metals (Gosselin and Hare 2004). Moreover, this insect is a potentially important source of contaminants for higher trophic levels such as fish.

We measured Cd influx and efflux at several levels within *H. limbata*, including the gut, because this organ is reported to be the major site at which Cd accumulates in this insect (Hare et al. 1991*a*,*b*), and various subcellular fractions, because the likelihood of toxic effects and the potential for trophic transfer is thought to depend on how metals are bound in cells (Cain et al. 2004; Campbell et al. 2005). We compared our measurements of Cd influx and efflux to the predictions of a bioaccumulation model to determine if it could reliably describe Cd exchange in this mayfly.

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Acknowledgments

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Methods

Study area—In June 2002, we collected nymphs of *H. limbata* at a single station in each of two rivers situated in largely forested drainage basins located in northwestern Quebec, Canada: the Allard River (49°49′35.9″N, 77°47′38.3″W), near Matagami in the James Bay region, and the Colombière River (48°08′59.2″N, 77°37′45.0″W), near Val d'Or in the Abitibi region. Parts of both rivers have been affected by activities associated with the mining of copper, gold, and zinc, including abandoned surface mines and mine tailings as well as atmospheric deposition from a metal smelter in Rouyn-Noranda, Québec. We chose these stations for our experiments because they harbored large populations of *H. limbata* and because preliminary measurements showed that Cd concentrations were much lower in nymphs from the Colombière River than in nymphs from the Allard River. During our experiment, the waters of these rivers (Colombière and Allard, respectively) were circumneutral (pH 6.6 and 7.3) and similar in their dissolved organic carbon concentrations (12 and 13 mg L^{-1}) (Masson and Campbell unpubl. data).

Reciprocal transplantation of H. limbata—In each river, divers collected ≈400 liters of surficial sediment (<5-cm depth) by dragging a large pail along the river bottom in an area where *H. limbata* nymphs were abundant. These sediments were mixed thoroughly in 80-liter plastic barrels; then, ≈9 liters was placed into each of a series of plastic containers (30 × 30 × 15 cm deep, surface area = 0.09 m^2). The 30 containers for a given river were covered with tight-fitting lids and pushed by divers into the river bottom (on 16 and 18 June 2002, in the Colombière and Allard Rivers, respectively) at the sites from which the sediments had been collected. Containers were left for 1 week to asphyxiate any remaining *H. limbata* (Warren et al. 1998); four control containers examined at the end of this period contained no live nymphs. The hermetic lids were removed from the containers and replaced by mesh lids (aperture = 1 mm) to allow the oxidation of the surficial sediments.

After 2 weeks, *H. limbata* nymphs were collected in each river by dragging a 1-mm mesh-aperture net along the river bottom, and nymphs of uniform size were selected for reciprocal transfer between the two rivers. The mean (±SD) lengths of nymphs (excluding cerci) collected from the Allard and Colombière Rivers were 15.4 ± 2.5 mm and 14.6 ± 1.1 mm, respectively. Nymphs originating from one river were transported to the other river, where 10 individuals were placed into each of the sediment-filled containers. Nymph densities in these containers were ≈110 nymphs m^{-2}, which is somewhat less than the mean reported for 22 North American lakes (160 nymphs m^{-2}) (Charbonneau and Hare 1998). Additional nymphs were introduced into containers filled with sediment from their own river (10 nymphs per container), and these containers were placed in their home river to serve as controls to determine if confinement affected their metal concentrations or growth rates. All containers were fitted with 1-mm mesh-aperture lids that prevented nymph exchange between containers and surrounding sediments. Transplanted nymphs were collected on nine occasions (days 0, 3, 6, 9, 12, 15, 21, 28, and 36), whereas *H. limbata* in control containers were collected on five occasions (days 0, 6, 9, 15, and 28). At each sampling time, divers collected two containers and removed the nymphs by sieving through a 1-mm mesh-aperture net. Lastly, 20 indigenous nymphs were collected on five occasions (days 0, 6, 9, 15, and 20) by dragging the river bottom near the containers, but slightly downstream, with a 1-mm mesh-aperture net. Calendar dates for day 0 were 7 July for nymphs transplanted from the Allard to the Colombière River and 12 July for nymphs transplanted in the opposite direction.

Nymphs were transported to the field laboratory and held in river water for 12 h to partially eliminate the sediment in their guts. They were then held in an anesthetic solution (carbonated water), and their length was measured under a dissecting microscope. Nymphs were dissected into two portions, the gut and remaining parts, which we refer to as the body. Guts that were not empty were opened to remove remaining sediment (Hare et al. 1989). The parts of four nymphs were pooled (i.e., four bodies or four guts [when there had been no mortality]) and placed on pieces of acid-washed Teflon sheeting held in polypropylene, freeze-resistant, acid-washed tubes (Wheaton). These tubes were stored in liquid nitrogen in the field laboratory and at −80°C on return to the central laboratory; analyses were completed between 1 and 10 months after collection.

Cd measurements in sediment and pore water—Pore-water samplers and labware used for Cd measurements in water and sediment were washed in 15% (vol/vol) HNO_3 for 24 h, rinsed seven times with ultrapure water, and dried in a laminar flow hood prior to use.

To compare Cd concentrations between rivers, we collected sediment samples from each river on the last sampling day (12 and 17 August 2002 in the Colombière and Allard Rivers, respectively). We also collected sediment from containers to compare Cd concentrations in containers with those in surrounding sediments. The 3-cm-diameter cores were extruded and sectioned at 1-cm intervals (to a depth of 10 cm); slices were placed in preweighed 20-mL plastic vials, held at 4°C in the field, and frozen on their return to the laboratory. Sediment samples were freeze-dried (FTS Systems) to constant weight and then digested in a mixture of 4% HNO_3 and 5% HCl at 85°C for 2 h (modification of method 200.2, U.S. Environmental Protection Agency 1992). Concentrations of Cd were measured by inductively coupled plasma–mass spectrometry (ICP-MS; Thermo Electron Corp., X7). Reference sediment from the Saint-Lawrence River (CRM 1-21 and 2-31, COREM and INRS-ETE, Québec, Québec, Canada) was digested at the same time, and Cd measurements were within 2% of certified values.

To collect Cd in pore waters, we used acrylic diffusion samplers (peepers) comprising a vertical series of ten 4-mL cells situated at 1-cm intervals (Hare et al. 1994). The samplers were held under a nitrogen atmosphere for 3 weeks prior to use, filled with ultrapure water (~18 MΩ cm), covered with a polysulfone membrane (Pall Gelman Sciences, HT-200; 0.2 μm), and then stored under a nitrogen atmosphere for at least 1 week prior to use to ensure removal of oxygen (Carignan et al. 1994). Pore-water samples for Cd

analyses were obtained by piercing the membrane with a Pipetman pipette (P5000) fitted with an acid-washed plastic tip and injected into prewashed and preacidified (40 μL of 2 mol L^{-1} Seastar HNO_3) 4-mL high-density polyethylene vials. All samples were stored at 4°C prior to analysis. Cd in pore waters was measured by ICP-MS (Thermo Electron Corp., X7). Blanks and certified samples (riverine water reference material, NIST 1640, U.S. National Institute of Standards and Technology, Gaithersburg, Maryland) were analyzed during each analytical run, and measured trace metal concentrations were within the certified range.

Fractionation and analysis of biological samples—Tubes containing biological samples were prewashed in 70% (vol/vol) HNO_3 for 1 h, rinsed seven times with ultrapure water, and dried in a laminar flow hood prior to use. During manipulation, biological samples were kept on ice to minimize enzymatic degradation of tissues.

Frozen samples of *H. limbata* were transferred into preweighed centrifuge tubes and weighed; guts were held in 1.5-mL polypropylene microcentrifuge tubes (Eppendorf), whereas bodies were held in 3-mL polyallomer centrifuge tubes (Beckman). These samples were homogenized in ice-cold isotonic buffer (Tris, 25 mmol L^{-1}, Omnipure 9210, 99.8% purity, adjusted to pH 7.2 with 35% trace metal-grade HCl; Couillard unpubl. data) at dilutions of 1 : 2 (wt/wt) for bodies and 1 : 5 (wt/wt) for guts. Homogenization was performed manually in the centrifugation tubes using a glass pestle; we determined by visual inspection that four and seven turns of the pestle were sufficient to homogenize guts and bodies, respectively.

We removed a 10-μL aliquot of homogenate to measure total Cd and then used the following procedure to obtain three subcellular fractions (modified from Wallace et al. 1998 and Campbell et al. 2005). The homogenate was centrifuged at 100,000 × g (Beckman TL-100 with a TLA-100.3 fixed-angle rotor) for 1 h at 4°C to yield a pellet containing exoskeleton (body only), granules, etc., which we refer to as the remainder. The supernatant was heated at 80°C for 10 min, cooled on ice for 1 h, and then centrifuged at 50,000 × g (Sorvall RC 5C Plus with an SS-34 rotor) for 10 min at 4°C to yield a second supernatant containing heat-stable moieties that should include the metal-binding protein metallothionein as well as glutathione, small peptides, and free amino acids. The pellet is expected to contain heat-denatured proteins. All pellets and homogenates were frozen, whereas the supernatants were acidified with an equal volume of concentrated HNO_3 (trace-metal grade) and held at 4°C. Although the cell fractions obtained with this type of technique are operationally defined, several researchers have reported that they give a reliable estimate of metal partitioning at the subcellular level (Wallace et al. 1998; Cain et al. 2004; Campbell et al. 2005).

All fractions were freeze-dried (FTS Systems) to constant weight and digested in their centrifugation tubes at room temperature in concentrated trace-metal grade HNO_3. Each milligram dry weight of sample (to a limit of 26 mg) was digested in 100 μL of acid for 5 d, which was followed by the addition of 40 μL of H_2O_2 for 24 h and then dilution with 860 μL of ultrapure water. Samples that weighed >26 mg were treated like 26-mg samples. Cd concentrations in all subcellular fractions were measured by ICP-MS (Thermo Electron Corp., X7). We submitted samples of similar weight of a certified reference material (lobster hepatopancreas, TORT-1, National Research Council of Canada) to the same digestion procedure during each run. The mean percentage of recovery of Cd in TORT-1 reference samples (n = 14) was 95% ± 5% (±SD) and was within the certified range for this metal. Digestion blanks indicated negligible Cd contamination during sample preparation, digestion, and analysis. The mean (±SD) sum of Cd in the subcellular fractions was close to the values measured for whole homogenate, that is, 103% ± 9% for the gut portion and 86% ± 9% for the body portion, suggesting that there was little loss of Cd during the fractionation procedure.

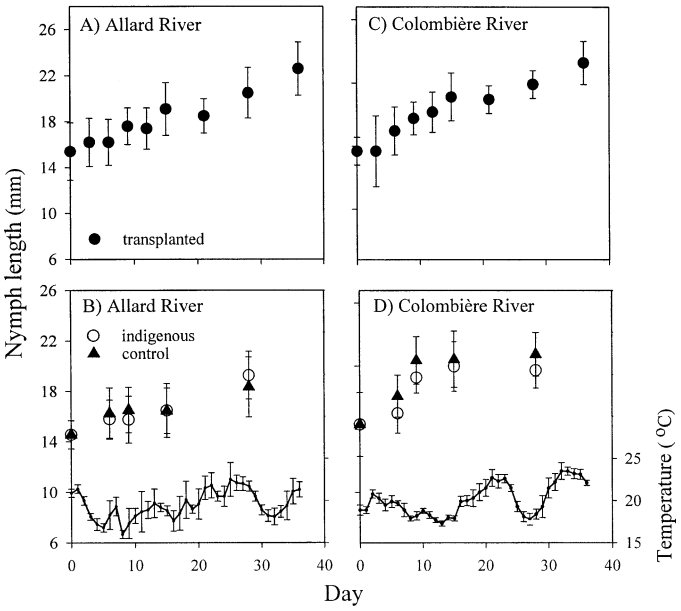

Fig. 1. Temporal changes in the mean length (±SD, n = 10–16) of *Hexagenia limbata* nymphs that were either transplanted (A, C; filled circles), placed in containers as controls (B, D; filled triangles), or indigenous to the river (B, D; open circles). River water temperatures during our experiments are shown in panels (B) and (D).

Results

Survival and growth of nymphs—For the Cd uptake experiment (nymphs transferred from the Colombière River to the Allard River), recovery was high (8–10 of the 10 nymphs introduced per container), and nymphs grew at a mean (±SD) rate of 0.19 ± 0.02 mm d^{-1} (Fig. 1A) during the 36-d period. Control nymphs in containers and indigenous nymphs from the Allard River (Fig. 1B) grew at similar rates (0.12 ± 0.02 mm d^{-1} and 0.16 ± 0.02 mm d^{-1}, respectively; $p > 0.05$, t-test). For the Cd loss experiment (nymphs transferred from the Allard River to the Colombière River), nymph growth rates (0.17 ± 0.02 mm d^{-1}) were similar to those in the Cd-influx experiment (Fig. 1C). Control nymphs in containers and indigenous nymphs from the Colombière

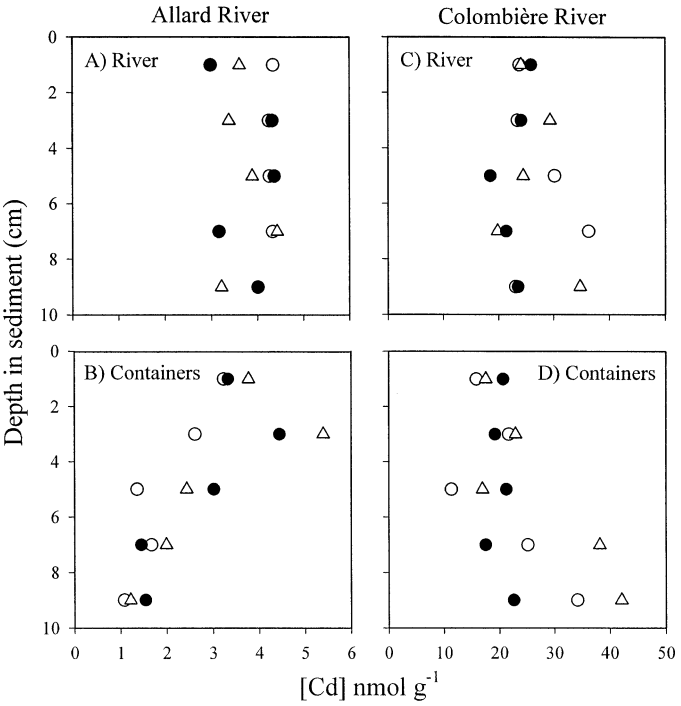

Fig. 2. Depth profiles of Cd concentrations (nmol g^{-1} dry weight) in sediment cores collected within (B and D) or near (A and C) our experimental containers. The various symbols are for replicate cores. Note that the scales for Cd concentrations differ.

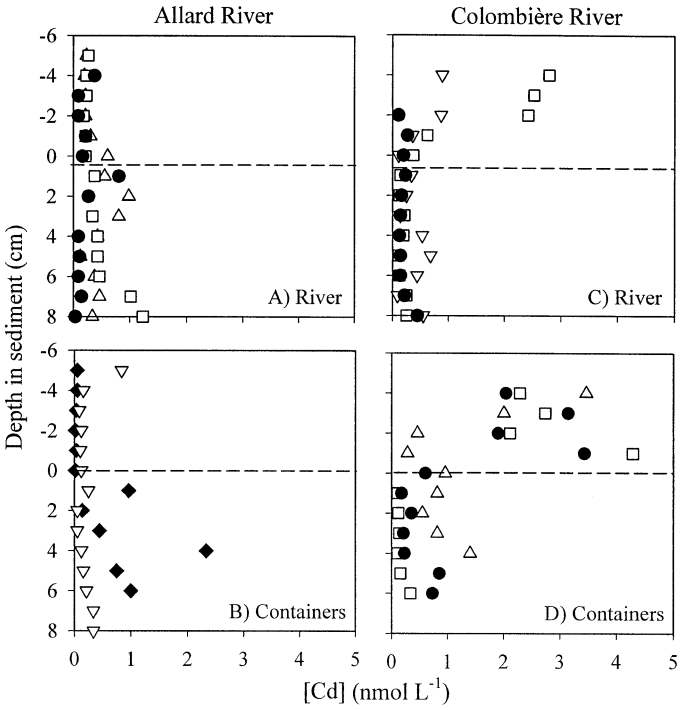

Fig. 3. Dissolved Cd profiles within (B and D) or near (A and C) our experimental containers. The various symbols are for replicate profiles. The horizontal broken line indicates the sediment–water interface.

River (Fig. 1D) had similar growth rates of 0.18 ± 0.08 mm d^{-1} and 0.16 ± 0.07 mm d^{-1}, respectively ($p > 0.05$, *t*-test). Nymph recovery from containers in the Colombière River was variable (3–10 of 10) because divers lost some nymphs during their insertion into the containers. The similarity in growth rates between nymphs in containers and indigenous nymphs suggests that the container environment was similar to that in nature. The similarity in nymph growth rates between rivers is consistent with the similarity in water temperatures between the two rivers (Fig. 1B,D).

[Cd] in water and sediments—Cd concentrations were lower in sediments from the Allard River (Fig. 2A,B) than in sediments from the Colombière River (Fig. 2C,D). Dissolved Cd concentrations were similar in the interstitial waters of the two rivers (Fig. 3A,B vs. 3C,D) but were higher in the overlying water of the Colombière River (Fig. 3C,D) than were the Cd concentrations measured in the Allard River (Fig. 3A,B). In both rivers, Cd concentrations in sediment (Fig. 2) and interstitial waters (Fig. 3) were similar between the containers and their surroundings, suggesting that sediment manipulation did not create experimental artifacts. Although depth profiles for sedimentary Cd in the containers were more variable (Fig. 2B,D) than those measured in the environs (Fig. 2A,C), Cd profiles in containers were vertical in the near-surface zone where *H. limbata* concentrate their burrowing and feeding activities (as determined from visual assessments in the field).

[Cd] in whole nymphs—Cd concentrations in whole *H. limbata* transferred to the Allard River increased over time, reaching those of indigenous nymphs from the Allard River in <2 weeks (Fig. 4A). For nymphs transferred to the Colombière River, Cd concentrations decreased by ≈90% over time but did not quite reach the low levels of indigenous nymphs from this river by the end of our 36-d experiment (Fig. 4B).

[Cd] in the nymph gut and body—Cd concentrations in the gut tissues of nymphs transplanted to the Allard River attained those of indigenous Allard River nymphs after a few weeks (Fig. 5A), whereas Cd concentrations in the body increased between days 0 and 3, after which they stabilized near those of indigenous nymphs (Fig. 5B). In the Cd-loss experiment, gut Cd concentrations in nymphs transplanted to the Colombière River decreased but did not quite reach those of indigenous nymphs from the Colombière River (Fig. 5C), whereas Cd concentrations in the bodies of these transplanted nymphs declined to reach those of indigenous nymphs from the Colombière River after several weeks (Fig. 5D).

Cd concentrations in the gut (Fig. 5A,C) far exceeded those in the body portion (Fig. 5B,D) of *H. limbata.* In terms of Cd burdens, indigenous nymphs that had higher Cd concentrations (those from the Allard River) had the majority (mean = ≈60%) of their Cd in the gut (Fig. 6A), whereas those with lower Cd concentrations (those from the Colombière River) had greater mean proportions of their Cd in the body than in the gut (Fig. 6C). Accordingly, in transplanted *H. limbata*, there was a change over time in the

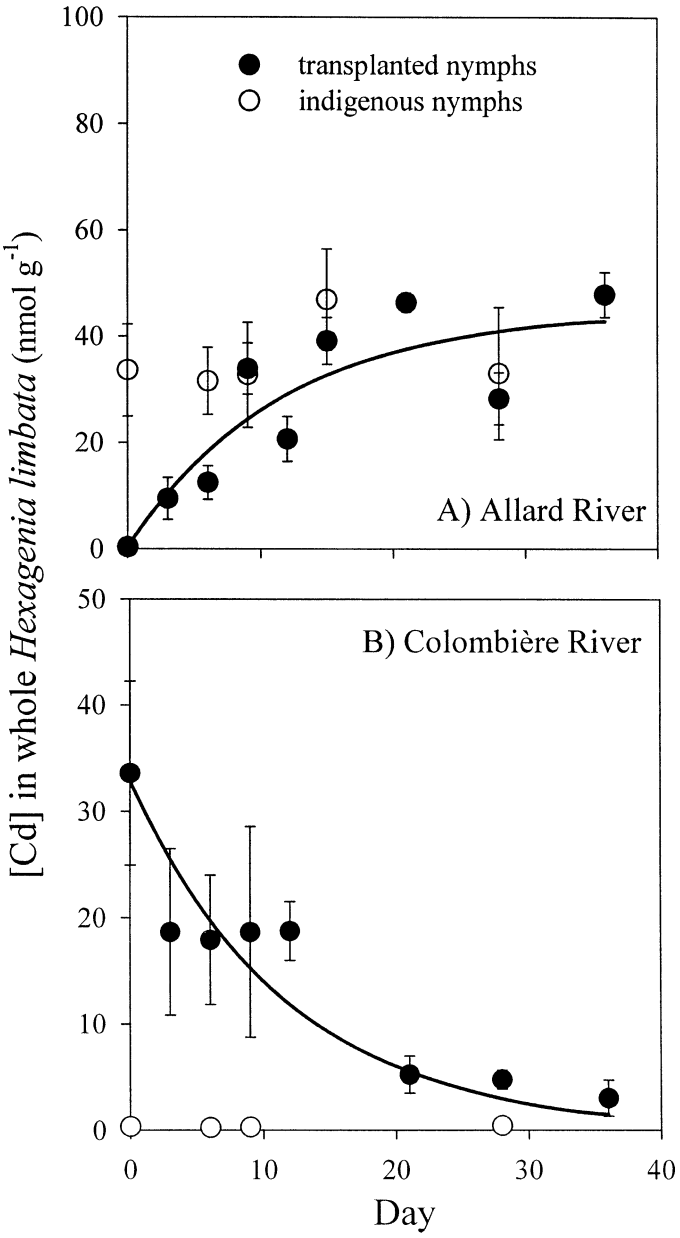

Fig. 4. Temporal changes in mean Cd concentrations (±SD, nmol g^{-1} dry weight, $n = 4$) of whole *Hexagenia limbata* either transferred (filled symbols) to the (A) Allard and (B) Colombière Rivers or indigenous to a given river (open symbols). Model curves were generated using Eq. 3.

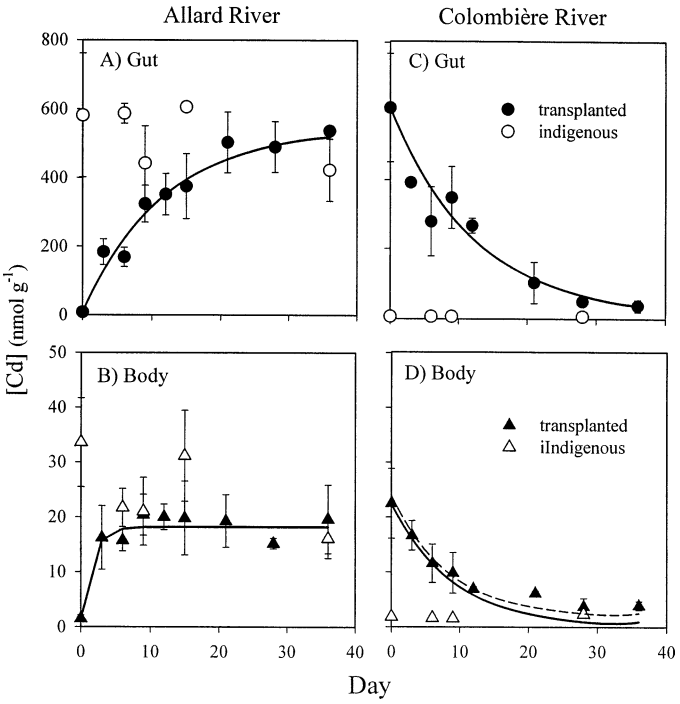

Fig. 5. Temporal changes in mean Cd concentrations (±SD, nmol g^{-1} dry weight, $n = 4$) in either the gut (A, C) or body (B, D) of *Hexagenia limbata* transferred to the Allard River (A, B) or to the Colombière River (C, D). Filled symbols represent transplanted nymphs and open symbols represent indigenous nymphs. Note differences in vertical scales for the gut and body. Model curves were generated using Eq. 3 with the exception of the broken line in D that was generated using Eq. 5.

major organ of Cd accumulation toward that measured in indigenous nymphs (Fig. 6B,D).

[Cd] in subcellular fractions of H. limbata—In the gut during the Cd-uptake experiment (transfer to the Allard River, Fig. 7A–C), Cd concentrations increased in the ''remainder'' fraction (e.g., exoskeleton, granules) early in the experiment (Fig. 7A) to reach those of indigenous nymphs from the Allard River, whereas those in the heat-denatured protein fraction showed few such trends (Fig. 7B). In contrast, Cd concentrations in the heat-stable protein fraction of the gut increased markedly within the first 2 weeks of the uptake experiment, eventually reaching those in indigenous nymphs from the Allard River (Fig. 7C). In all nymphs, Cd concentrations in the heat-stable protein fraction far exceeded those in the other fractions (compare vertical scales in Fig. 7A–C). During the Cd-loss experiment (transfer to the Colombière River, Fig. 7D–F), Cd concentrations in the gut declined somewhat in the remainder fraction (Fig. 7D) to reach those of indigenous nymphs from the Colombière River, remained stable in the heat-denatured protein fraction (Fig. 7E), but declined substantially in the heat-stable protein fraction to reach those in indigenous *H. limbata* from the Colombière River (Fig. 7F).

In the body during the Cd-uptake experiment (Fig. 8A–C), Cd concentrations increased slightly in the remainder fraction (Fig. 8A) but did not quite reach the mean of those in the bodies of indigenous nymphs from the Allard River, whereas those in the heat-denatured protein fraction increased from days 0 to 3, at which time they equaled those of indigenous nymphs from the Allard River (Fig. 8B). Cd concentrations in the heat-stable protein fraction also increased to reach those in the body portion of indigenous nymphs from the Allard River within ≈2 weeks (Fig. 8C). During the Cd-loss experiment (Fig. 8D–F), Cd concentrations in the body changed little in the remainder fraction (and were somewhat higher than those in the indigenous nymphs from the Colombière River; Fig. 8D) but declined in both

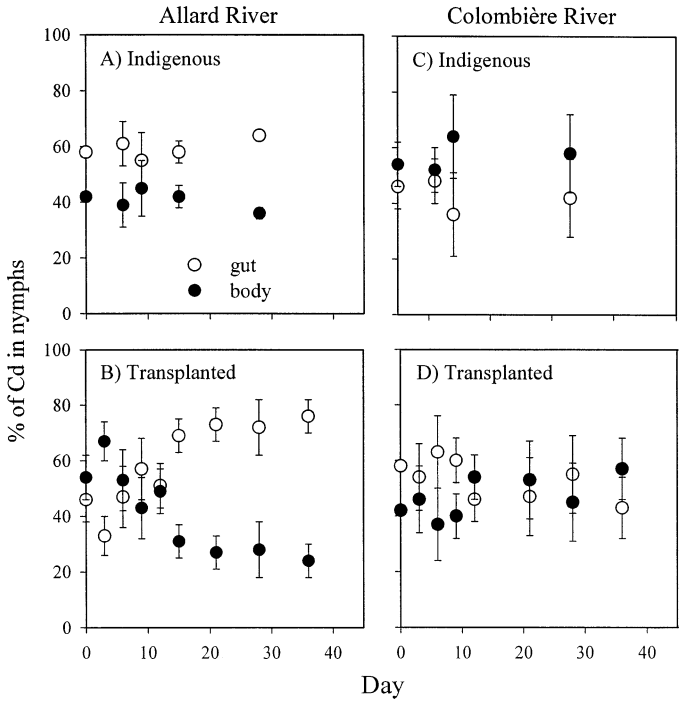

Fig. 6. Mean contributions (% ±SD, $n = 4$) of Cd in parts of indigenous (A, C) and transplanted (B, D) *Hexagenia limbata* to their total Cd burdens in the Allard (A, B) and Colombière (C, D) Rivers. Open circles represent the nymph gut, whereas filled circles represent the body.

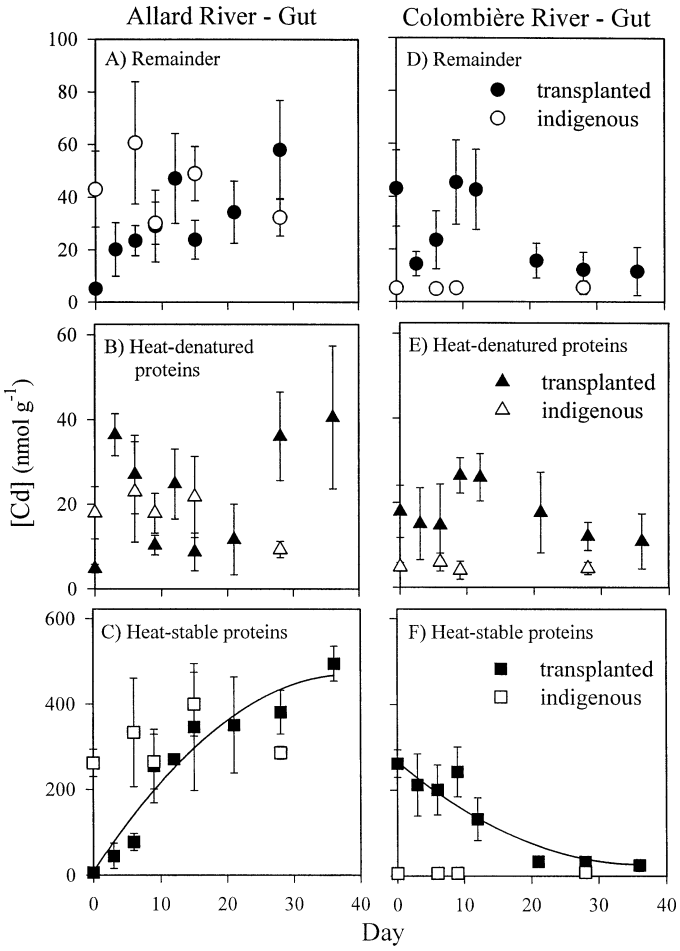

Fig. 7. Temporal changes in mean gut Cd concentrations (±SD, nmol g^{-1} dry weight, $n = 4$) in the following subcellular fractions of *Hexagenia limbata* during the Cd-uptake experiment (A–C) or the Cd loss experiment (D–F): remainder (A, D); heat-denatured cytosolic proteins (B, E); heat-stable cytosolic proteins (C, F). Filled symbols represent nymphs transplanted between rivers, whereas open symbols represent nymphs indigenous to the rivers. Model curves were generated using Eq. 3.

protein fractions to reach those of indigenous nymphs (Fig. 8E,F).

The burden of Cd in the gut of indigenous low-Cd nymphs from the Colombière River was equally distributed among the three subcellular fractions (Fig. 9C). When these nymphs were transplanted to the Allard River, the proportion of Cd associated with the heat-stable protein fraction increased rapidly such that ~80% of the total came to be associated with this fraction (Fig. 9B), as in indigenous *H. limbata* from this river (Fig. 9A). For nymphs transplanted to the Colombière River (the Cd-loss experiment), the proportion of gut Cd in the heat-stable protein fraction fell somewhat (Fig. 9D) but did not quite reach that of indigenous nymphs in this river (Fig. 9C).

In the body portion of the indigenous nymphs from the Colombière River, only ~10% of their Cd was in the heat-stable protein fraction (triangles in Fig. 10C). When these nymphs were transplanted to the Allard River, the proportion of Cd in this fraction increased (Fig. 10B) to reach those of indigenous nymphs from the Allard River (~40%, Fig. 10A) within about 2 weeks' time. For *H. limbata* nymphs transplanted to the Colombière River (the Cd-loss experiment), the proportion of body Cd in the heat-stable protein fraction declined (Fig. 10D) to reach that of indigenous nymphs in this river (Fig. 10C). Changes in the proportion of Cd in the heat-denatured protein fraction were minor, whereas changes in the remainder fraction were substantial, driven in large part by changes in the heat-stable protein fraction (Fig. 10A–D).

Discussion

Cd concentrations in nymphs were not directly related to those in either the sediment or the water of our study rivers, which suggests that both the bioavailability of metals in exposure media (Bryan and Langston 1992; Ankley 1996; Croteau et al. 2002*b*) and the burrowing and feeding behavior of animals (Lee et al. 2000; Wang et al. 2001) need to be considered to relate metal concentrations in animals to those in their environs. The similarity in steady-state [Cd] between nymphs in containers and the environs suggests that bioavailable Cd concentrations in sediments were similar between the containers and their surroundings.

Cd in H. limbata—The following model (Hare et al. 1991*a*) can be used to describe changes in Cd concentrations in *Hexagenia* or its parts, $[Cd]_{Hexagenia}$,

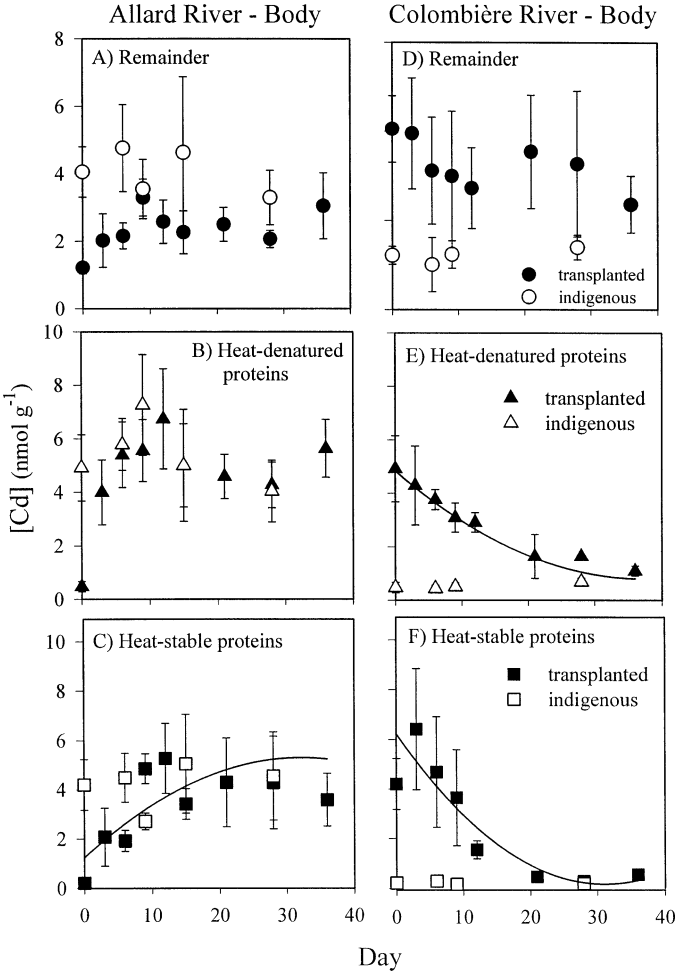

Fig. 8. Temporal changes in mean body Cd concentrations (±SD, nmol g^{-1} dry weight, $n = 4$) in the following subcellular fractions of *Hexagenia limbata* during the Cd-uptake experiment (A–C) or the Cd-loss experiment (D–F): remainder (A, D); heat-denatured cytosolic proteins (B, E); heat-stable cytosolic proteins (C, F). Filled symbols represent nymphs transplanted between rivers, whereas open symbols represent nymphs indigenous to the rivers. Model curves were generated using Eq. 3.

$$\frac{d[\mathrm{Cd}]_{Hexagenia}}{dt} = k_I[\mathrm{Cd}]_{\mathrm{environs}} - k_E[\mathrm{Cd}]_{Hexagenia} \qquad (1)$$

(influx) (efflux)

where k_I and k_E are first-order rate constants for Cd influx and efflux, respectively. The term $[\mathrm{Cd}]_{\mathrm{environs}}$ represents bioavailable [Cd] in the insect's environs, whether the Cd is in the sediment, the water, or both (the relative importance of water and food as Cd sources for *Hexagenia* is unknown). If we assume that $[\mathrm{Cd}]_{\mathrm{environs}}$ was constant during our 36-d experiment, then Cd influx can be represented as an apparent uptake rate constant (k_I') in Eq. 1, as shown below.

$$k_I\,[\mathrm{Cd}]_{\mathrm{environs}} = k_I' \qquad (2)$$

Substituting Eq. 2 into Eq. 1 and integrating the resulting equation yields:

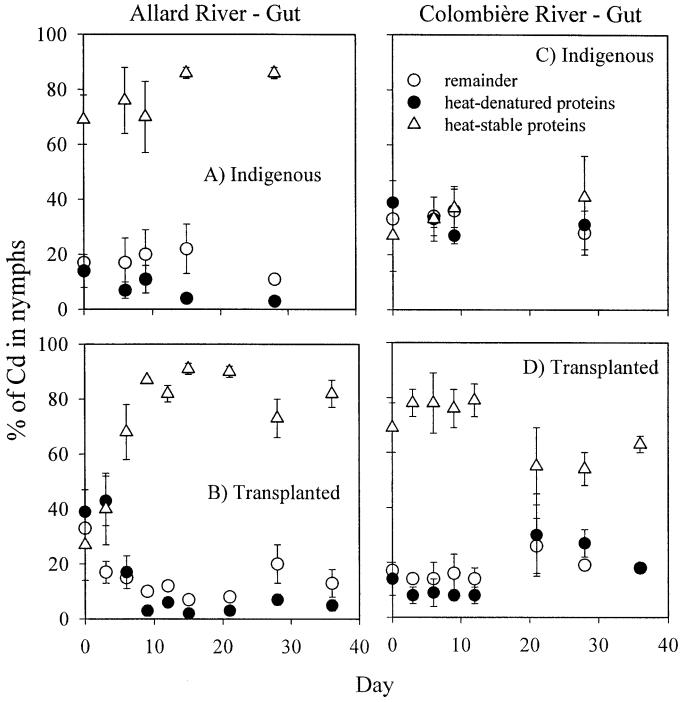

Fig. 9. Temporal changes in the mean relative contributions (±SD, $n = 4$) of the three subcellular fractions to total Cd burdens in the gut of *Hexagenia limbata* that were either indigenous to the (A) Allard or the (C) Colombière Rivers or (B, D) transplanted between these rivers. Fractions are represented as follows: remainder, open circles; heat-denatured cytosolic proteins, filled circles; heat-stable cytosolic proteins, triangles.

$$[\mathrm{Cd}]_{Hexagenia} = \frac{k_I'}{k_E} - \left(\frac{k_I'}{k_E} - [\mathrm{Cd}]^0_{Hexagenia}\right)e^{k_E t} \qquad (3)$$

Using Eq. 3 and data from our Cd-uptake experiment, we estimated, by iteration, k_I' and k_E for whole *H. limbata* and for its constituent parts (the gut and the body). We then used these estimates of k_I' and k_E (Table 1) along with appropriate values of $[\mathrm{Cd}]^0_{Hexagenia}$ to generate model curves for both our Cd-uptake and Cd-loss experiments, as shown in Figs. 4 (whole nymphs) and 5 (nymph parts).

Model curves for the Cd-uptake experiments fit our observed data well (Figs. 4A, 5A,B), suggesting that the model we used, although simple, is sufficient to characterize Cd accumulation by *H. limbata*. Likewise, model curves fit our Cd-loss data well (Figs. 4B, 5C,D), which is encouraging given that the rate constants k_E and k_I' were estimated from our data for Cd uptake. Exceptionally, the fit of the model curve for Cd loss from the body portion (Fig. 5D) was somewhat less exact. We attribute this lack of fit to a very rapid Cd uptake by the nymph body (Fig. 5B), which led to an overestimation of the apparent uptake rate constant k_I' for this insect part (Table 1). This overestimation could be related to the presence of iron oxyhydroxide deposits on the exoskeleton of *H. limbata* from the Colombière River; when these nymphs were transferred to the Allard River, Cd would have sorbed rapidly onto these deposits. Cd sorption onto the exoskeleton was not, however, the sole process involved, because the concentration of heat-stable proteins increased

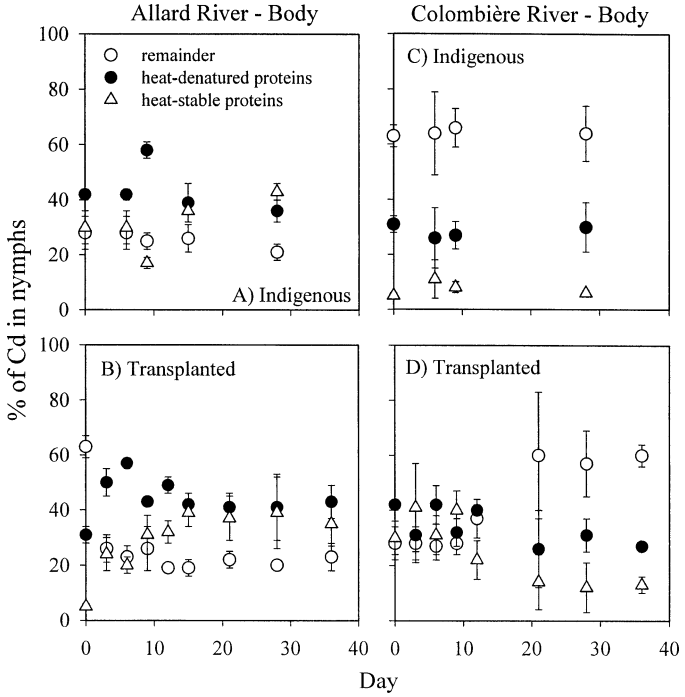

Fig. 10. Temporal changes in the mean relative contributions (±SD, $n = 4$) of three subcellular fractions to total Cd burdens in the body of *Hexagenia limbata* that were either indigenous to the (A) Allard or the (C) Colombière Rivers or (B, D) transplanted between these rivers. Fractions are represented as follows: remainder, open circles; heat-denatured cytosolic proteins, filled circles; heat-stable cytosolic proteins, triangles.

in the body portion of these nymphs (Fig. 8C). Cd efflux from the body portion (Fig. 5D) was likely due solely to physiological Cd loss (note the decline in heat-stable proteins in the body portion in Fig. 8F), since there were no visible deposits of iron oxyhydroxides on the exoskeleton of nymphs transferred from the Allard River. To eliminate possible bias due to metal sorption in the Cd-uptake experiment, we determined a second value of k_E for the body using data from our Cd-loss experiment. For this purpose, we assumed that during our Cd-loss experiment, Cd uptake by nymphs was negligible because bioavailable $[Cd]_{environs}$ was likely very low in the Colombière River (given the very low [Cd] in indigenous nymphs from this river). In this case, Eq. 1 reduces to:

$$\frac{d[Cd]_{Hexagenia}}{dt} = -k_E[Cd]_{Hexagenia} \quad (4)$$

which can be integrated to:

$$[Cd]_{Hexagenia} = [Cd]^0_{Hexagenia}e^{-k_E t} \quad (5)$$

where the superscript zero refers to initial conditions. The value of the loss rate constant k_E derived in this manner is given in Table 1 and was used in Eq. 5 to generate a second model curve for Cd loss from the body portion (broken line in Fig. 5D). The fit of this model curve is improved over that generated using data from the uptake experiment, suggesting that this value of k_E better describes physiological Cd loss than that derived from the uptake experiment. We also used Eq. 5 to estimate the biological half-life ($t_{½}$) of Cd, which is ~1 week for the whole insect and its constituent parts (Table 1).

The value of the Cd loss rate constant k_E (Table 1) for whole *H. limbata* (0.09 d^{-1}) is similar to, but somewhat lower than, those reported for copepods (0.1–1.3 d^{-1}) (Wang and Fisher 1998; Xu et al. 2001) and higher than those reported for bivalves (0.01–0.03 d^{-1}) (Roditi and Fisher 1999; Chong and Wang 2001) and gastropods (0.001–0.005 d^{-1}) (Wang and Ke 2002). The values of the loss rate constants for the gut and body portions that we measured in our field study (0.11 and 0.13 d^{-1}, respectively) are similar to those reported from a laboratory study on *Hexagenia rigida* (0.10 and 0.09 d^{-1}, respectively), which suggests that species of *Hexagenia* differ little in this respect and that results in the laboratory for this genus can be representative of those obtained in the field. The similarity in these Cd-loss rate constants is echoed by that of the biological half-lives for Cd in the gut and body of these species, which are 7–8 d for both *H. limbata* and *H. rigida* (Table 1). Half-lives for Cd on the order of 1 to several weeks have been reported for aquatic arthropods (Timmermans et al. 1992; Wang and Fisher 1998) and juvenile fish (Baines et al. 2002), whereas half-lives for bivalves are generally much longer and range from months to years (Roditi and Fisher 1999; Baudrimont et al. 2003). The similarity of the rate constants for Cd loss from the gut and

Table 1. Estimated values (±SE; $n = 9$) of model constants for Cd exchange in nymphs of *Hexagenia limbata* from our field study compared to those reported for *H. rigida* nymphs that were exposed in the laboratory to Cd-amended lake sediment (Hare et al. 1991*a*). Two values are given for constants related to Cd loss from the body portion, those estimated from out Cd-uptake experiment and those estimated from our Cd-loss experiment (*see text for explanation*). HSP, heat-stable proteins; N/A, not available; exper., experiment.

			Our field study of *H. limbata*						Laboratory study of *H. rigida*	
Term	Units	Symbol	Whole nymph	Gut	Gut (HSP fraction)	Body (uptake exper.)	Body (loss exper.)	Body (HSP fraction)	Gut	Body
Efflux rate constant	d^{-1}	k_E	0.086 (±0.033)	0.11 (±0.01)	0.049 (±0.015)	0.56 (±0.11)	0.13 (±0.01)	0.22 (±0.41)	0.098 (±0.034)	0.088 (±0.023)
Biological half-life	d	$t_{1/2}$	7	6	12	5	8	3	7	8
Apparent influx rate constant	nmol g^{-1} d^{-1}	k_I'	3.4 (±0.6)	45 (±5)	28 (±5)	11 (±3)	NA	0.92 (±0.19)	N/A	N/A

body (Table 1) suggests that uptake rates control the relative Cd concentrations in these parts.

In a matter of weeks, Cd concentrations in nymphs transplanted to the Allard River (Cd-uptake experiment) attained a steady state that was close to the concentrations measured in indigenous nymphs from the Allard River. These results are consistent with those reported for *H. limbata* colonizing Cd-amended sediments in open containers on a lake bottom, where nymph Cd concentrations reached a plateau after ~18 d (Warren et al. 1998). The results of laboratory experiments also suggest that rapid Cd exchange rates are typical for aquatic insects. Thus, mayflies (Aoki et al. 1989; Hare et al. 1991*a*) and phantom midges (Croteau et al. 2001) reach a steady state in their Cd concentrations within weeks of an increase in Cd exposure. Given this rapidity of Cd exchange, insects would likely be useful for monitoring Cd within weeks of a change in exposure concentrations. In contrast, the Cd concentrations in some bivalves are reported to take years to attain those of indigenous animals (Couillard et al. 1995; Baudrimont et al. 2003).

Indigenous low-Cd nymphs from the Colombière River had a lower proportion of their Cd in the gut portion than did the more contaminated nymphs from the Allard River, which suggests that the proportion of Cd in the gut of *H. limbata* is directly related to Cd concentrations in this insect (Hare et al. 1991*b*). If we assume that little Cd is transferred among parts of this mayfly, as shown by Inza et al. (2001), then our results suggest that Cd is taken up from both water, into the body, and food (sediment), into the gut, and that food becomes an increasingly important source of Cd in more Cd-contaminated environments.

The majority of the Cd in *H. limbata* is in the cytosol (our two protein fractions) rather than bound to the exoskeleton, granules, or cell membranes (the remainder fraction). The cytosol has also been reported to contain the majority of the Cd present in field-collected aquatic insects (Suzuki et al. 1988; Cain et al. 2004) but not in annelids (Wallace et al. 1998) and mollusks (Bonneris et al. 2005), in which greater proportions of Cd tend to be bound in granules. Because consumers more readily assimilate metal from the cytosolic fraction of their food than from noncytosolic fractions such as granules (Reinfelder and Fisher 1991; Wallace and Lopez 1997), the majority of the Cd in *H. limbata* is likely easily assimilated by its predators.

In the gut cytosol of *H. limbata* from the Allard River, where nymphs have high Cd concentrations, the majority of the Cd was associated with the heat-stable protein fraction, likely metallothionein. Numerous investigators have reported that this low-molecular-weight (6–7 kDa), cysteine-rich protein plays a major role in metal metabolism and detoxification in indigenous populations of aquatic invertebrates (Mason and Jenkins 1995; Baudrimont et al. 2003). Although we did not measure metallothionein directly, it is reported to be important in binding both essential and nonessential metals in several genera of aquatic insects, including the mayflies *Hexagenia* (Couillard unpubl. data) and *Baetis* (Aoki et al. 1989), as well as the phantom midge *Chaoborus* (Croteau et al. 2002*a*). Furthermore, Cd concentrations in lake-dwelling mollusks and fish are reported to be directly related to those of metallothionein-like proteins in these animals (Campbell et al. 2005). Because most of the Cd that entered *H. limbata* was detoxified, little was found in the heat-sensitive protein fraction; thus, we would not expect this metal to have produced direct toxic effects. On the one hand, it could be argued that if the energetic cost of producing metal-binding proteins was sufficiently high, there could be have been indirect deleterious effects on nymphs. On the other hand, nymph growth rates were similar among all treatment levels, suggesting that the cost of Cd detoxification was negligible. This ability to detoxify Cd could explain in part the presence of *H. limbata* in Cd-contaminated habitats (Hare et al. 1991*b;* Gosselin and Hare 2004).

Cd concentrations in nymphs transplanted to the Colombière River declined substantially but did not reach those of indigenous nymphs from the Colombière River during our 36-d experiment. This result implies the existence of more than one Cd pool in nymphs. Our data on subcellular partitioning suggest that there is a fast-exchanging pool of Cd bound to heat-stable proteins such as metallothionein and a slow-exchanging pool bound to either or both of the remaining fractions (remainder, heat-denatured proteins).

In conclusion, although our estimates for the elimination rate constant k_E (Eq. 1) can be compared to those for other metals and types of animals, our estimates of the uptake rate constant k_I' are specific to our study site, since they include $[Cd]_{environs}$ (Eq. 2). Knowing where *H. limbata* takes up its Cd (water or sediment) would allow us to determine whether we should consider free Cd ion concentrations in water, some measure of sedimentary Cd, or both to predict $[Cd]_{Hexagenia}$. However, as we have seen, evaluating metal bioavailability in ambient sediment is no simple task, and we suggest that sediment in the gut would better represent the particles chosen by an animal. By digesting gut sediment in natural or artificial digestive fluid (Voparil and Mayer 2004), we could obtain more meaningful measurements of bioavailable sedimentary Cd. Such information would be useful in developing a biomonitoring model that could be applied to *Hexagenia* from a range of sites differing in their $[Cd]_{environs}$. Lastly, although the simplicity of the one-compartment model we used to describe changes in Cd concentrations in *H. limbata* (Eq. 1) is likely to be appreciated by environmental managers, with improvements in knowledge about this insect's metal sources (water vs. food) and its methods for managing Cd at the cellular level, it could prove useful for some purposes to consider several conceptual compartments. For example, a two-compartment model might include Cd uptake from water into the body portion and from food into the gut with appropriate rate constants for Cd uptake and loss from each of these compartments.

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