

Cadmium sources and exchange rates for *Chaoborus* larvae in nature

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Abstract

Although freshwater insects are known to accumulate trace metals in the laboratory from both water and food, the relative importance of metal sources for these animals, as well as the rate at which they take up and eliminate their metal, has not been measured in nature. We describe a novel in situ approach that allowed us to determine that trophic transfer is the main source of cadmium for larvae of a common lake-dwelling animal, the phantom midge *Chaoborus punctipennis*. We transferred *C. punctipennis* larvae from a low-cadmium to a high-cadmium lake, where they were exposed in 64- μ m-mesh mesocosms to the prevailing high-Cd concentrations in water and to various quantities of prey collected from the Cd-rich lake. Our experimental design ensured exposure of *C. punctipennis* larvae to realistic Cd concentrations in water and in a natural mixture of prey types. Our results indicate that larvae take up their Cd mainly from prey. Thus models of metal dynamics and effects on these invertebrates are likely to be more realistic if they include food as a metal source. Using the same mesh mesocosm design, we also determined that *C. punctipennis* larvae transferred from a high-Cd to a low-Cd lake lost their Cd slowly. Combining our information on Cd uptake and loss from *C. punctipennis* allowed us to model Cd exchange between this insect and its surroundings.

Aquatic systems directly influenced by mining, metal smelting, and other industrial activities have been contaminated by the thousands with potentially toxic trace metals such as cadmium, copper, and lead (Pacyna et al. 1995). Trace metals are also transported long distances in the atmosphere (Nriagu and Pacyna 1988), thereby reaching aquatic ecosystems distant from local sources (Franzin et al. 1979; Verta et al. 1986). The wide dispersion and potential toxicity of metals require that their impact on aquatic organisms be evaluated.

The biological effects of metals on aquatic animals are most often assessed by exposing individuals in the laboratory to aqueous metal in the absence of metal-contaminated food. Exposures of this type assume by design that metal uptake from food is negligible (Luoma 1995). Rigorous demonstrations to support this assumption are few, owing in large part to the technical difficulties involved in unambiguously separating food and water as metal sources to animals (Kay 1985; Fisher and Reinfelder 1995). One exception is a recent laboratory experiment in which water and food (a planktonic crustacean) were successfully separated as cadmium (Cd) sources for the predatory insect *Chaoborus punctipennis* (Munger and Hare 1997). In this experiment, the predator was shown to accumulate Cd solely from its prey, which brings into question the realism of experiments in

which such animals are exposed to this metal in water only. However, the conclusions of laboratory experiments cannot be readily extrapolated to reliably model the transfer of metals along food webs in ecosystems. The handling of animals can produce experimental artifacts, and conditions in the field (metal bioavailability, food webs) are more complex than those in the laboratory (Taylor 1983; Kay 1985; Bothwell et al. 1994).

In nature, Cd concentrations in *Chaoborus* larvae have been related to those in water (Hare and Tessier 1996, 1998; Croteau et al. 1998). However, such a relationship cannot be used to affirm that this insect takes up its metal directly from water, because a correlation could also occur if metal concentrations in food are correlated with those in water. Here we use a novel experimental approach to determine whether the Cd taken up by larvae of *Chaoborus punctipennis* in nature comes from their food or from water. Our approach also allowed us to estimate Cd-influx and -efflux rates from this predator, so as to model Cd exchange between the predator and its environment. Reliable measurements of metal sources to animals in nature would improve our ability both to model metal movements along food webs and to relate metal concentrations in animals to those in their environment (a prerequisite to using animals as metal biomonitors).

Methods

Experimental design—We transferred larvae between low- and high-Cd lakes, where they were held in mesh mesocosms (64- μ m-mesh aperture) that allowed the free passage of lake water but restricted the movement of planktonic crustaceans that are the major food source for late instars of this insect (Moore 1988). Larvae in mesocosms were offered various quantities of crustacean prey and their uptake or loss of Cd was measured. We chose lakes for our study (Table 1) based on (1) the presence of native populations of *C. punctipennis*, (2) their similar pH, and (3) the higher con-

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Table 1. Cadmium content of fourth-stage larvae of *Chaoborus punctipennis* (mean \pm SD), pH, and dissolved Cd, Ca, and organic matter (DOC) concentrations in the high-Cd lake, Crowley Lake (46°23'N, 80°59'W), and in the two low-Cd lakes, Lake St. Joseph (46°55'N, 71°40'W) and Lake au Cèdre (46°54'N, 71°43'W).

Variable	High-Cd lake (Crowley)	Low-Cd lake (St. Joseph)	Low-Cd lake (au Cèdre)
Cd content of <i>C. punctipennis</i> (ng larva ⁻¹ \pm SD)	2.9 \pm 0.3	0.2 \pm 0.1	0.02 \pm 0.01
Total dissolved Cd (nM)	1.0	0.15	<0.1
pH	6.4	6.4	6.7
DOC (mg C L ⁻¹)	1.3	2.9	4.9
Ca (μ M)	69	97	51

centrations of Cd in lake water and indigenous *C. punctipennis* larvae from one of the lakes (Crowley Lake), likely a result of its location within 10 km of the mining and metal-refining complex at Sudbury, Ontario (Gunn et al. 1995). Because pHs were similar among lakes and the high-Cd lake had much higher dissolved Cd concentrations and lower concentrations of organic ligands (dissolved organic carbon; Table 1), free Cd-ion concentrations were undoubtedly much higher in the high-Cd lake than in the other study lakes (Hare and Tessier 1996).

Mesocosms (Fig. 1) were filled by the passive entry of lake water through the Nitex netting making up their sides.

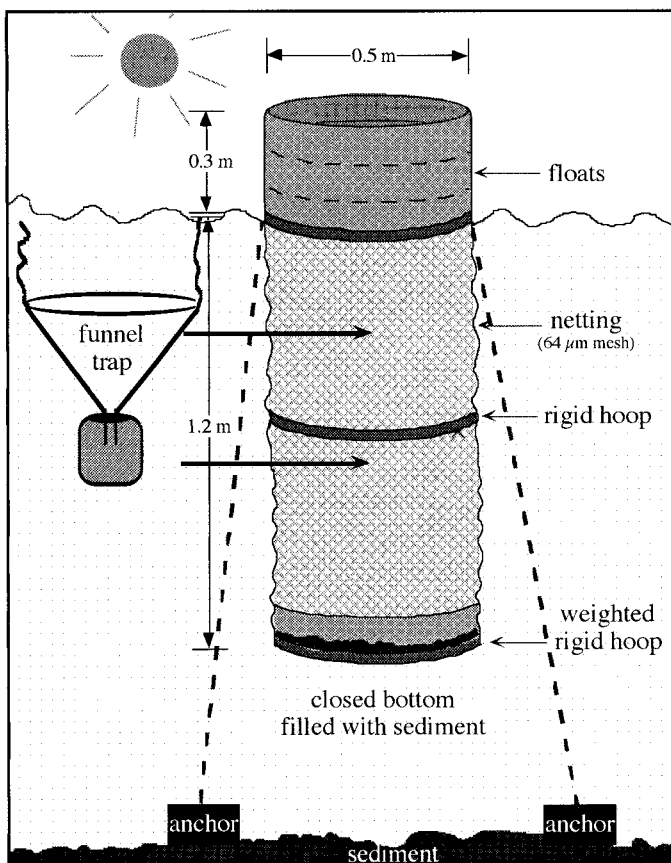


Fig. 1. Drawing of a mesocosm and the funnel trap used in our in situ experiments.

Each mesocosm was anchored individually in the littoral zone (3-m depth) and buoyed at the surface to preclude the entry of water and prey by wave action. Final instar larvae of *C. punctipennis* were collected with a plankton net (64- μ m-mesh aperture) at night and transported to the laboratory where they were isolated, placed in bottles with lake water at 4°C, shipped by air to the study site, and placed in waiting mesocosms within 24 h of collection. Several centimeters of sediment collected with a grab in the lake containing the mesocosms had been placed on the bottom of each mesocosm as a daytime refuge for these vertically migrating larvae (we estimate that three indigenous *C. punctipennis* were introduced inadvertently into each mesocosm with the added lake sediment, based on the enumeration of animals in four grab samples).

Zooplankton offered as prey was collected by horizontal tows of a 64- μ m-mesh plankton net at a depth of 3 m in the lake containing the mesocosms. In a nearby laboratory, potential prey were sieved through 500- μ m-mesh netting to eliminate those larger than the mouth diameter of *C. punctipennis* (450 μ m). A subsample of remaining prey was separated with a plankton splitter, narcotized with a drop of chloroform, and counted under a microscope at a magnification of 25 \times . On the basis of such estimates of prey density, we prepared lots of various prey densities for addition to the mesocosms. We measured prey quantities daily in each mesocosm by counting zooplankton in a 2.5-liter water sample collected with a 75-cm-long Plexiglas tube (3.25-cm diameter). Densities of prey in the mesocosms were adjusted if necessary by adding freshly collected prey from the lake.

We exploited the migratory behavior of *C. punctipennis* to collect larvae from the mesocosms with a minimum of disturbance. Larvae were captured at night during their downward migration by placing a funnel trap (Fig. 1) in each mesocosm during the day when larvae were in the sediment. The larvae collected from each mesocosm were allowed to defecate their gut contents for 24 h in a 500-mL high-density polyethylene container filled with 64 μ m of filtered lake water. Cadmium losses from *C. punctipennis* larvae are negligible for depuration periods of up to several days in the laboratory (L. Hare, unpubl. data) and are consistent with the low elimination rate of Cd observed in the efflux experiment (low prey : predator ratio) of this study. Depurated larvae were dried in a lyophilizer, weighed on a microbalance, digested in concentrated Aristar nitric acid in an autoclave,

and analyzed for Cd by graphite furnace atomic absorption spectrometry (method as in Hare et al. 1989). Repeated measurements of Cd in small samples of a certified reference material (lobster hepatopancreas) were within the range of acceptable analytical variation (coefficient of variation \approx 5%, $n = 10$). Water samples for the measurement of Cd, major ions, and organic carbon were collected in each study lake using diffusion samplers (collection and analyses are described in Hare and Tessier 1998).

Cadmium influx experiment—Larvae of *C. punctipennis* were collected in early September in the low-Cd Lake St. Joseph and transferred to the high-Cd Crowley Lake where 200 individuals were placed in each of 15 mesocosms. All individuals were exposed in the mesocosms to the prevailing high aqueous Cd concentrations and to a range of densities of Cd-rich crustacean zooplankton collected from Crowley Lake. Prey to predator ratios in mesocosms (three replicates at 0, 25, 75, 225, or 675 prey:predator) were chosen to bracket the ratio measured in Crowley Lake at the time of the experiment (225 prey:predator, as estimated by nighttime vertical hauls of a 64- μ m-mesh net). Considering the results of a previous laboratory study (Munger and Hare 1997), we hypothesized that the Cd content of animals exposed to the prevailing high-Cd concentrations in lake water only would not increase, whereas the addition of indigenous prey from the Cd-rich lake would lead to increases in predator Cd content. The mean coefficient of variation of counts of plankton samples collected from the mesocosms was 16%, as estimated from triplicate samples on days 4, 8, 12, and 16 of the experiment. Fifteen *C. punctipennis* taken from net hauls in each mesocosm and from Crowley Lake on night 13 were dissected and their gut contents examined (method as in Swift and Fedorenko 1973). Fifteen larvae were collected from each mesocosm (three pooled samples of five larvae) at 4-d intervals during our 16-d experiment for the measurement of larval mass and Cd content.

Cadmium efflux experiment—We collected fourth instar *C. punctipennis* larvae from Cd-rich Crowley Lake in early June and transferred them to low-Cd Lake au Cèdre. Larvae were placed in mesocosms and fed low-Cd prey from Lake au Cèdre at one of two densities to determine if feeding rate influenced Cd efflux. The low prey:predator treatment level (35 prey:predator) was obtained by adding $6,900 \pm 3,500$ prey (SD, $n = 16$) and 200 *C. punctipennis* in three replicate mesocosms while the high prey:predator treatment level (210 prey:predator) was obtained by the addition of $62,000 \pm 32,700$ prey (SD, $n = 28$) and 300 *C. punctipennis* in six replicate mesocosms. The prevailing prey:predator ratio in Lake au Cèdre at the time of the experiment was between our two treatment levels (115 ± 7 ; SD, $n = 3$). One sample of five *C. punctipennis* larvae per mesocosm was collected using funnel traps at intervals of 1–4 d over the 32-d duration of the experiment for measurement of larval weight and Cd content.

Results

Reliability of experimental approach—*Chaoborus punctipennis* larvae were observed in the water column of the

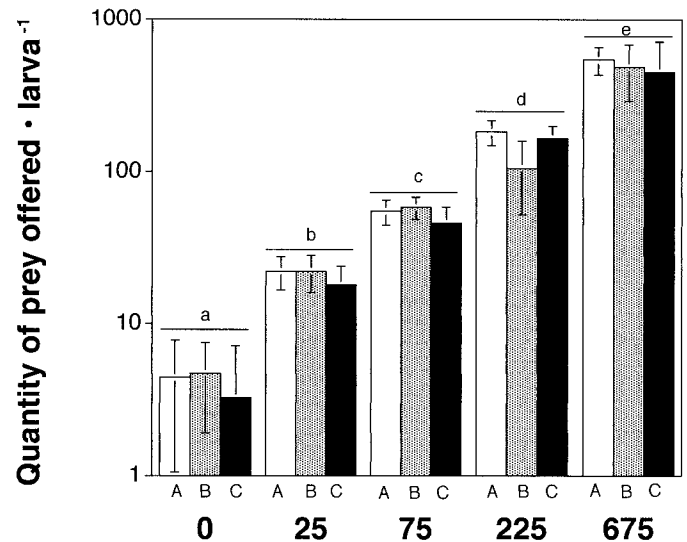


Fig. 2. Quantities of crustacean prey offered to *C. punctipennis* larvae during the Cd-influx experiment. Mean values (\pm 95% CI) are shown for replicate mesocosms (A, B, C) of the five nominal prey:predator ratios (0, 25, 75, 225, and 675 prey:predator). The value for a given mesocosm is the mean of 14–27 measurements during our 16-d experiment. Different letters (a–e) indicate significant differences among treatment level means ($P < 0.05$ Kruskal-Wallis test and Dunn's method).

mesocosms at night but not during the day (presumably taking refuge in the sediment), indicating that their normal migratory behavior was not altered by handling or confinement in mesocosms. Furthermore, larval feeding behavior did not appear to be suppressed as some individuals from all treatment levels were found with prey in their gut. There was no visible development of periphyton on the mesocosm netting during our experiment: an absence of fouling is also supported by the similar chlorophyll *a* concentrations in lake and mesocosm water when measured at 4-d intervals by fluorometry (Welschmeyer 1993) during our experiment (overlap of 95% CI at all times). These observations suggest that water and phytoplankton moved freely across the mesocosm walls during our experiments.

Cadmium influx experiment—Measurements of prey densities in mesocosms confirmed that they were close to targeted values and that they differed significantly among treatment levels (Fig. 2). The proportions of copepods (mainly the calanoid *Diaptomus minutus*) and rotifers (mainly *Keratella cochlearis*) in mesocosms were not significantly different from those in Crowley Lake (Fig. 3A), except for the lowest prey:predator ratio (25 prey:predator). In mesocosms, there was a tendency toward lower proportions of copepods at low prey densities (Fig. 3A), which suggests that at these densities the predator was able to reduce copepod numbers more effectively than at high prey densities. The proportions of cladocerans (mainly *Diaphanosoma birgei* and *Bosmina longirostris*) in mesocosms at all treatment levels were significantly lower than the proportion measured in the surrounding lake (Fig. 3A). The higher predator densities in mesocosms than in the lake may have led to greater

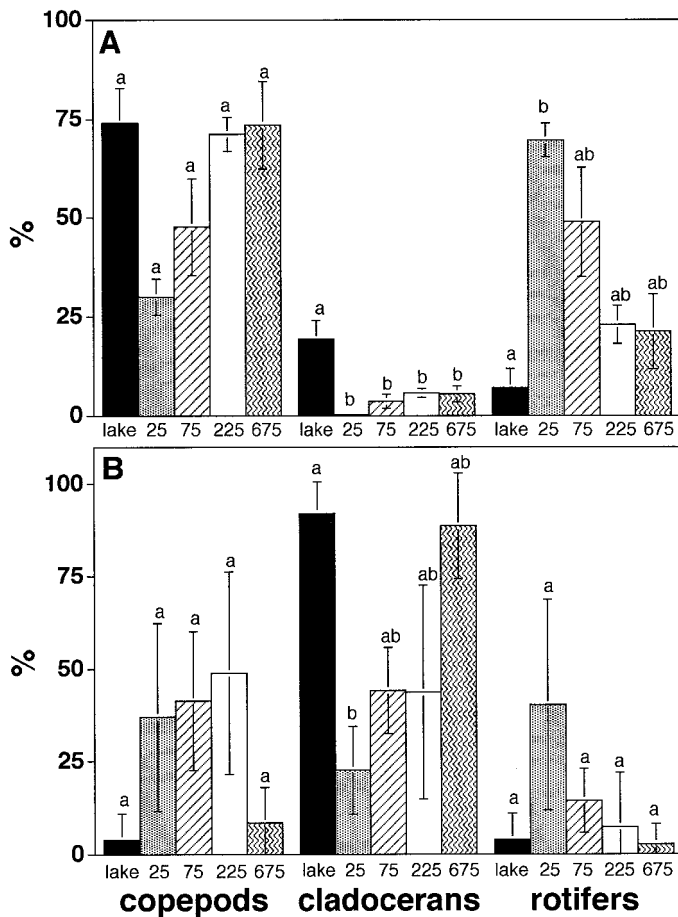


Fig. 3. Percentage of total numbers of zooplankton represented by copepods, cladocerans, and rotifers during the Cd-influx experiment (A) in the lake (means of days 0, 10, and 13) and in mesocosms (on day 16) at prey:predator ratios of 25, 75, 225, and 675, and (B) in the crops of *C. punctipennis* larvae collected on night 13 (lake and mesocosms). Different letters (a and b) indicate a significant difference ($P < 0.05$ based on Kruskal-Wallis and Student-Newman-Keuls method). All values are means $\pm 95\%$ CI in (A) $n = 3$ measurements mesocosm $^{-1}$ and in (B) $n = 7-16$ *C. punctipennis* larvae. The 0 prey treatment level is not included because of the very small prey densities.

predation pressure on cladocerans in mesocosms. Cladocerans appear to have been more vulnerable to predation by *C. punctipennis* larvae than were the other prey types; indeed, although cladocerans represented from 25–90% of the animals identified in predator crop contents (Fig. 3B), they accounted for <20% of the prey community (Fig. 3A).

The mean weight of larvae varied significantly among treatment levels, and larval weight increased with increasing numbers of prey offered (Fig. 4A). We expressed larval Cd in terms of mass of Cd taken up per individual rather than Cd concentration, so that the changes measured in larval weight would not confound interpretation of larval Cd uptake. Larvae exposed to Cd simultaneously in prey and water showed a significant increase ($P < 0.05$) in their Cd content over time, whereas the Cd content of larvae exposed to Cd in water only (0 prey added) remained constant (Fig. 4B). Because small numbers of crustacean prey (<5 per predator)

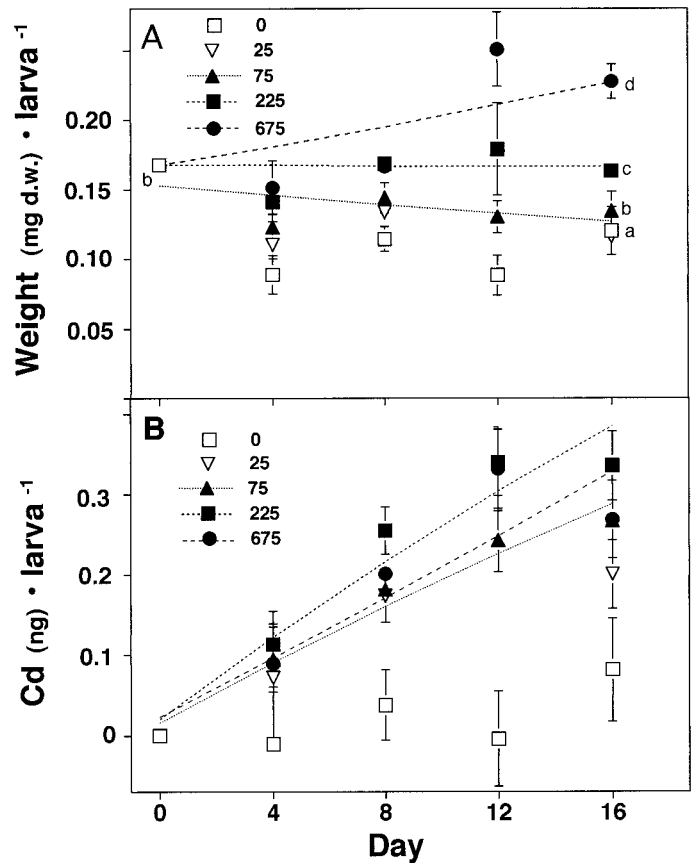


Fig. 4. Temporal changes in (A) weight and (B) Cd content of *Chaoborus punctipennis* larvae exposed to water and crustacean prey from Cd-rich Crowley Lake at the nominal prey:predator ratios of 0, 25, 75, 225, and 675. Model curves, obtained with the parameters given in Table 2, are represented by lines, and experimental data are represented by symbols. The vertical axis in (B) represents increases in larval Cd content above their value at the beginning of the experiment (0.219 ± 0.006 [\pm SE] ng Cd, $n = 3$). Values are means (\pm SE) for the three mesocosms at each treatment level (the value for a given mesocosm is the mean of one to five pooled samples of five *C. punctipennis*). In (A), different letters (a–d) indicate a significant difference among treatment levels ($P < 0.05$ ANOVA and Student-Newman-Keuls, based on a comparison of experimental data from days 4–16 for each treatment level). Larval weights and Cd contents at the 25 prey:predator treatment level were not included in statistical analyses because of a missing data point for day 12. In (B), the slope of the line for the nominal ratio of 0 prey:predator is not significantly different from zero ($P > 0.05$). Larval weight (mg d.w. larva $^{-1}$) and Cd content (ng Cd larva $^{-1}$) in the indigenous larvae of Crowley Lake were 0.23 ± 0.01 (\pm SE, $n = 8$) and 2.9 ± 0.3 (\pm SE, $n = 8$), respectively.

were found in the mesocosms to which no prey had been added (they probably passed through the netting at early life stages), as well as in the guts of larvae from this treatment level, the Cd values for the nominal 0 prey treatment level have been corrected for the contribution of food. We multiplied the slope of the relation between the Cd content of larvae and the number of prey offered by the number of prey found in the 0 prey treatment level to obtain values used to correct for the contribution of food. Given the small numbers

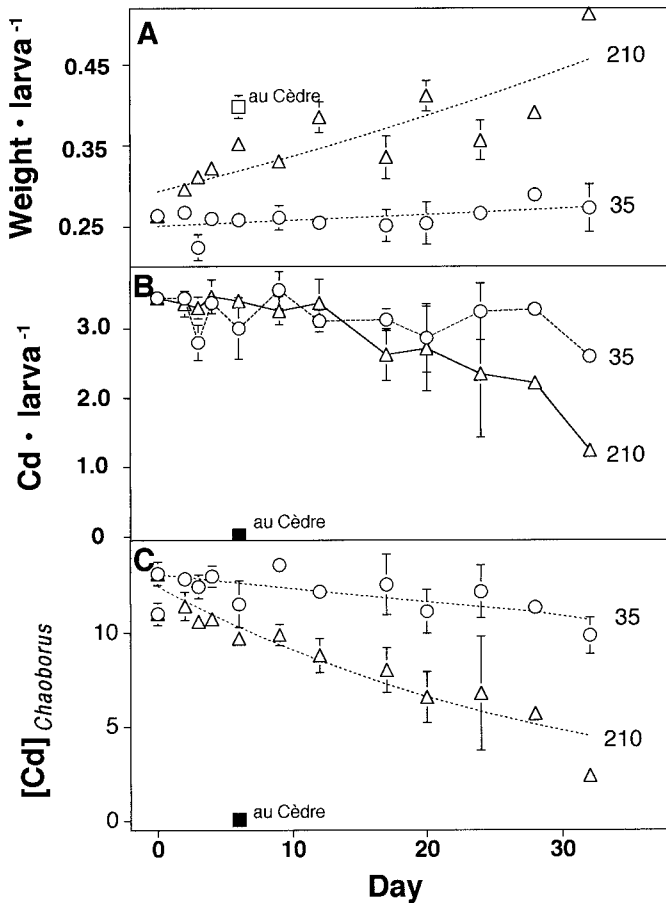


Fig. 5. Temporal changes in (A) weight (mg d.w. larva⁻¹), (B) Cd content (ng Cd larva⁻¹), and (C) Cd concentration (μg Cd g⁻¹ d.w.) of *Chaoborus punctipennis* larvae transferred from the Cd-rich Crowley Lake and exposed to Cd-poor crustacean prey in mesocosms deployed in uncontaminated Lake au Cèdre at nominal densities of 35 and 210 prey : predator. Model curves, obtained with the parameters given in Table 2, are represented by lines in A and C, and experimental data are represented by symbols. Values are means (±SE) for one to six samples of five *C. punctipennis* at each treatment level. The solid squares indicate values for indigenous Lake au Cèdre *C. punctipennis* larvae. In (A), the slope of the line for the nominal density of 35 prey : predator is not significantly different from zero ($P > 0.05$).

of prey involved, the Cd contents of larvae in the 0 prey nominal treatment level were reduced by <15% (a mean of 0.02 ng larva⁻¹) on application of this correction.

Cadmium efflux experiment—The weight of *C. punctipennis* larvae transferred from high-Cd Crowley Lake to low-Cd Lake au Cèdre increased rapidly when they were offered prey at high prey : predator ratios (Fig. 5A). In contrast, the weight of larvae offered low prey densities (35 prey : predator) did not increase significantly during our experiment (Fig. 5A; $P > 0.05$). Larvae lost Cd slowly during our 32-d experiment (Fig. 5B), and we estimate the Cd loss rates for the high and low prey : predator ratios to be 0.059 and 0.016 ng Cd predator⁻¹ d⁻¹, respectively.

Modeling cadmium exchange—We fit a kinetic bioaccumulation model to our experimental data to estimate the Cd growth and efflux rate constants, the efficiency with which the Cd ingested is retained by the predator (i.e., assimilation efficiency), the biological half-life of Cd, and the Cd concentration in the predator at steady state.

If we treat *C. punctipennis* as a single compartment, the rate of change of Cd concentrations in this animal ($d[Cd]_a/dt$) can be expressed (Thomann 1981) as the difference between metal entering and leaving the animal, i.e.,

$$\frac{d[Cd]_a}{dt} = k_u[Cd]_w + AE \cdot IR \cdot [Cd]_f - k_e[Cd]_a - k_g[Cd]_a \quad (1)$$

where k_u (L g⁻¹ d⁻¹), k_e (d⁻¹), and k_g (d⁻¹) are rate constants for Cd uptake, Cd efflux, and larval growth, respectively; $[Cd]_w$ (ng L⁻¹), $[Cd]_a$ (ng g⁻¹ dry weight [d.w.]), and $[Cd]_f$ (ng g⁻¹ d.w.) are Cd concentrations in water, in *C. punctipennis*, and in food (zooplankton), respectively; and AE (g Cd retained g⁻¹ Cd ingested) and IR (g prey ingested g⁻¹ of body weight d⁻¹) are assimilation efficiency and ingestion rate, respectively. The four composite terms on the right-hand side of Eq. 1 represent uptake from water, uptake from food, loss by efflux, and loss by growth, respectively. Our experimental results (Fig. 4B) suggest that Cd uptake from water by *C. punctipennis* larvae is negligible in nature, which agrees also with the results of a laboratory study for this species (Munger and Hare 1997). For this reason, we can neglect Cd uptake from water, and Eq. 1 reduces to

$$\frac{d[Cd]_a}{dt} = AE \cdot IR \cdot [Cd]_f - k_e[Cd]_a - k_g[Cd]_a \quad (2)$$

Because we chose to express our Cd bioaccumulation data in terms of Cd burden, we can recast the equation to express the rate of change in the Cd burden (dQ_{Cd}/dt) of *C. punctipennis* as

$$\frac{dQ_{Cd}}{dt} = AE \cdot IR \cdot [Cd]_f \cdot W - k_e[Cd]_a \cdot W \quad (3)$$

where Q_{Cd} (ng) is the quantity of Cd in the body and W is the larval weight, changes in which are exponentially related to time (Winberg 1971), i.e.,

$$W = W^0 e^{k_g t} \quad (4)$$

where W is the weight at time t (mg larva⁻¹), W^0 is the initial weight (mg larva⁻¹), and t is time (d). Equation 4 and integrated forms of Eqs. 2 and 3 can be used to estimate the rate constants k_g and k_e , as well as the assimilation efficiency (AE). First, we estimated k_g (Table 2) by nonlinear regression of the *Chaoborus* weight over time (Eq. 4) using the data in Figs. 4A and 5A. For the Cd efflux experiment, uptake of cadmium can be neglected because the transferred *C. punctipennis* larvae were maintained in a low-Cd lake and were fed Cd-poor food. Given this assumption, the integrated form of Eq. 2 becomes

$$[Cd]_a = [Cd]_a^0 e^{-(k_g + k_e)t} \quad (5)$$

where $[Cd]_a^0$ is the initial Cd concentration in the larvae. The value of the term ($k_g + k_e$) was estimated by nonlinear regression of the experimental data shown in Fig. 5C using

Eq. 5. Knowing k_g (from Eq. 4; see Table 2), k_e was obtained by subtraction. Integration of Eq. 3 gives

$$Q_{Cd} = \frac{AE \cdot IR \cdot [Cd]_f \cdot W^0}{k_g + k_e} (e^{k_g t} - e^{-k_e t}) + Q_{Cd}^0 e^{-k_e t} \quad (6)$$

Assimilation efficiencies (AE, Table 2) were obtained by fitting Eq. 6 to the data points in Fig. 4B for prey to predator ratios of 75, 225, and 675 (the only ratios for which complete data sets were available). For this purpose, the values of k_g and k_e were fixed; k_g values of -0.012 , -0.0005 , and 0.02 d^{-1} were used for the 75, 225, and 675 prey:predator treatment levels, respectively (see Table 2), whereas a k_e value of 0.018 d^{-1} was used for larvae from both the 225 and 675 prey:predator treatment levels and 0.003 d^{-1} was used for larvae exposed to 75 prey:predator (Table 2). The IR values used in Eq. 6 are given in Table 2; they are the means of the ingestion rates measured for each treatment level at days 4, 8, 12, and 16. The value of $[Cd]_f$ ($10.8 \mu\text{g g}^{-1}$) used in Eq. 6 is the mean of the Cd concentrations in four replicate samples of 50–100 cladocerans or copepods from Crowley Lake (Munger and Hare unpubl. data).

After a sufficiently long exposure time, Cd concentrations in *C. punctipennis* larvae should reach a steady state value, at which time $d[Cd]_a/dt$ equals zero. Under these conditions, Eq. 2 becomes

$$[Cd]_{ss} = \frac{AE \cdot IR \cdot [Cd]_f}{k_e + k_g} \quad (7)$$

Values for $[Cd]_{ss}$ of 3.2 and 3.7 ng Cd larva $^{-1}$ (Table 2) were calculated for the uptake experiment at the two prey to predator ratios (75 and 225 prey:predator, respectively) using the values of AE, IR, and k_g from the influx experiment as well as those for k_e from the efflux experiment (values given in Table 2) and by assuming a mean dry weight of $0.22 \text{ mg larva}^{-1}$ and a value for $[Cd]_f$ of $10.8 \mu\text{g g}^{-1}$ (Munger and Hare unpubl. data). The value of $[Cd]_{ss}$ for the treatment level of 675 prey:predator was not calculated given the lack of a k_e value at a treatment level close to 675 prey:predator. The biological half-life of Cd in larvae (Table 2), $t_{1/2}$, was obtained from Eq. 8, which was in turn derived from Eq. 5:

$$t_{1/2} = \frac{\ln 0.5}{k_e + k_g} \quad (8)$$

Model curves of growth, Cd elimination, and Cd uptake obtained with the constants given in Table 2 are compared with our experimental data in Figs. 4 and 5.

Discussion

Because food is the major Cd source for *C. punctipennis* larvae in nature, the strong relationship reported to exist between Cd concentrations in this insect and those in water (Hare and Tessier 1996; Croteau et al. 1998; Hare and Tessier 1998) must be indirect; that is, the metal must be taken up from water at a lower level in the food chain leading to the predator. Consequently, food-related variables (e.g., type of food consumed, feeding rate, and assimilation efficiency) could be considered as a means to improve the relationship between Cd in *Chaoborus* and Cd in its surroundings.

Our findings that food is the major Cd source for *C. punctipennis* agree with those obtained by Stephenson and Turner (1993) for the freshwater amphipod *Hyalella azteca*; this taxon is reported to take up 60% of its Cd from its periphyton food in nature. Food is also reported to be an important Cd source in the laboratory for mites and caddisfly larvae (Timmermans et al. 1992), as well as polychaetes (Selck et al. 1998). However, these conclusions are in contrast with those of several laboratory investigations in which aquatic animals are reported to take up the majority of their Cd from water (Williams and Giesy 1978; Kayser 1982; Wang and Fisher 1998). Such differences among taxa could be explained in part by differences in the relative numbers of Cd uptake sites on the gut versus those on respiratory surfaces and the affinity of these sites for the metal (Hare 1992). However, the conclusions of many laboratory studies should be accepted with caution (Table 3) because (1) Cd concentrations in artificial exposure media often largely exceed those at even highly contaminated sites, (2) a consumer's food and the consumer itself are often exposed to different Cd concentrations, (3) Cd speciation in water, and thus Cd bioavailability, are usually not controlled, (4) food is usually not exposed to Cd for a sufficient length of time to reach an internal steady state (as discussed in Munger et al. 1999), (5) consumers are stressed by unnatural experimental conditions, and (6) a natural mixture of food is not usually offered to consumers. We believe that our innovative experimental design involving the in situ exposure of aquatic animals to natural Cd concentrations in water and to a realistic mixture of food types has allowed us to avoid the pitfalls of many laboratory studies.

The data points in Fig. 4 suggest plateaus in larval Cd content around $0.55 \text{ ng predator}^{-1}$ (initial larval Cd content of 0.22 ng plus the increase of 0.32 ng during our experiment). However, assuming a similar feeding behavior in larvae confined in mesocosms to those in Crowley Lake, a plateau in larval Cd content seems unlikely because the Cd content of fed larvae at the end of our experiment was $<20\%$ of that measured in indigenous larvae from Crowley Lake ($2.9 \text{ ng larva}^{-1}$). Moreover, our one-compartment model suggests that the steady state Cd concentration in larvae from the mesocosms should be $3.2\text{--}3.7 \text{ ng larva}^{-1}$ (Table 2). The apparent plateaus could be explained by a decline in feeding rates during our experiment associated with a decline in lake temperature (a drop of 9°C was recorded during the experiment).

We observed an inverse relationship between values of AE and IR in our experiment (Table 2); that is, a high IR ($128 \text{ prey consumed predator}^{-1} \text{ d}^{-1}$ when 675 prey predator $^{-1}$ were offered) was accompanied by a low AE (2%) and a low IR ($9 \text{ prey consumed predator}^{-1} \text{ d}^{-1}$ when 75 prey predator $^{-1}$ are offered) was associated with a high AE (18%). In other studies, inverse relationships have also been reported between IR and the AE of phosphorus by *Chaoborus trivittatus* (Giguère 1981) and of Cd by marine mussels (Wang et al. 1995).

The range of Cd AE values that we calculated for larvae of *C. punctipennis* feeding on a mixed zooplankton community in our field experiment (2–18%, Table 2) encompasses the value of 4% reported for this species feeding ad

Table 2. Estimated values of various model parameters for Cd exchange in larvae of *Chaoborus punctipennis*. Error terms are standard errors.

Parameter	Symbol	Estimated values Elimination experiment (prey : predator ratio)		
		35		210
Growth rate constant (d ⁻¹)	k _g	0.003 ± 0.001		0.014 ± 0.003
Elimination rate constant (d ⁻¹)	k _e	0.003 ± 0.002		0.018 ± 0.005
Biological half-life (d)	t _{1/2}	117		22
		Uptake experiment (prey : predator ratio)		
		75	225	675
Growth rate constant (d ⁻¹)	k _g	-0.012 ± 0.009	-0.0005 ± 0.0007	0.02 ± 0.01
Assimilation efficiency (%)	AE	18	13	2
Larval Cd at steady-state (ng larva ⁻¹)	[Cd] _{ss}	3.2	3.7	—
Ingestion rate (g prey g ⁻¹ d ⁻¹)	IR	0.064	0.21	0.91

libitum on a cladoceran in the laboratory (Munger and Hare 1997). However, these AE values are somewhat lower than those reported for this predator feeding on cladocerans or copepods in another laboratory experiment (AEs of 27 and 33%, respectively; Munger and Hare unpubl. data). Given the reported influence of factors such as ingestion rate (this study; Munger and Hare unpubl. data), food quality (Reinfelder and Fisher 1991; Wang and Fisher 1996), and temperature (M.-N. Croteau, INRS-Eau, unpubl. data) on metal assimilation efficiency, such differences among studies are to be expected.

Literature values for Cd assimilation efficiencies by other freshwater invertebrates vary from being similar to those we measured for *C. punctipennis*, i.e., 1% and 4% for the detritivorous crustacean *Asellus aquaticus* (van Hattum et al. 1989) and the predatory insect *Mystacides* spp. (Timmermans et al. 1992), respectively, to being much higher than those we measured, i.e., 61% and 80% for periphyton-feeding *Hyaella*

azteca (Stephenson and Turner 1993) and the predatory mite *Limnesia maculata* (Timmermans et al. 1992), respectively. Cadmium assimilation efficiencies reported for marine invertebrates are 25% for mussels feeding on diatoms (Wang et al. 1995) and 30% for copepods feeding on algae (Reinfelder and Fisher 1991). Although differences in gut morphology and physiology among animal groups could be used to explain differences in their Cd AE, factors related to the experimental protocols used by various investigators, as discussed above, are also likely to be involved.

Estimated values of the elimination rate constant (k_e) increased with increasing food density. This increase could be explained if a greater food consumption led to either a higher metabolic rate, and thus a higher loss rate of Cd, or to differences in digestive processes, as has been observed in mussels (Wang et al. 1995). The biological half-life (t_{1/2}) of Cd decreased from 117 to 22 d with increasing food density (Table 2). The latter value of 22 d is probably more repre-

Table 3. Classification of freshwater studies on the relative importance of water and food as Cd sources according to their adherence (Y) or not (N) to six key methodological criteria (as discussed in the text).

Animal	Criterion number*						Metal source	Reference
	1	2	3	4	5	6		
Crustaceans								
<i>Moina macrocopa</i>	N	N	?	?	N	N	W	Hatakeyama and Yasuno 1981
<i>Daphnia magna</i>	N	?	?	?	N	N	W	Carney et al. 1986
<i>Asellus aquaticus</i>	N	N	N	?	N	N	W	van Hattum et al. 1989
<i>Procambarus acutus</i>	N	N	N	?	N	N	W, F	Giesy et al. 1980
<i>Hyaella azteca</i>	Y	Y	Y	Y	Y	Y	F	Stephenson and Turner 1993
Mite								
<i>Limnesia maculata</i>	N	Y	?	?	N	N	F	Timmermans et al. 1992
Insects								
<i>Mystacides</i> spp.	N	Y	?	?	N	N	F	Timmermans et al. 1992
<i>Chaoborus punctipennis</i>	Y	Y	Y	Y	N	N	F	Munger and Hare 1997
<i>Chaoborus punctipennis</i>	Y	Y	Y	Y	Y	Y	F	This study

* (1) environmentally realistic Cd concentrations in the exposure media; (2) all trophic levels exposed to the same Cd concentration in water; (3) control of aqueous Cd speciation; (4) realistic metal bioavailability in food; (5) realistic experimental conditions; (6) natural mixture of food; ? information was not available in the publication; W, water; F, food.

sentative of the $t_{1/2}$ of Cd in larvae from Crowley Lake because it was obtained for a mesocosm prey:predator ratio close to that in the lake. Our value of 22 d is the same as that reported for the isopod *Asellus aquaticus* (van Hattum et al. 1989), but is higher than the one reported for larvae of the aquatic insect *Hexagenia rigida* (8 d; Hare et al. 1991) and lower than that for the mussel *Mytilus edulis* (67 d; Wang et al. 1996).

The present study illustrates that our approach can be used to address the previously intractable problem of separating metal sources available to aquatic animals in nature. Although we studied a planktonic system, our approach could be extended to study benthic animals by using sediment-filled trays covered with netting to retain animals. Our experimental results suggest that, in addition to being an important channel for the movement of nutrients from zooplankton (Neill and Peacock 1980) to fish (Pope et al. 1973), *Chaoborus* larvae represent a means by which trace metals such as Cd could be transferred between trophic levels. In more general terms, the results of our experiment indicate that models designed to trace the dynamics and fate of metals in communities of aquatic animals should not be based solely on metal uptake from water, but are likely to be improved by including metal transfer along trophic pathways. Our results also suggest that food should not be excluded a priori as a Cd source for animals in laboratory experiments and toxicity tests (Luoma 1995), because doing so would likely result in the underestimation of Cd accumulation and toxicity with a concomitant risk for the environment.

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