Dynamics of Cadmium, Lead, and Zinc Exchange between Nymphs of the Burrowing Mayfly *Hexagenia rigida* (Ephemeroptera) and the Environment

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Radioisotopes of cadmium, lead, and zinc added in trace amounts to lake sediments were used to measure the uptake and efflux of these metals from various body parts of nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera). Total metal concentrations in *Hexagenia* and its environment were held constant. A simple model permitted the estimation of rate constants that were used to generate model curves which corresponded closely to the measured trends in trace metal uptake and efflux. There was no measurable accumulation of radioisotopes in gill tissues, suggesting that the gills were not the major organ of metal uptake in *Hexagenia* in this experiment. On the other hand, net uptake of ^{109}Cd and ^{65}Zn by the gut exceeded that by all other body parts in both quantity and concentration terms, suggesting that the primary source of these metals to *Hexagenia* is sediment consumed as food. The rate of exchange of ^{65}Zn was slower than that of ^{109}Cd. ^{210}Pb differed from the other two metals in that it was not detected in the gut, but was found mainly on the body surface.

Des radioisotopes du cadmium, du plomb et du zinc ont été ajoutés en très petites quantités à des sédiments lacustres pour mesurer l'influx et l'efflux de ces métaux traces dans différentes parties du corps de la larve de l'éphémère fouisseuse *Hexagenia rigida* (Ephemeroptera) dans des conditions de quasi état stationnaire (i.e. dans des conditions où les concentrations environnementales et animales de métaux traces demeuraient inchangées). À l'aide d'un modèle simple on a estimé les constantes de vitesse pour la prise en charge et l'élimination des métaux. La dynamique d'échange, telle que simulée avec ces constantes de vitesse, se comparait avantageusement avec l'influx et l'efflux mesurés. Il n'y a pas eu d'accumulation mesurable des radioisotopes dans les branchies, ce qui suggère que les branchies ne sont pas l'organe majeur d'entrée de ces métaux chez *Hexagenia* dans cette expérience. La concentration et la quantité de ^{109}Cd et de ^{65}Zn dans l'intestin excédaient celles des autres parties du corps, ce qui suggère que la source principale de ces métaux pour *Hexagenia* est la nourriture (sédiments). Le taux d'échange du ^{65}Zn était plus faible que celui du ^{109}Cd. Le ^{210}Pb n'a pas été détecté dans l'intestin mais a été retrouvé largement adsorbé sur le corps des larves.

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Organisms are used increasingly as contaminant biomonitors in aquatic systems. The choice of an appropriate biomonitor requires a knowledge of the rates of exchange of contaminants between the candidate organism and its environment. In slow-growing organisms, the biological half-time of pollutants may be on the order of many months (e.g. large bivalve molluscs; Luten et al. 1986; Marigomez and Ireland 1989). Such organisms would be of limited use in short-term biomonitoring studies. In contrast, small, active, fast-growing species, such as insects, have the potential to respond much more rapidly to changes in ambient pollutant concentrations. In the laboratory, aquatic insects achieve within a few days apparent steady state with various concentrations of dissolved mercury (Saouter et al. 1989) and cadmium (Clubb et al. 1975; Dressing et al. 1982; Yamamura et al. 1983). Exchange rates in nature are, however, little known.

Rates of exchange of trace metals between benthic animals and their surroundings are often measured at concentrations far higher than those found in nature. Exchange mechanisms could differ at high and low metal concentrations. In addition, deposit feeders are often studied in water alone which precludes feeding (and uptake of metals by this route) and could alter the uptake

pattern of trace metals from water (Luoma 1983). In general, sediment-bound trace metals are less easily taken up by organisms than those in water; however, high sediment metal concentrations can result in a greater uptake from this source (Luoma 1983). Especially lacking are measurements of pollutant exchange between *freshwater* invertebrates and their environment.

Measurements of contaminant dynamics are also required for the assessment of losses from animals during the laboratory depuration of gut contents prior to contaminant analysis (Hare et al. 1989). The placement of active burrowing organisms in conditions of stress (without sediments) and in the near absence of a pollutant could lead to pollutant loss and a corresponding underestimate of organism pollutant concentrations.

The objective of the present study was to measure the exchange rates (influx and efflux) of trace metal contaminants (cadmium, lead, and zinc) between a freshwater benthic insect, the sediment-feeding mayfly *Hexagenia rigida* (McDunnough) (Ephemeroptera), and its environment. Radioisotopes of the trace metals, added in small quantities to sediments in laboratory microcosms, were used to follow trace metal movements. Exchange rates were measured in individual body parts in order to identify the sites of trace metal storage and exchange and to determine the probable environmental source of pollutants, i.e. from water or food.

Methods

Preparation of Radioactive Sediment and Microcosms

Sediment was collected in January 1987 from a site inhabited by *Hexagenia* (depth 15 m) in a lake subject to aerial fallout from a smelter located 30 km to the west at Rouyn-Noranda in western Quebec (Lake Joannès, 48°11′N, 78°41′W). In the laboratory, approximately 600 μCi (1 μCi = 37 kBq) of each of the radioisotopes ^{109}Cd, ^{210}Pb, and ^{65}Zn was added to 15 L of sediment, which was then mixed using a paddle fitted onto a power drill. The efficacy of the mixing technique was confirmed by the small variation measured among counts of radioactivity in sediment samples (coefficients of variation ≤4% among 10 sediment cores). The sediments were kept in a closed container for 10 mo prior to use in order to obtain uniform specific activities among the various sediment fractions. Since this long equilibration time exceeded the half-life of ^{65}Zn, an additional 600 μCi of ^{65}Zn was added 2 wk prior to the start of the biological uptake experiment to ensure that measurable levels would be obtained in nymphs.

One half litre of radioactive sediment was placed in each of 27 cylindrical (20 cm high, 12 cm diameter), transparent plastic containers ("Frig-O-Seal") which were subsequently filled with 1.5 L of spring water (20 and 5 ppm Ca and Mg, respectively). Nine additional containers were prepared in the same manner using unlabeled sediment from the original batch, for later use in the measurement of radioisotope efflux. All containers were kept covered in the dark at 20°C throughout the experiment. Oxygen levels in the water were maintained at near 100% saturation by constant aeration and pH varied little (7.0–7.5). The containers were left for 2 wk prior to the introduction of nymphs to allow for the oxidation of surficial sediments, as occurs in nature.

The use of radioisotopes allowed the study of trace metal movements among the body parts of the insects without measurably increasing environmental metal concentrations. In addition, variability was reduced as all individuals began the experiment with only background radiation levels.

Rearing and Introduction of Nymphs

Eggs of *H. rigida* taken from adults collected near Lake Winnipeg, Manitoba (supplied by M. Freisen, Freshwater Institute, Winnipeg, Man.), were hatched and the nymphs reared (according to Freisen 1981) at Bordeaux, France, in sediments collected from the nearby Garonne River. After transport to Canada, nymphs were maintained in sediments from Lake St. Joseph, Que. (46°55′N, 71°40′W) for 1 mo prior to use. Nymphs were sieved from the sediment and grouped into four weight classes (ranging from 15 to 60 mg live weight), and one individual from each class was placed into each of the 27 microcosms containing radioactive sediments. This procedure insured that there was little difference among replicates both in terms of mean nymph size (mean wet weight (±SD) = 35.7 ± 1.7 mg) and the range and distribution of nymph sizes (mean coefficient of variation of nymph wet weights (±SD) = 35.1 ± 2.6%).

Influx and Efflux Experiments

The concentration of trace metals in nymphs was measured in three replicate containers at each of 2, 4, 8, 16, 32, and 50 d (influx experiment). After 50 d of exposure, nymphs in the nine remaining radioactive microcosms were removed and redistributed in the nine microcosms containing unlabeled sediment. A uniform size distribution of nymphs was maintained as described above. Trace metal concentrations in the nymphs were then measured after 3, 8, and 16 d (efflux experiment).

On each sampling day, three containers were selected at random and radioactivity was measured, as described below, in both sediment (three cores) and water (10-mL samples, unfiltered and filtered with a 0.4-μm Nuclepore filter). In order to measure the distribution of metals among sediment fractions, the surficial layers (1 cm) of cores collected from containers sampled at days 4, 16, and 50 were subjected to a sequential extraction procedure based on the methods of Tessier et al. (1979) and Belzile et al. (1989). This procedure involves the use of a series of reagents of increasing strength that serve to extract trace metals from increasingly refractory sediment fractions.

Nymphs were removed from the sediments by sieving. Of the four nymphs introduced initially, three or four remained alive at the time of sampling with the exception of one microcosm on day 32 which contained only one nymph (not included in data analyses). Radioactive gut contents were eliminated by placing nymphs for 3 h in beakers containing nonradioactive Lake Joannès sediment to which fluorescent dye particles had been added. Nymphs were removed and examined under a microscope and ultraviolet light to ensure that dye particles were visible along the entire length of the gut.

For dissection, individuals were placed in a small amount of deionized water, an incision was made between the thorax and the head to sever the gut and immobilize the animal, and the body surface was gently scraped as clean as possible of adhering deposits. The gills were removed and the abdomen was opened ventrally in order to remove the gut. For the few individuals that had not depurated the radioactive sediments, the gut was opened to permit their removal. Each body part (gills, gut, remainder) was placed on a small preweighed piece of acid-washed Teflon, frozen at −40°C, dried in a lyophilizer,

and weighed. Details of the dissection procedure are given in Hare et al. (1989). The weight of the gut, without contents, was estimated from body weight using a relationship developed for *Hexagenia limbata* (Hare et al. 1989), i.e. $Y = 0.0952X - 0.0482$, where Y and X are the dry weights (milligrams) of the gut and body, respectively ($r^2 = 0.97$, $n = 10$; for body weights from 0.5 to 15.0 mg). Losses of radioactive metal during dissection represented <5% of the total trace metal burden of all individuals measured on day 50.

Samples were digested individually in screw-cap Teflon vials in concentrated Aristar-grade nitric acid (100 μL·1.0 mg body weight^{-1}) in a water bath at 70°C for 48 h. Digested samples were transferred to either an acid-washed polypropylene 1.5-mL snap-cap scintillation vial (small samples) or a 10-mL polystyrene test tube (larger samples) and diluted with deionized water to a final acid concentration of 16.7% (5:1 v/v). Deionized water for all uses was obtained from a Milli-Q3RO/Milli-Q2 system (Millipore Corp.).

Analyses

Radioactive trace metals were measured in an "LKB Wallac 1282 Compugamma" gamma particle counter (NaI(Tl) well type). Correction was made for radioactive decay and for background radiation. Only net values >3 SD of the mean of 10 blank samples were retained. Counts of ^{109}Cd and ^{210}Pb were corrected for interference from each of the other radioisotopes present (counts of ^{65}Zn were unaffected by the other metals). Samples of gill tissue did not in most cases exceed background values, even when the gills of all individuals from one sampling day were pooled. Counts of ^{210}Pb in the gut were also below background once corrected for interference from the other trace metals. Counting efficiencies (^{109}Cd 60%, ^{210}Pb 3%, ^{65}Zn 9%) were determined in order to convert counts per minute (CPM) values to disintegrations per minute (DPM).

To determine if steady-state conditions prevailed during the experiment, total trace metal concentrations in nymphs were measured by atomic absorption spectrophotometry, either by flame atomization (Zn; Varian AA-575) or in a graphite tube furnace (Cd; Varian AA-1275 with GTA-95 graphite tube atomizer). Correction was made for the contribution of gut contents to trace metal concentrations in gut tissues using the calculation method given in Hare et al. (1989). Measures of similar-sized samples of a certified reference material (lobster hepatopancreas, TORT-1, National Research Council of Canada, Ottawa, Ont.) varied little over time (coefficient of variation 3–7%; $n = 10$ for each metal) and were close to the expected certified values (within 3.5–6.0% of expected means; $n = 10$ for each metal). Total lead could not be accurately measured with the digestion method used.

Results

Distribution of Radioisotopes in Sediments and Water

A radioisotope can be used to follow the movement of its nonradioactive counterpart provided that the system is at steady state, both over time and with respect to the distribution of the isotopes. This is a necessary condition if the biological uptake of the two forms is to be comparable.

There was little or no change in specific activity *within* each sediment fraction during the course of the experiment, suggesting that the bioavailability of the isotopes of each metal varied little over time (Fig. 1). In addition, isotope distributions

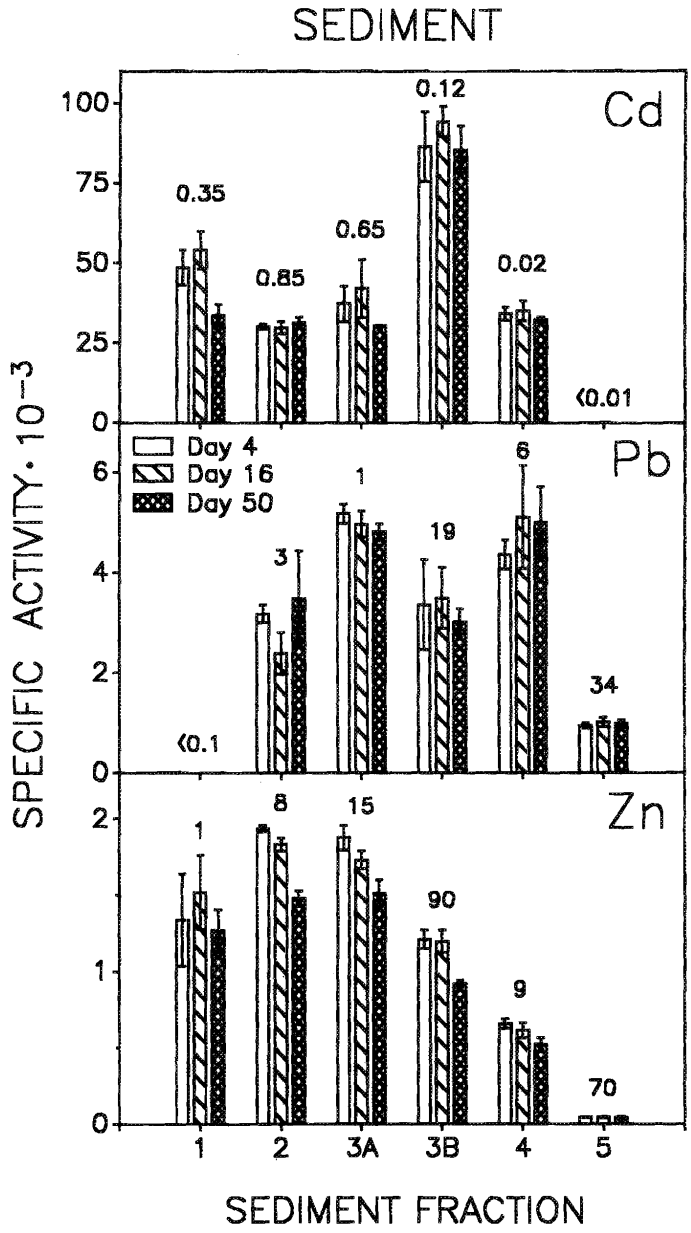

FIG. 1. Mean (±SD) specific activities (DPM radioactive metal·μg total metal^{-1}) of Cd, Pb, and Zn in fractions of surficial sediments collected on days 4, 16, and 50 of the experiment. Mean total metal concentrations (μg·g^{-1}) are indicated above the histograms for each fraction. Fractions correspond to successive extractions of the sediment with (1) $MgCl_2$, (2) acetate buffer, (3A) $NH_2OH{\cdot}HCl$ at room temperature, (3B) $NH_2OH{\cdot}HCl$ at 96°C, (4) H_2O_2/HNO_3, and (5) $HNO_3/HClO_4$.

among sediment fractions were largely at steady state by the beginning of the experiment, as evidenced by the similarity among the specific activities of the more weakly bound sediment fractions (Fig. 1). The strength of metal/sediment associations approximately increases with increasing fraction number. In contrast, in the most tightly bound fraction (5) the specific activities of Pb and Zn were low and ^{109}Cd was not detectable. This indicates that little radioactive metal had been incorporated into fraction 5 which is mainly not bioavailable. In fraction 4 (extraction with $HNO_3/HClO_4$), the specific activity of Zn was somewhat lower than in preceding fractions, a consequence perhaps of the late addition of a portion of the

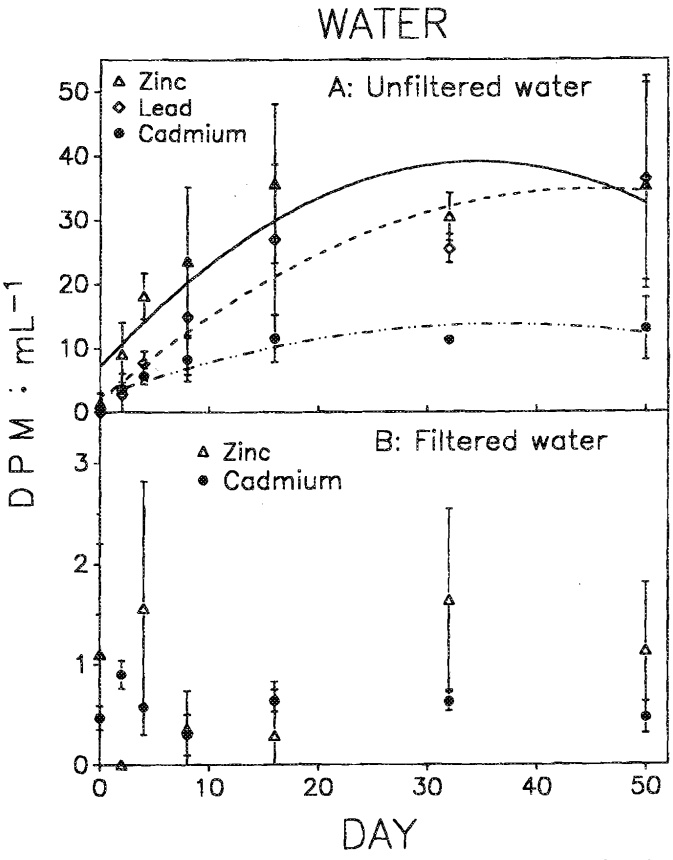

FIG. 2. Changes over time in the concentrations ($\bar{x} \pm$ SD) of radioactive trace metals in (A) unfiltered and (B) filtered microcosm water. Pb was not detected in filtered water.

radioactive Zn. In fraction 3b (extraction with $NH_2OH \cdot HCl$) the specific activity of Cd was anomalously high. However, since the bulk of the Cd was associated with previous fractions (85% of ^{109}Cd in fractions 1–3a), this difference probably had little biological importance. These observations suggest that the radioisotopes had attained a steady state with respect to their nonradioactive counterparts.

All of the radioisotopes were distributed uniformly with depth in sediment cores collected both early (2 d) and late (32 d) in the exposure period (coefficient of variation <7% among all core subsamples from three depths on both sampling days). This suggests that nymphs feeding at different depths in the sediment would not have been exposed to different total radioisotope concentrations.

The quantity of radioactive sediment suspended in the water increased during the early part of the experiment (Fig. 2A), as a consequence of the burrowing and feeding activities of the nymphs. However, the radioactivity of filtered water did not increase (Fig. 2B), indicating that trace metals adsorbed onto particles in the water did not readily pass into solution.

Uptake and Depuration of Trace Metals

As expected, total concentrations of Cd and Zn in body parts did not show significant trends over time (Fig. 3). Thus the radioactive trace metal dynamics observed can be considered to be representative of those at steady state.

Animals took up the radioisotopes of Cd, Pb, and Zn rapidly during the early part of the experiment, after which time net uptake slowed as an apparent steady state between radioisotope influx and efflux was reached (Fig. 4). The general form of the net uptake curves is similar for the gut and for the body (animal minus gut and gills) for both ^{109}Cd and ^{65}Zn. Radioactive Pb in the gut was below the detection limit, as were all three radioisotopes in the gills.

The concentrations of Cd and Zn were always much higher in the gut than in the body (Fig. 3 and 4), suggesting that the major entry site of Cd and Zn is the gut. Furthermore, the ratio of ^{65}Zn in the gut to that in the body declined during the first 1–2 wk of the experiment (Fig. 5), suggesting an initial entry via the gut and a subsequent transfer to the body. Such a trend was not observed for Cd (Fig. 5), suggesting that there was little movement of Cd from the gut to the body or, and perhaps more likely, that such movement occurred but was too rapid to be detected given the time scale of the experiment. Gut metal concentrations greatly exceeded those in the sediment, i.e. the ratios of gut/sediment concentrations at near zero net uptake were Cd 45 and Zn 15. Pb was not detected in the gut. In contrast, body metal concentrations were somewhat lower than those in the sediment (ratio body/sediment: Cd 0.35, Zn 0.50, and Pb 0.20).

Animals labeled for 50 d and then transferred to nonradiolabeled sediments lost radioactive trace metals from both the gut and the body (Fig. 6).

Discussion

Kinetics of Metal Exchange

Nymphs readily gained and lost radioisotopes in the two phases of the experiment, indicating a continuous exchange of Cd, Pb, and Zn between *Hexagenia* and its environment. A model based on first-order kinetics for both influx and efflux can be used to describe these exchanges for either the body or the gut. According to this simple model, net uptake can be written as follows:

$$(1) \quad \frac{d[M]_a}{dt} = k_I \cdot [M]_e \text{ (influx)} - k_E \cdot [M]_a \text{ (efflux)}$$

where k_I and k_E are first-order rate constants for the influx and efflux of trace metals, respectively, and $[M]_e$ and $[M]_a$ represent the concentrations of trace metal in the external environment and in animal tissues, respectively. Equation (1) holds for the "cold" metal (M) or for the radioisotopes (*M). The values of the constants k_E and k_I can be estimated using the experimental data. First, k_E is obtained from the efflux experiments for which Eq. (1) reduces to

$$(2) \quad \frac{d[*M]_a}{dt} = -k_E \cdot [*M]_a$$

since $[*M]_e$ is virtually 0 in the efflux experiment. The latter equation is readily integrated to

$$(3) \quad [*M]_a = [*M]_a^0 \, e^{-kEt}$$

where the index zero refers to initial conditions. The values of k_E obtained from regression of $\ln [*M]_a$ versus time (t) are given in Table 1.

The rate constants k_I can be estimated from the data obtained early in the influx experiments, i.e. from those data for which $[*M]_a$ is low enough so that the second term in Eq. (1) is much smaller than the first one. Equation (1) then reduces to

$$(4) \quad \frac{d[*M]_a}{dt} = k_I \cdot [*M]_e = k'_I$$

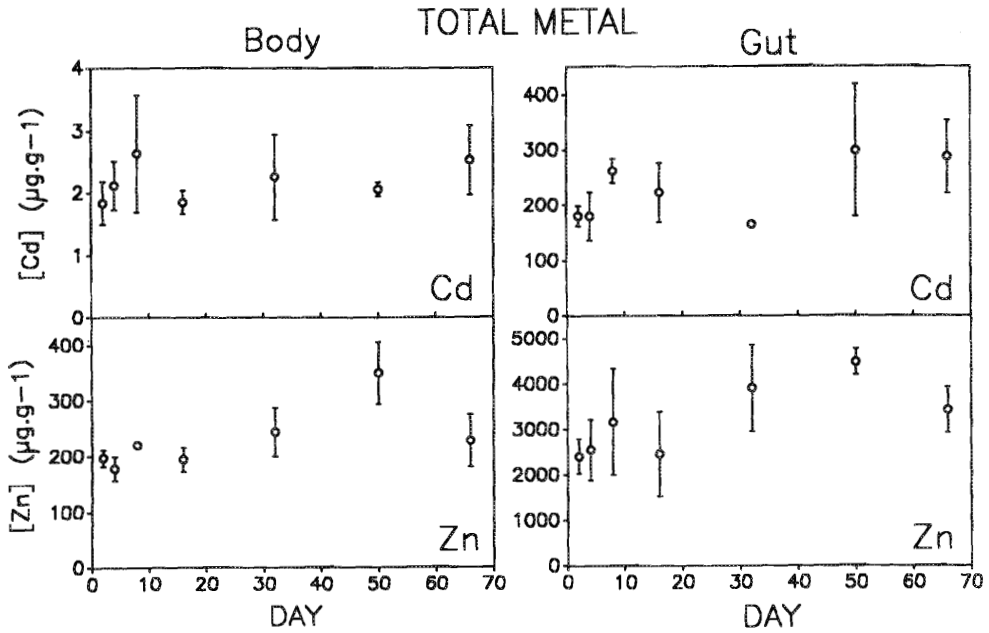

FIG. 3. Changes throughout the course of the experiment in the total concentrations ($\bar{x} \pm$ SD; μg·g dry weight^{-1}) of Cd and Zn in the body and gut of *Hexagenia*. Nymphs were transferred from radiolabeled to nonradiolabeled sediments on day 50. The mean and standard deviations of values for three microcosms (two on day 32) are presented on each sampling day.

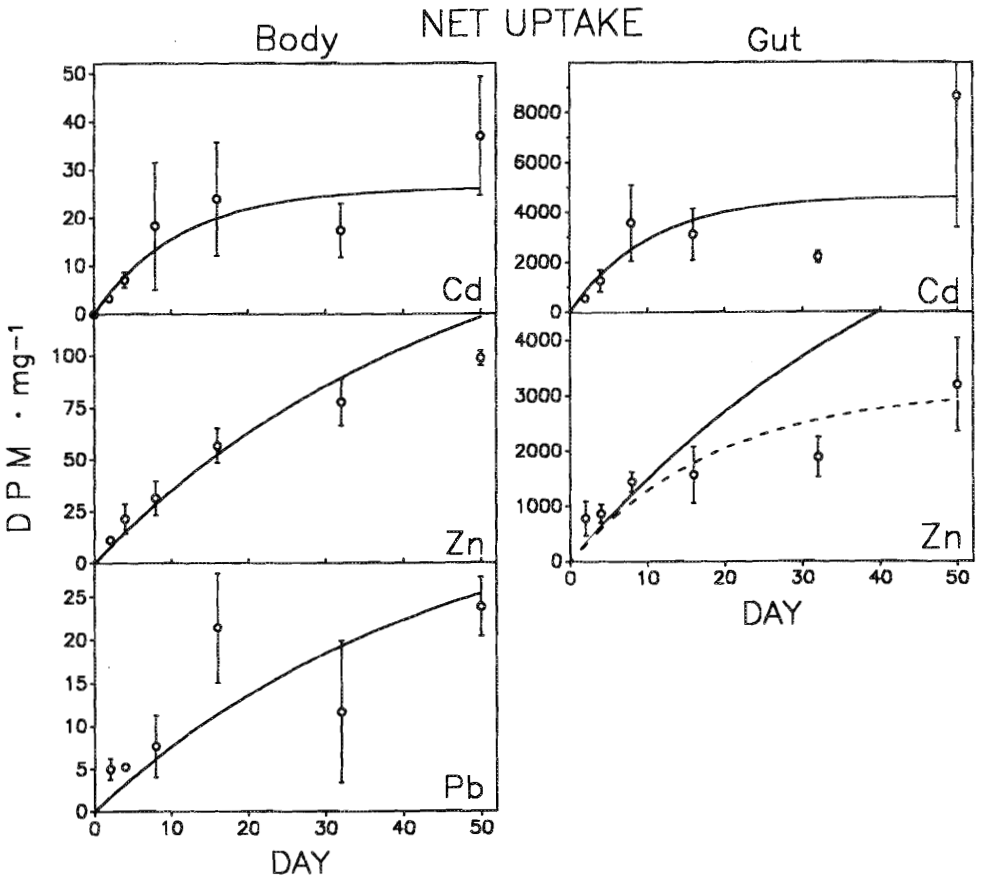

FIG. 4. Net uptake over time of radioactive Cd, Zn, and Pb in the body and gut of *Hexagenia* held in radiolabeled lake sediments for 50 d. The mean and standard deviations of concentrations (DPM·mg dry weight^{-1}) for three microcosms (two on day 32) are presented on each sampling day. Model curves were generated according to Eq. (6); curves for Zn in the gut were generated either with k_E derived from experimental data from days 0–16 (solid curve) or from days 0–8 (broken curve).

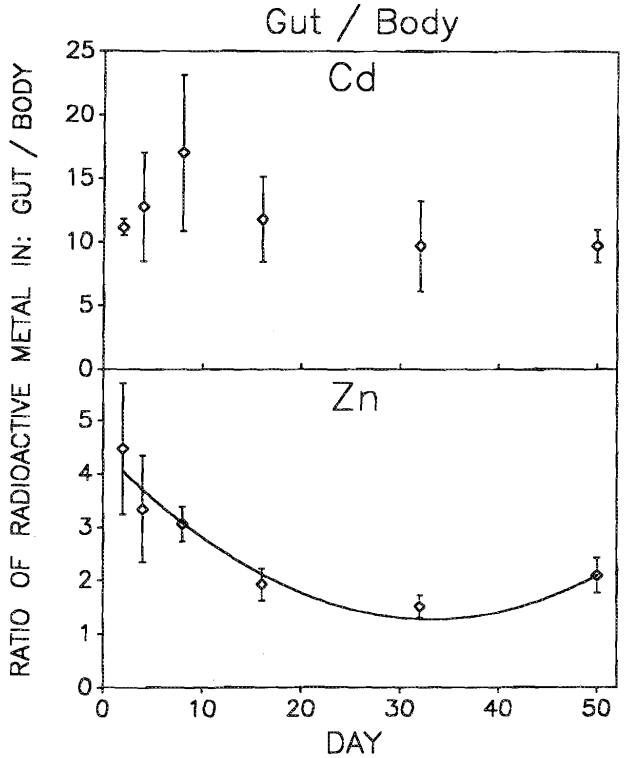

FIG. 5. Changes over time in the ratios of the quantities of radioactive Cd and Zn in the body to those in the gut of *Hexagenia* held in radiolabeled lake sediments for 50 d. The mean and standard deviations of values for three microcosms (two on day 32) are presented on each sampling day. A curvilinear regression for Zn data was generated by the software package Sigmaplot.

Since $[*M]_e$ was almost constant during the experiment because the animals took up only a tiny fraction of the radioisotopes added to the system, the influx has been represented as a single term k'_I in Eq. (4). Integration of Eq. (4) leads to

$$(5) \quad [*M]_a = k'_I\, t.$$

As expected from Eq. (5), approximately straight lines are observed early in the uptake experiments (days 0–8, Fig. 4), the slopes of which were taken as estimates of the apparent rate constant k'_I (Table 1). Model curves were generated (Fig. 4 and 6) from the k'_I and k_E values given in Table 1 and the appropriate values of $[*M]_a^0$ by solving Eq. (1) for $[*M]_a$:

$$(6) \quad [*M]_a = k'_I / k_E - (k'_I / k_E - [*M]_a^0)e^{-kEt}$$

The net uptake of Zn by the gut was best described by a model curve generated with a value of k_E estimated without the last data point in the efflux experiment (i.e. day 16, broken curves in Fig. 4 and 6).

The internal consistency of the values of k'_I and k_E can be checked in the following manner. By definition, $[*M]_a$ at steady state is constant over time and thus Eq. 1 becomes

$$\frac{d[*M]_a}{dt} = k_I \cdot [*M]_e \text{ (influx)} - k_E \cdot [*M]_a \text{ (efflux)} = 0$$

or

$$(7) \quad k_I \cdot [*M]_e = k_E \cdot [*M]_a^{ss}$$

where $[*M]_a^{ss}$ is the steady-state metal concentration in a body part. Since $k_I \cdot [*M]_e$ was defined in Eq. (4) as k'_I, Eq. (7) can be rewritten as

$$(8) \quad \frac{k'_I}{k_E} = [*M]_a^{ss}.$$

The similarity of the ratios of the influx and efflux constants, as given in Table 1, to the measured steady-state concentrations in the various body parts, as shown in Fig. 4, is reassuring. As a final check, the ratios of the influx and efflux constants (i.e. $[*M]_a^{ss}$) can be divided by the sediment radioisotope concentrations ($[*M]_{sed}$) to obtain steady-state bioconcentration factors (Table 1). These values should be comparable with bioconcentration factors of "cold" metals (ie. $[M]_a/[M]_{sed}$), as given in Table 1. Although the two bioconcentration factors are of similar magnitude, the radioisotope bioconcentration factors are consistently lower than those for the "cold" metal, suggesting that a portion of the total metal present initially in the nymphs had a slower exchange rate with the environment than did the remainder. The notion of internal "pools" of a trace metal differing in exchange rates has been proposed for several marine invertebrates (e.g. Bryan and Hummerstone 1973). The location (intra- versus extracellular, e.g. Andersen and Baatrup 1988) and form (e.g. granules, Brown 1982; protein bound, Yamamura et al. 1983) in which slowly exchanging pools of metals might be sequestered in aquatic insects are not yet known.

The biological half-times ($t_{1/2}$) of each metal in each body part can be estimated from Eq. (3). These correspond to about 1 wk for Cd and longer times (2 wk to 1 mo) for Zn and Pb (Table 1). These data suggest that if live organisms are left in metal-free water for several days in order to remove contaminated gut contents (Hare et al. 1989), losses of Cd might be substantial while those of Zn and Pb should be less.

In general, the model curves fit the experimental data well, suggesting that this simple model is sufficient to characterize the rates of net metal uptake by *H. rigida* in the experimental system. Apparent steady states should be reached in 1–3 mo. This suggests a time frame for the turnover of the pools of exchangeable trace metals under the experimental conditions which were comparable with those in the littoral zone of a temperate lake during summer. It is to be expected that at other temperatures, metabolic and feeding rates would change (Zimmerman and Wissing 1978) with concomitant effects on trace metal exchange rates (Mishima and Odum 1963; Van Dolah et al. 1987; Douben 1989).

It is acknowledged that the direct extrapolation of laboratory results to nature must be made with caution, e.g. mean nymph weight did not increase during the present experiment, suggesting that the animals may have been stressed. In nature, trace metal exchange rates could also vary with changes in environmental conditions (Van Dolah et al. 1987), the concentrations of other metals (Wicklund et al. 1988), as well as with an individual organism's history of pollutant exposure (Bryan and Hummerstone 1971). Furthermore, extrapolations from one metal to another must be made with caution, since the dynamics of metals such as Zn, which is an essential micronutrient, are likely to differ from those of other metals such as Cd and Pb, which are metabolic poisons (Amiard et al. 1987; Rainbow 1988).

Metal Dynamics in the Gills and Gut

Exchange rates were measured for individual body parts in order to estimate trace metal sources; metals in the gills or gut

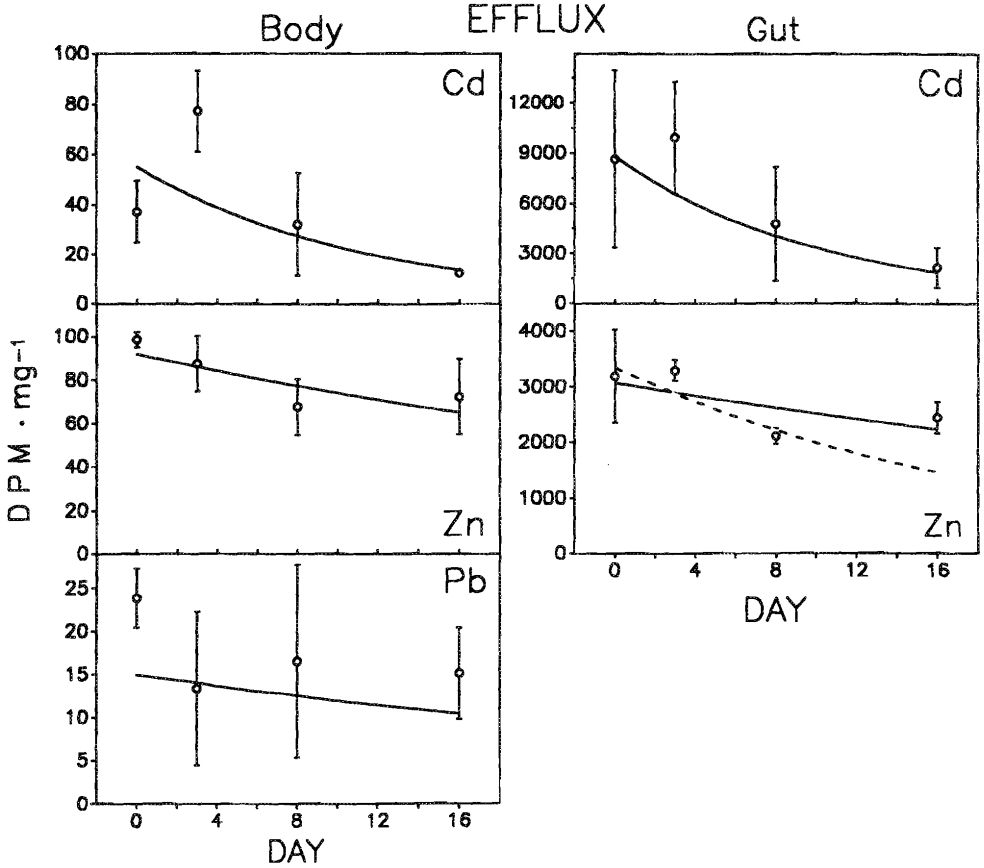

FIG. 6. Efflux of radioactive Cd, Zn, and Pb from the body and gut of *Hexagenia* in nonradiolabeled lake sediment. Nymphs had been previously held in radiolabeled lake sediments for 50 d. The mean and standard deviations of concentrations (DPM·mg dry weight^{-1}) for three microcosms are presented on each sampling day. Model curves were generated according to Eq. (3); curves for Zn in the gut were generated either with k_E derived from experimental data from days 0–16 (solid curve) or from days 0–8 (broken curve).

TABLE 1. Estimated values (±SE) of various model terms for trace metal exchange in nymphs of *H. rigida*. Sediment trace metal concentrations, $[M]_{sed}$ and $[*M]_{sed}$, are the sum of metals in sediment fractions 1–4 (i.e. "residual" trace metals (fraction 5) omitted).

		Body			Gut		
Term	Symbol	Cd	Zn	Pb	Cd	Zn	Zn[a]
Efflux rate constant (d^{-1})	k_E	0.088 (±0.028)	0.022 (±0.011)	0.022 (±0.051)	0.098 (±0.034)	0.020 (±0.010)	0.052 (±0.021)
Biological half-time (d)	$t_{1/2}$	8	32	31	7	35	14
Apparent influx rate constant (DPM·mg^{-1}·d^{-1})	k'_1	2.3 (±0.7)	3.9 (±0.6)	0.85 (±0.24)	450 (±90)	160 (±30)	160 (±30)
k'_1/k_E		25	175	38	4600	8000	3100
$(k'_1/k_E)/[*M]_{sed}$		0.35	1.15		60	50	20
$[M]_a/[M]_{sed}$		1.1	1.9		110	27	27

[a]Alternate values for gut Zn are based on data from days 0, 3, and 8 only (i.e. day 16 omitted).

are likely to have been taken up from water or food, respectively. Uptake from these sources is thought to be both independent and additive (Giesy et al. 1980; Hattum et al. 1989). The large volumes of water passing over the gills of *Hexagenia* could favour the uptake of trace metals from water. However, radioactivity in the gills of *Hexagenia* never exceeded back-

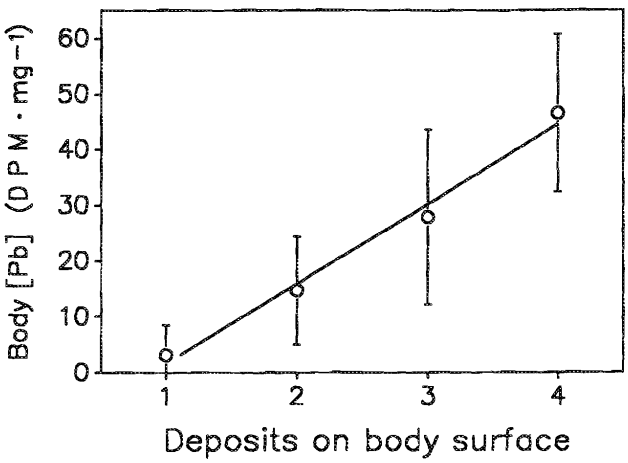

FIG. 7. Relationship between the concentration of Pb (DPM·mg dry weight^{-1}) associated with the body of *Hexagenia* and the age of its exoskeleton, as estimated from the extent of deposits on the body surface using a visual scale from 1, no deposit, to 4, a heavy deposit ($r^2 = 0.64$, $n = 59$ organisms sampled on days 16–66, $\bar{x} \pm$ SD).

ground levels of Cd, Pb, and Zn. In nature, aquatic insects also tend to have low concentrations of these trace metals in respiratory structures (L. Hare, A. Tessier, and P. G. C. Campbell, in review).

The radioactivities of Cd and Zn in the gut of *Hexagenia* were greater at all times than those in the body, both in quantity and in concentration. Furthermore, the influx rate constants are about 2 orders of magnitude higher for the gut than for the body (Table 1), suggesting that gut tissues are the major site of storage and entry of these metals and that they are taken up largely from sediment consumed as food.

External versus Internal Localisation of Metals

Negative relationships between insect body size and trace metal concentrations have been taken to indicate that a large proportion of the metals is adsorbed externally (Smock 1983). In the present study, highly significant correlations were not observed for nymphs on any sampling day for any of the metals studied (except for Cd on day 50, $r^2 = 0.5$), *suggesting* that in our study the surface adsorption of these metals is quantitatively unimportant.

The inference of surface adsorption from body size – metal concentration relationships is based on the assumption that metabolic rates do not vary with body size, which is generally not the case (Zimmerman et al. 1975; Zimmerman and Wissing 1978). Secondly, it is assumed that the rate of surface adsorption is rapid compared with the time span of the larval or nymphal instar. This may not be the case for organisms which pass through many instars (such as mayflies like *Hexagenia*; Fink 1980) and/or where metal association with the body surface occurs by continuous adsorption. The need for this second assumption can be circumvented if the age of the nymphal skin (i.e. time since the previous moult) and not the size of the insect is used in such comparisons.

In the present experiments, the extent of iron oxide deposition on the body surfaces of *Hexagenia* was used as an index of nymphal skin age. These deposits were partially removed prior to dissection; however, removal was not complete, since a strong correlation was observed between the concentration of Fe in and on the body after dissection (data not shown) and a visual estimate of the extent of iron oxide coverage prior to dissection ($r^2 = 0.64$; visual scale from 1, no deposit, to 4, heavy deposit). There was little or no correlation between the visual scale and concentrations of Cd and Zn associated with the body ($r^2 = 0.16$ and 0.01, respectively), suggesting, as concluded above, that only minor proportions of these metals are adsorbed on the body surface. However, a strong positive relationship *was* observed for Pb (Fig. 7). Consistent with these trends, body Fe concentrations were highly correlated with those of Pb ($r^2 = 0.7$) but not Cd and Zn ($r^2 = 0.01$ for both).

These results suggest that much of the body Pb was located externally, in association with iron oxides, a fact which was not apparent when body size rather than an index of skin age was considered. Consequently, much of the variability among replicate measures of Pb (see Fig. 4 and 6 and Table 1) can be related to differences among nymphs in the time since their previous moult, i.e. the age of their skin. The differences observed among metals are consistent with the fact that Pb is more strongly bound to iron oxyhydroxides, which characterize the deposits, than are Cd and Zn (Leckie et al. 1983). These results suggest that Pb in sediments may have little direct toxic effect on *Hexagenia*; however, indirect effects of Pb on the sediment microflora consumed by *Hexagenia*, or on its predators, could be important.

Conclusions

A model based on first-order kinetics was adequate to describe the exchange of Cd, Pb, and Zn between nymphs of *H. rigida* and their surroundings. The exchange rates measured suggest that aquatic insects such as *Hexagenia* may be useful in monitoring changes over weeks or months. Since Cd and Zn appear to be taken up largely by the gut from sediment consumed as food, concentrations of these metals in *Hexagenia* would most likely reflect those in the sediment. In contrast, Pb associated with nymphs is largely bound with iron oxides adhering to their body surface. Given the fairly rapid exchange rates measured for Cd, depuration of nymphs prior to Cd analysis should be limited to a few days.

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References

AMIARD, J. C., C. AMIARD-TRIQUET, B. BERTHET, AND C. METAYER. 1987. Comparative study of the patterns of bioaccumulation of essential (Cu, Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. J. Exp. Mar. Biol. Ecol. 106: 73–89.

ANDERSEN, J. T., AND E. BAATRUP. 1988. Ultrastructural localization of mercury accumulations in the gills, hepatopancreas, midgut, and antennal glands of the brown shrimp. *Crangon crangon*. Aquat. Toxicol. 13: 309–324.

BELZILE, N., P. LECOMTE, AND A. TESSIER. 1989. Testing readsorption of trace elements during partial chemical extractions of bottom sediments. Environ. Sci. Technol. 23: 1015–1020.

BROWN, B. E. 1982. The form and function of metal-containing 'granules' in invertebrate tissues. Biol. Rev. 57: 621–667.

BRYAN, G. W., AND L. G. HUMMERSTONE. 1971. Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of heavy metals. I. General observations and adaptation to copper. J. Mar. Biol. Assoc. U.K. 51: 845–863.

1973. Adaptation of the polychaete *Nereis diversicolor* to manganese in estuarine sediments. J. Mar. Biol. Assoc. U.K. 53: 859–872.

CLUBB, R. W., A. R. GAUFIN, AND J. L. LORDS. 1975. Acute cadmium toxicity studies upon nine species of aquatic insects. Environ. Res. 9: 332–341.

DOUBEN, P. E. T. 1989. Uptake and elimination of waterborne cadmium by the fish *Noemacheilus barbatulus* L. (stone loach). Arch. Environ. Contam. Toxicol. 18: 576–586.

DRESSING, S. A., R. P. MAAS, AND C. M. WEISS. 1982. Effect of chemical speciation on the accumulation of cadmium by the caddisfly, *Hydropsyche* sp. Bull. Environ. Contam. Toxicol. 28: 172–180.

FINK, T. J. 1980. A comparison of mayfly (Ephemeroptera) instar determination methods, p. 367–380. *In* J. F. Flannagan and K. E. Marshall [ed.] Advances in Ephemeroptera biology. Plenum Press, New York, NY.

FRIESEN, M. K. 1981. *Hexagenia rigida* (McDunnough), p. 127–142. *In* S. G. Lawrence [ed.] Manual for the culture of selected freshwater invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. 54.

GIESY, J. P., J. W. BOWLING, AND H. J. KANIA. 1980. Cadmium and zinc accumulation and elimination by freshwater crayfish. Arch. Environ. Contam. Toxicol. 9: 683–697.

HARE, L., P. G. C. CAMPBELL, A. TESSIER, AND N. BELZILE, 1989. Gut sediments in a burrowing mayfly (Ephemeroptera, *Hexagenia limbata*): their contribution to animal trace element burdens, their removal, and the efficacy of a correction for their presence. Can. J. Fish. Aquat. Sci. 46: 451–456.

HATTUM, B. VAN, P. DE VOOGT, L. VAN DEN BOSCH, N. M. VAN STRAALEN, E. N. G. JOOSSE, AND H. GOVERS. 1989. Bioaccumulation of cadmium by the freshwater isopod *Asellus aquaticus* (L.) from aqueous and dietary sources. Environ. Pollut. 62: 129–151.

LECKIE, J. O., D. T. MERRILL, AND W. CHOW. 1983. Trace element removal from power plant wastestreams by adsorption/coprecipitation with amorphous iron oxyhydroxide. AIChE Symp. Ser. 81(243): 28–43.

LUOMA, S. N. 1983. Bioavailability of trace metals to aquatic organisms — A review. Sci. Total Environ. 28: 1–22.

LUTEN, J. B., W. BOUQUET, M. M. BURGGRAAF, AND J. RUS. 1986. Accumulation, elimination, and speciation of cadmium and zinc in mussels, *Mytilus edulis*, in the natural environment. Bull. Environ. Contam. Toxicol. 37: 579–586.

MARIGOMEZ, J. A., AND M. P. IRELAND. 1989. Accumulation, distribution and loss of cadmium in the marine prosobranch *Littorina littorea* (L.). Sci. Total Environ. 78: 1–12.

MISHIMA, J., AND E. P. ODUM. 1963. Excretion rate of Zn^{65} by *Littorina irrorata* in relation to temperature and body size. Limnol. Oceanogr. 8: 39–44.

RAINBOW, P. S. 1988. The significance of trace metal concentrations in decapods, p. 291–313. *In* A. A. Fincham and P. S. Rainbow [ed.] Aspects of decapod crustacean biology. Clarendon Press, Oxford Symp. Zool. Soc. London 59.

SAOUTER, E., F. RIBEYRE, AND A. BOUDOU. 1989. Bioaccumulation of mercury compounds ($HgCl_2$ and CH_3HgCl) by *Hexagenia rigida* (Ephemeroptera). Heavy metals in the Environment, Seventh Int. Conf., Geneva, Proceedings, Vol. 1: 378–381.

SMOCK, L. A. 1983. Relationships between metal concentrations and organism size in aquatic insects. Freshwater Biol. 13: 313–321.

TESSIER, A., P. G. C. CAMPBELL, AND M. BISSON. 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51: 844–851.

VAN DOLAH, F. M., T. C. SIEWICKI, G. W. COLLINS, AND J. S. LOGAN. 1987. Effects of environmental parameters on the elimination of cadmium by eastern oysters, *Crassostrea virginica*. Arch. Environ. Contam. Toxicol. 16: 733–743.

WICKLUND, A., P. RUNN, AND L. NORRGREN. 1988. Cadmium and zinc interactions in fish: effects of zinc on the uptake, organ distribution, and elimination of ^{109}Cd in the zebrafish, *Brachydanio rerio*. Arch. Environ. Contam. Toxicol. 17: 345–354.

YAMAMURA, M., K. T. SUZUKI, S. HATAKEYAMA, AND K. KUBOTA. 1983. Tolerance to cadmium and cadmium-binding proteins induced in the midge larva *Chironomus yoshimatsui* (Diptera, Chironomidae). Comp. Biochem. Physiol. 75C: 21–24.

ZIMMERMAN, M. C., AND T. E. WISSING. 1978. Effects of temperature on gut-loading and gut-clearing times of the burrowing mayfly, *Hexagenia limbata*. Freshwater Biol. 8: 269–277.

ZIMMERMAN, M. C., T. E. WISSING, AND R. P. RUTTER. 1975. Bioenergetics of the burrowing mayfly, *Hexagenia limbata*, in a pond ecosystem. Verh. Int. Ver. Limnol. 19: 3039–3049.