Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*[1]

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Abstract: We measured substantial differences in Cd accumulation among four species of the phantom midge *Chaoborus* that were exposed in the laboratory to the same Cd concentration in naturally contaminated prey. The two large-bodied species accumulated more Cd than did the two small-bodied species, in spite of the fact that all species ingested prey at the same rate. To determine why this was the case, we fitted our experimental data to a bioaccumulation model that allowed us to compare the species with respect to their rate constants for growth and Cd efflux, their Cd assimilation efficiency, and their Cd concentrations at steady state. Differences among species were explained mainly by the fact that the small-bodied species assimilated a much lower proportion of the Cd that they ingested with prey (≈6%) than did the large-bodied species (45 and 58%). A comparison between Cd concentrations measured in *Chaoborus* species in the field and predictions from the model suggests that differences in Cd concentrations among coexisting *Chaoborus* species in nature are explained by differences both in the rate at which they assimilate Cd and in their feeding habits.

Résumé : Nous avons mesuré des différences substantielles dans l'accumulation du Cd chez quatre espèces de *Chaoborus* (Mouche-fantôme) qui avaient été exposées en laboratoire à des quantités similaires de Cd contenues dans des proies naturellement contaminées. Au terme de cette expérience, les deux espèces de grande taille avaient accumulé plus de Cd que les deux espèces de petite taille et ce, malgré un taux d'ingestion des proies similaire chez les quatre espèces. Afin de déterminer les causes de cette accumulation différentielle du Cd entre espèces, nous avons appliqué à nos données expérimentales un modèle de bioaccumulation théorique. Ce modèle nous a permis de comparer les espèces en regard de leurs constantes de taux de croissance et de perte de Cd, de leur efficacité d'assimilation du Cd et de leurs concentrations de Cd à l'état stationnaire. Les différences mesurées dans l'accumulation du Cd entre les espèces furent expliquées principalement par le fait que les espèces de petite taille assimilaient de leurs proies une proportion de Cd beaucoup plus faible (≈6%) que les espèces de grande taille (45 et 58%). La comparaison des prédictions de notre modèle aux concentrations de Cd mesurées chez des espèces de *Chaoborus* récoltées en milieu naturel suggère que les différences observées en milieu naturel dans les concentrations de Cd entre les espèces coexistantes sont attribuables au fait que les espèces diffèrent entre elles tant dans leur efficacité d'assimilation du Cd que dans leur régime alimentaire.

Introduction

Interest in trace metal accumulation by aquatic organisms stems in part from their potential use as biomonitors (Phillips and Rainbow 1993). In both marine and freshwater systems, bivalve mollusks are widely used as sentinel animals for monitoring contaminant levels (Elder and Collins 1991; Rainbow and Phillips 1993). However, most bivalve species are absent from waters of pH below 6 (Pennak 1989) and their metal concentrations could be subject to the confounding influence of a reproductive cycle (Langston and Spence 1995). Moreover, the use of many freshwater bivalves for monitoring purposes is limited by the dependence of their larval stage on the presence of suitable host fish (Clarke 1981) and their sensitivity to trace metals (Couillard et al. 1993). The phantom midge *Chaoborus* has been proposed as an alternative metal biomonitor in lakes (Hare and Tessier 1996). Larvae of this insect occur over a wide range of chemical conditions (Hare and Tessier 1996, 1998; Croteau et al. 1998), are widely distributed (Borkent 1981), and can be abundant (Yan et al. 1985). Furthermore, *Chaoborus* larvae are able to accumulate and tolerate high concentrations of trace metals (Hare and Tessier 1996, 1998; Croteau et al. 1998) and their metal content does not change during a reproductive cycle (Hare and Campbell 1992).

The use of an animal as a biomonitor depends on the establishment of a reliable relationship between the animal's metal concentration and the concentration of the metal in its surroundings. Hare and Tessier (1996) tested such a relationship for larvae of *Chaoborus punctipennis* in a large number of lakes. They demonstrated that Cd concentrations in this insect are best described by the free-ion activity model, provided that competition for biological uptake sites between H ions and free Cd ions are explicitly taken into account. Further studies showed that several species of *Chaoborus* could be grouped together for biomonitoring purposes, obviating the need for microscopic examination of individuals

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(Croteau et al. 1998; Hare and Tessier 1998). However, the performance of this generic model was reduced compared with that for a single species (*C. punctipennis*) because Cd concentrations differed among coexisting *Chaoborus* species (Croteau et al. 1998; Hare and Tessier 1998). Specific differences in Cd concentrations have also been reported among sympatric congeners of a marine copepod (*Calanus* spp.; Ritterhoff and Zauke 1997).

Cadmium bioaccumulation can be influenced by physical (e.g., temperature), chemical (e.g., trace metal speciation), ecological (e.g., diet, life history), and physiological factors (e.g., Cd assimilation efficiency, Cd loss rate). Few studies have compared the processes that govern metal uptake and loss among related animal species. In the present study, we used a novel experimental approach to determine if larvae of four species of the phantom midge *Chaoborus* accumulate Cd differently when environmental and ecological factors are held constant. That is, we wished to determine if there are physiological differences among species that could influence their accumulation of this metal. We exposed the various *Chaoborus* species to naturally contaminated prey of the same type and Cd content in a controlled environment. Then, using a bioaccumulation model, we estimated and compared key food-related parameters, such as Cd assimilation efficiency and Cd loss rate, among species. Lastly, we compared model predictions with Cd concentrations measured in field-collected *Chaoborus*.

Methods

Our measurements of Cd concentrations and dynamics were performed on four *Chaoborus* species that are widespread in North America (Borkent 1981): *C. albatus*, *C. americanus*, *C. flavicans*, and *C. punctipennis*.

Collection of field samples

For our Cd uptake experiment, larvae of four species of *Chaoborus* were collected in early September 1998 from three low-Cd lakes, two of which were located near Québec City and one (Hélène) in the Rouyn-Noranda region upwind from a metal smelter (Table 1). For our Cd loss experiment, larvae of four *Chaoborus* species were collected in mid-September 1998 from three high-Cd lakes located in the Rouyn-Noranda area (Table 1). Larvae for the experiments were sampled after sunset by hauling a 250-μm plankton net horizontally in the water column. Water samples were collected using in situ diffusion samplers (peepers) similar to those described by Carignan et al. (1985). These Plexiglas samplers comprise eight compartments of 4 mL each that were filled with ultrapure water (Milli-Q system water, >18 Mohm·cm^{-1}) and separated from the lake water by a 0.2-μm polysulfone membrane (Gelman HT-200 membrane). After preparation, each sampler was sealed in a clean plastic bag prior to its placement in a lake. Two diffusion samplers were suspended 1 m above the bottom in the epilimnion of each lake. After a 3-day equilibration period, the diffusion samplers were retrieved and water was collected immediately for the measurement of Cd.

Samples (4 mL) for Cd analyses were removed from compartments in each diffusion sampler by piercing the membrane with a pipette fitted with an acid-cleaned tip. These samples were injected into preacidified (53 μL of 1.35 N Anachemia nitric acid) high-density polyethylene (HDPE) bottles (4 mL capacity). On installation and retrieval dates, pH was measured with a portable pH meter (Hanna instruments, microprocessor model HI9024/HI9025) in water samples collected at the depth of the diffusion samplers using a Van Dorn bottle.

For comparison of Cd concentrations among *Chaoborus* species, larvae were obtained from 17 Canadian Shield lakes containing two to four species of the genus (Table 2). Larvae were collected either with an Ekman grab in sediment during the day (1992 and 1993 samples) or with a 250-μm-mesh-aperture plankton net during the night (1996 and 1997 samples). In the laboratory, *Chaoborus* larvae were sorted according to species (Saether 1972) and final (fourth) instar larvae were differentiated by head capsule length, as given in Larow and Marzolf (1970) (*C. punctipennis*), Fedorenko and Swift (1972) (*C. americanus*), and Parma (1971) (*C. flavicans*). Given the similar size and morphology of *C. albatus* and *C. punctipennis* larvae (they belong to the same subgenus; Borkent 1979), we assumed that head capsule lengths were the same for these two species. Larvae were held in filtered lake water for 24 h prior to metal analysis to allow them to defecate their gut contents. Five to 20 individuals of a given species were pooled, to minimise potential individual variations in Cd, and placed on a piece of preweighed acid-washed Teflon sheeting and then frozen until analysis.

Internal Cd distribution

To measure the internal distribution of Cd in larvae, *Chaoborus* from several lakes (i.e., Lake Marlon for *C. punctipennis* and *C. albatus* larvae, Lake Caron for *C. flavicans* larvae, and Lake Turcotte for *C. americanus* larvae (Table 1)) were dissected under a microscope into gut and remaining body parts; organs from 10–15 larvae of a given species were pooled to make a single sample, and five such samples were prepared for each *Chaoborus* species. Larvae were not given time to defecate their gut contents prior to dissection because a preliminary experiment conducted at 5°C showed that there was no significant difference in Cd concentrations between larvae that were allowed to eliminate their gut contents for 24 h and those that were not (Fig. 1).

Cadmium uptake experiment

Twenty-five final-instar larvae of each *Chaoborus* species collected from low-Cd lakes (i.e., Lake Hélène for *C. punctipennis* and *C. albatus*, Lake Laflamme for *C. flavicans*, and Lake Bertrand for *C. americanus* (Table 1)) were placed individually into 30-mL HDPE bottles filled with filtered (64 μm) water from Cd-contaminated Lake Marlon (Table 1). From this same lake, we collected the widespread calanoid copepod *Skistodiaptomus oregonensis* using a 64 μm mesh size plankton net to offer as prey to *Chaoborus* larvae. We verified daily the efficacy of our prey sorting by identifying prey to be offered to *Chaoborus* in three randomly selected vials (nominally holding 25 prey each). All individuals examined were calanoid copepods, of which 90% were *S. oregonensis* and 10% were the similar-sized *Epischura lacustris*. Prey were mainly adults (36%) and copepodite V (45%), with the remainder being either copepodite IV or copepodite III. Adults and copepodite V should be eaten in similar numbers, given their similar body width (0.25 ± 0.02 (SD) mm), and readily, given their small width compared with the mouth diameter of *Chaoborus* (0.45 mm for fourth instars of our smallest species, *C. punctipennis*; Moore 1988).

Each *Chaoborus* larva was offered 25 fresh prey daily, which is in excess of its needs (larvae of a given species consumed an average of 12–44% of prey offered). *Chaoborus* larvae were held in the dark at 5°C throughout the Cd uptake experiment. Both filtered lake water and prey were renewed every 24 h by transferring each larva to a new bottle filled with freshly collected filtered lake water and copepods. Formalin (5% final solution) was added to the previous 24-h-exposure bottles to preserve uneaten copepods for later counting under a microscope. On the basis of these samples, we calculated daily ingestion rates for each *Chaoborus* larva.

Table 1. Location of the lakes from which we collected final-instar *Chaoborus* larvae for the uptake and loss experiments as well as their mean (±SD) Cd concentrations.

Lake (region)	Location	pH	[Cd] (nM)	Species	Cd concentration ($\mu g \cdot g^{-1}$)
Cd uptake experiment					
Bertrand (Québec City)	46°58′N, 72°01′W	5.01	0.36	*C. americanus*	1.44±0.17
Laflamme (Québec City)	47°19′N, 71°07′W	6.71	0.09	*C. flavicans*	0.46±0.02
Hélène (Rouyn-Noranda)	48°13′N, 79°10′W	7.76	0.06	*C. punctipennis*	0.18±0.04
				C. albatus	0.22±0.05
Cd loss experiment					
Turcotte (Rouyn-Noranda)	48°18′N, 79°04′W	5.26	8.06	*C. americanus*	14.4±1.0
Caron (Rouyn-Noranda)	47°56′N, 78°58′W	7.18	0.82	*C. flavicans*	6.0±1.0
Marlon (Rouyn-Noranda)	48°16′N, 79°04′W	7.48	1.08	*C. punctipennis*	4.9±1.9
				C. albatus	3.2±1.4

Note: Also given are lake water pH and total dissolved Cd concentration.

Table 2. Location of the study lakes and year of sampling as well as the Cd concentrations in final-instar *Chaoborus* larvae collected in the spring (May–June).

			Cd concentration ($\mu g \cdot g^{-1}$)			
Region and lake	Location	Year	*C. punctipennis*	*C. albatus*	*C. flavicans*	*C. americanus*
Sudbury, Ont.						
Crooked	46°25′N, 81°02′W	1997	3.2±0.3 *a*	3.8	4.0±0.6 *b*	4.0±0.2 *b*
Crowley	46°23′N, 80°59′W	1997	10.9±0.3 *a*	np	8.6±0.4 *b*	np
Forest	46°23′N, 81°00′W	1997	8.3±0.3 *a*	np	4.9±0.2 *b*	np
Hazen*	46°25′N, 80°59′W	1996	1.1±0.1 *a*	np	1.6±0.1 *b*	2.6±0.4 *c*
Jonny	46°26′N, 81°02′W	1997	11.9±0.7 *a*	np	np	13.6±0.8 *b*
Pine*	46°22′N, 81°02′W	1996	3.9±1.2 *a*	3.4±0.1 *a*	np	np
Tilton	46°22′N, 81°04′W	1997	7.6±0.8 *a*	np	6.6±0.5 *a*	np
Rouyn-Noranda, Qué.						
Bousquet	48°14′N, 78°34′W	1997	2.0±0.1 *a*	np	3.0±0.4 *b*	np
Caron	47°56′N, 78°58′W	1996	4.5±0.3 *a*	np	7.4±0.5 *b*	np
Desperiers*	48°11′N, 79°09′W	1996	1.1±0.2 *a*	np	2.8±0.3 *b*	np
Duprat	48°20′N, 79°08′W	1997	1.4±0.1 *a*	np	1.3±0.1 *a*	np
Hélène†	48°13′N, 79°10′W	1993	0.24±0.03 *a*	0.31±0.04 *a*	np	np
Joannès†	48°11′N, 78°41′W	1992	2.6±0.3 *a*	np	3.4±0.5 *a*	np
Marlon	48°16′N, 79°04′W	1997	5.6±0.5 *a*	4.9±0.5 *a*	np	np
Surimeau*	48°08′N, 79°19′W	1996	2.7±0.2 *a*	np	1.3±0.1 *b*	np
Turcotte*	48°18′N, 79°04′W	1996	3.1±0.1 *a*	np	np	9.9±0.7 *b*
Vaudray	48°07′N, 78°42′W	1997	2.9±0.2 *a*	np	4.5±0.2 *b*	np

Note: Cd concentration values are means ± 95% confidence interval of usually several (n = 1–8) samples of pooled final-instar larvae. np, not present. Means of sympatric species followed by a different letter are significantly different (t test, $p < 0.05$).

*Data from Croteau et al. (1998).

†Data from Hare and Tessier (1998).

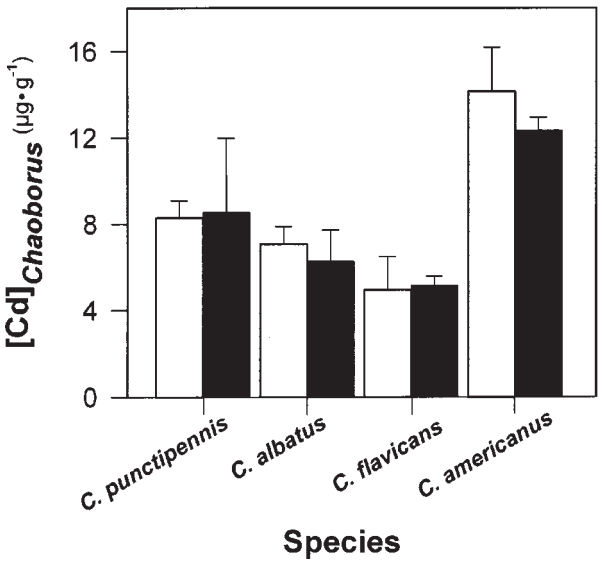
On each day, Cd concentrations were measured in three replicate samples of 100–150 prey. Pooled prey samples were held on preweighed acid-washed Teflon sheeting in microcentrifuge tubes and frozen at –4°C until analysis. At the beginning of the experiment and after 2, 4, 7, 10, and 14 days of Cd exposure, five undepurated larvae (see Fig. 1) of each *Chaoborus* species were placed individually on a piece of preweighed acid-washed Teflon sheeting and frozen.

Cadmium loss experiment

Fifty final-instar larvae of each *Chaoborus* species collected from high-Cd lakes (i.e., Lake Marlon for *C. punctipennis* and *C. albatus*, Lake Caron for *C. flavicans*, and Lake Turcotte for *C. americanus* (Table 1)) were placed individually into 30-mL HDPE bottles filled with filtered (64 μm) water from low-Cd Lake Hélène (Table 1). These *Chaoborus* larvae were fed ad libidum bulk zooplankton (mainly copepods) collected from low-Cd Lake Hélène using a 64-μm-mesh-size plankton net. *Chaoborus* larvae were held in the dark at 5°C throughout the Cd loss experiment. Both filtered lake water and prey were renewed every 24 h by transferring each larva to a new bottle filled with freshly collected filtered lake water and bulk plankton. On each day, three replicate samples of bulk zooplankton (≈1–2 mg wet weight) were placed on a piece of preweighed acid-washed Teflon sheeting and frozen until analysis. At times 0, 6, 12, 18, 24, and 36 h and 2, 3, 5, 7, and 10 days, five undepurated larvae (see Fig. 1) of each *Chaoborus* species were placed individually on pieces of preweighed acid-washed Teflon sheeting and frozen until analysis.

In the lakes from which we collected our *C. americanus* for both Cd uptake and loss experiments, we found two body sizes of *C. americanus* larvae, both of which had the same head capsule length (corresponding to that of the fourth instar). This suggests that there are two coexisting generations of larvae of different ages (≈1 and 2 years of age). For our experiments, we chose the smaller

Fig. 1. Mean Cd concentrations (+95% confidence interval) in larvae of the four *Chaoborus* species both soon after their capture for use in our Cd loss experiment (gut contents present, open bars) and following a 24-h depuration period (gut contents absent, solid bars).



of the two larval sizes so as to minimise the risk of emergence during our experiment and so that their age would be close to those of the other three species (<1 year).

Analyses

To minimise inadvertent trace metal contamination, labware, water-sampling materials, vials, and Teflon sheeting were soaked in 15% nitric acid and rinsed in ultrapure water prior to use.

Total dissolved Cd concentrations were measured by flameless atomic absorption spectrophotometry (Perkin-Elmer model SIMAA 6000). Certified reference water samples (riverine water reference material, NRCC) were also analysed for Cd during each analytical run and measured trace metal concentrations were within the certified range.

Previously frozen *Chaoborus* larvae and zooplankton samples were freeze-dried (FTS Systems™), weighed (Mettler ME30 electronic microbalance), and digested in concentrated nitric acid (Aristar grade). For samples of *Chaoborus* collected before 1998, digestions were carried out either in thick-walled, screw-cap Teflon vials in an autoclave at 120°C for 3 h or in Teflon bombs in a microwave oven. Cooled digested samples were diluted to volume with ultrapure water. Samples of both *Chaoborus* and copepods collected in 1998 were digested at room temperature in 4-mL HDPE vials with concentrated nitric acid (100 µL·mg dry weight sample^{-1}) for 7 days. Hydrogen peroxide (40 µL·mg dry weight sample^{-1}) was added 24 h prior to final dilution with ultrapure water (760 µL·mg dry weight sample^{-1}). Samples of similar weight of a certified reference material (lobster hepatopancreas, TORT-1, NRCC) were submitted to the same digestion procedures during each analytical run. Cadmium concentrations measured in TORT-1 were within the certified range and recoveries of Cd in spiked samples were within ±10% of the amount added. Cadmium concentrations in animals were analysed by flameless atomic absorption spectrophotometry (Varian Spectra AA-30). The software SYSTAT 10 was used for all statistical analyses. We used *t* tests to compare Cd concentrations between the gut and remaining organs as well as to compare estimates for each model term among *Chaoborus* species. Estimates for each model term in the Cd uptake and Cd loss experiments were made by nonlinear regression.

Results and discussion

Internal Cd distribution

A comparison of the distribution of Cd between the gut and remaining organs confirmed that the four *Chaoborus* species that we studied are similar in having the bulk of their Cd localised in gut tissues (*t* test, $p < 0.001$) (Fig. 2). This internal distribution pattern suggests that all of the species take up the bulk of their Cd from prey. This suggestion is borne out for one of our study species, *C. punctipennis*, by laboratory and field studies demonstrating that larvae of this species accumulate almost all of their Cd from food (Munger and Hare 1997; Munger et al. 1999). Because the Cd present in *Chaoborus* larvae likely originates from their food, then differences in Cd concentrations among sympatric species could be explained by the type and quantity of prey that each species consumes. In addition, factors unrelated to food type and ingestion rate, such as Cd assimilation efficiency, Cd elimination rate, and animal growth rate, could vary among species and thereby explain the differences in Cd concentrations among *Chaoborus* species that are reported to occur in nature (Croteau et al. 1998; Hare and Tessier 1998).

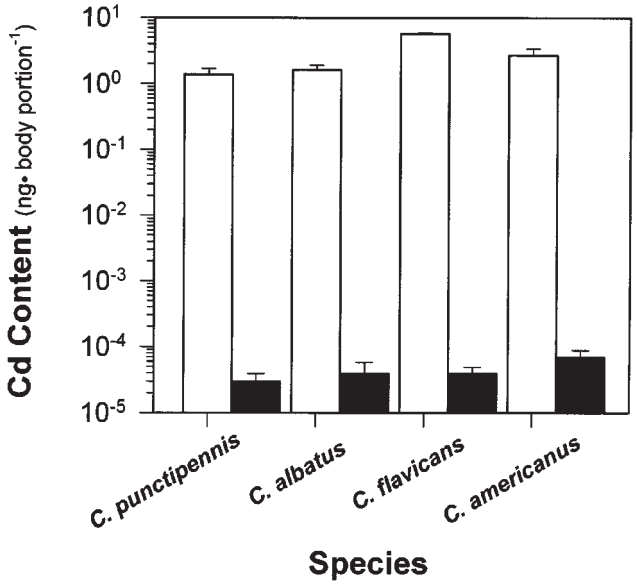
Modelling Cd bioaccumulation

We tested the hypothesis that if all species consume the same prey type at the same rate, their Cd concentrations will increase to the same extent. We did this by feeding four *Chaoborus* species the calanoid copepod *S. oregonensis* collected from the same Cd-rich lake. We used naturally contaminated prey so that Cd accumulation by the predator would be representative of that that occurs in the field; the availability of Cd from food is reported to depend on the exposure history of the food particles (Wallace and Lopez 1996). Despite the fact that the *Chaoborus* species took up their Cd from the same prey type, they differed significantly (*t* test, $p < 0.001$) in the rates at which they accumulated Cd, i.e., 9 ng Cd·g^{-1}·day^{-1} for *C. albatus*, 16 ng Cd·g^{-1}·day^{-1} for *C. punctipennis*, 74 ng Cd·g^{-1}·day^{-1} for *C. americanus*, and 109 ng Cd·g^{-1}·day^{-1} for *C. flavicans* (SD < 1 for all species). To explain this difference among species, we examined variables other than prey type and prey Cd concentration that are known to influence metal uptake: ingestion rate (IR), Cd assimilation efficiency (AE), Cd efflux rate, and *Chaoborus* growth rate (Wang and Fisher 1996; Munger et al. 1999; Munger and Hare 2000).

Mean ingestion rates (grams dry weight copepods consumed per gram dry weight predator per day) (Table 3) did not differ significantly among *Chaoborus* species during our Cd uptake experiment (*t* test, $p > 0.05$) (with the exception of a slightly lower value for *C. albatus* (*t* test, $p = 0.004$)). This similarity in ingestion rates among predator species suggests that prey ingestion rate cannot explain the differences in Cd accumulation rates that we observed among species in our Cd uptake experiment. To assess the influence of assimilation efficiencies, Cd efflux rates, and larval growth rates on the differences that we measured among *Chaoborus* species, we fit the following bioaccumulation model to our experimental data.

If we treat larvae as a single compartment and assume that their Cd uptake from water is negligible, as discussed above,

Fig. 2. Mean Cd contents (+ 95% confidence interval) in the gut (open bars) and remaining body parts (solid bars) of larvae of the four *Chaoborus* species.



we can express the rate of change in larval Cd concentrations as the difference between Cd entering and leaving larvae provided that we take into account Cd dilution due to animal growth (Thomann 1981):

$$(1)\qquad \frac{d[Cd]_{Chaoborus}}{dt} = \underbrace{(AE \times IR \times [Cd]_{Food})}_{\text{Cd uptake from food}} - \underbrace{(k_e[Cd]_{Chaoborus})}_{\text{Cd loss by efflux}} - \underbrace{(k_g[Cd]_{Chaoborus})}_{\text{Cd dilution by growth}}$$

where AE is the Cd assimilation efficiency (grams Cd retained per gram Cd ingested), $[Cd]_{Food}$ and $[Cd]_{Chaoborus}$ (micrograms Cd retained per gram dry weight) are the Cd concentrations in prey and in *Chaoborus* larvae, respectively, and k_e and k_g (per day) are the rate constants for Cd efflux and animal growth, respectively.

Furthermore, we assumed that larval growth can be represented by the exponential function (Spacie and Hamelink 1985)

$$(2)\qquad W = W_0 e^{k_g t}$$

where W_0 is the initial weight (milligrams dry weight per larva) and t is time (days). We then used eq. 2 and the integrated form of eq. 1 to estimate for each *Chaoborus* species the rate constants k_e and k_g as well as the Cd assimilation efficiency, as described below.

First, we used eq. 2 and our measurements of changes in larval mass during the Cd uptake experiment (Fig. 3*a*) to estimate, by nonlinear regression, values of W_0 and the growth rate constant k_g. Values of k_g were highest for the large-bodied species but low or negligible for the small-bodied species (Table 3). Low k_g values are not surprising given the low temperature (5°C) at which we conducted our experiments (Davies and Tribe 1969). Under similar feeding conditions but at higher temperatures, k_g values are substantially greater for these *Chaoborus* species (M.-N. Croteau, unpublished data). Our k_g values are in the range of those found for *C. punctipennis* (–0.001 to 0.020·day^{-1}) by Munger et al. (2000) and for a variety of soil arthropods (0.003–0.027·day^{-1}) by Crommentuijn et al. (1994). The fact that growth rate constants were higher for the large-bodied species suggests that this parameter cannot explain the higher increases in Cd concentrations of these species in our experiments; greater larval growth lowers Cd concentrations by dilution of the Cd in the added body tissues.

To estimate values of the Cd loss rate constant k_e, it was first necessary to estimate, by nonlinear regression, values of the growth rate constant for the loss experiment (k_g^*) (Table 3) using eq. 2 and our measurements of changes in larval mass during the Cd loss experiment (Fig. 3*b*). We then assumed that Cd uptake was negligible during our Cd loss experiment. This assumption is reasonable because we fed high-Cd *Chaoborus* larvae low-Cd prey; Cd concentrations in prey offered during the Cd loss experiment were 10 times lower than in those offered during the Cd uptake experiment (Table 3). This assumption allowed us to simplify eq. 1, which in its integrated form becomes

$$(3)\qquad [Cd]_{Chaoborus} = [Cd]^{0*}_{Chaoborus} e^{-(k_g^* + k_e)t}$$

Where $[Cd]^{0*}_{Chaoborus}$ is the Cd concentration in larvae (micrograms Cd per gram dry weight) at the beginning of the Cd loss experiment. Using our measurements of Cd concentrations in *Chaoborus* during the Cd loss experiment (Fig. 4) and the k_g^* values from Table 3, we estimated the loss rate constant k_e by nonlinear regression (eq. 3). The Cd loss rate constants for our four *Chaoborus* species (0.003–0.037·day^{-1}) (Table 3) are in the range of those reported for a variety of invertebrates, i.e., 0.003–0.018·day^{-1} for *C. punctipennis* (Munger et al. 1999), 0.012·day^{-1} for mussels (Roditi and Fisher 1999), and 0.017–0.048·day^{-1} for soil arthropods (Crommentuijn et al. 1994). Differences in the loss rate constant among species are reported to depend on the physiology of the species involved (Crommentuijn et al. 1994). The fact that *C. americanus* larvae had the highest Cd loss rate constant does not explain why they accumulated more Cd than did the small-bodied species in the Cd uptake experiment.

Using the values of k_g and k_e from Table 3, the ingestion rates calculated daily for each *Chaoborus* larva of each species, as well as the mean daily Cd concentration measured in food offered during the uptake experiment, we estimated Cd assimilation efficiencies for the four species (Table 3) by fitting our data points (Fig. 5) to the integrated form of eq. 1:

$$(4)\qquad [Cd]_{Chaoborus} = \frac{AE \times IR \times [Cd]_{Food}}{k_g + k_e} \times \left(1 - e^{-(k_g + k_e)t}\right) + [Cd]^0_{Chaoborus} e^{-(k_g + k_e)t}$$

For the overall mean Cd concentration in prey offered to *Chaoborus* ($[Cd]_{Food}$), we used a value of 10.6 ± 0.3 (SD) μg·g^{-1} as given in Table 3. We note that this Cd concentration in prey is much higher than the highest Cd concentration that we measured in *Chaoborus* at the end of our uptake experiment, i.e., 1.8 μg·g^{-1} for *C. americanus*. This apparent "biodilution" of Cd in our experimental food chain

Table 3. Values of Cd accumulation parameters measured (±95% confidence interval) or estimated (±SE) from our experiments with the various *Chaoborus* species.

Parameter	Abbreviation or symbol	*C. punctipennis*	*C. albatus*	*C. flavicans*	*C. americanus*
Cd uptake experiment					
Initial *Chaoborus* weight (mg·larva^{-1}) (±95% CI)	W_0	0.23±0.04 *a* (n = 5)	0.24±0.02 *a* (n = 5)	0.66±0.15 *b* (n = 5)	0.44±0.07 *c* (n = 5)
Initial *Chaoborus* Cd concentration (μg·g^{-1}) (±95% CI)	$[Cd]^0_{Chaoborus}$	0.18±0.04 *a* (n = 5)	0.22±0.05 *a* (n = 5)	0.46±0.02 *b* (n = 5)	1.44±0.15 *c* (n = 5)
Mean prey Cd concentration (μg·g^{-1}) (±95% CI)	$[Cd]_{Food}$	10.6±0.3 *a* (n = 49)	10.6±0.3 *a* (n = 49)	10.6±0.3 *a* (n = 49)	10.6±0.3 *a* (n = 49)
Mean ingestion rate (g prey·g predator^{-1}·day^{-1}) (±95% CI)	IR	0.052±0.009 *a* (n = 25)	0.038±0.005 *b* (n = 25)	0.057±0.008 *a* (n = 25)	0.046±0.007 *ab* (n = 25)
Growth rate constant (day^{-1}) (±SE)	k_g	0.0134±0.0082 *ab* (n = 25)	–0.0012±0.0092 *b* (n = 25)	0.0232±0.0058 *a* (n = 25)	0.0253±0.0046 *a* (n = 25)
Cd assimilation efficiency (%) (±SE)	AE	5.7±1 *a* (n = 25)	6.9±1 *a* (n = 25)	45±4 *b* (n = 25)	58±4 *c* (n = 25)
Cd loss experiment					
Initial *Chaoborus* weight (mg·larva^{-1}) (±95% CI)	W_0^*	0.22±0.04 *a* (n = 5)	0.29±0.04 *a* (n = 5)	0.61±0.18 *b* (n = 5)	0.24±0.03 *a* (n = 5)
Initial *Chaoborus* Cd concentration (μg·g^{-1}) (±95% CI)	$[Cd]^{0*}_{Chaoborus}$	8.29±0.81 *a* (n = 5)	7.07±0.83 *a* (n = 5)	4.95±1.55 *b* (n = 5)	14.1±2.05 *c* (n = 5)
Final *Chaoborus* Cd concentration (μg·g^{-1}) (±95% CI)	$[Cd]^{10*}_{Chaoborus}$	8.78±3.29 *ac* (n = 5)	4.63±1.67 *ab* (n = 5)	4.92±0.38 *b* (n = 5)	8.90±1.03 *c* (n = 5)
Mean prey Cd concentration (μg·g^{-1}) (±95% CI)	$[Cd]^*_{Food}$	1.1±0.1 *a* (n = 36)	1.1±0.1 *a* (n = 36)	1.1±0.1 *a* (n = 36)	1.1±0.1 *a* (n = 36)
Growth rate constant (day^{-1}) (±SE)	k_g^*	0.015±0.0118 *a* (n = 55)	–0.0051±0.0129 *a* (n = 54)	–0.0091±0.0112 *a* (n = 55)	–0.0019±0.0091 *a* (n = 55)
Loss rate constant (day^{-1}) (±SE)	k_e	0.0029±0.0164 *a* (n = 55)	0.0253±0.0172 *a* (n = 54)	0.0038±0.0090 *a* (n = 55)	0.0368±0.0085 *a* (n = 55)
Cd exchange rates (ng Cd·g^{-1}·day^{-1})					
Cd uptake rate	UR	$t_{0,10}$ = 3.2	$t_{0,10}$ = 2.6	$t_{0,10}$ = 28	$t_{0,10}$ = 29
Cd loss rate	LR	$t_{0,10}$ = 154, 163	$t_{0,10}$ = 143, 94	$t_{0,10}$ = –26	$t_{0,10}$ = 493, 344
Net change in Cd	ΔCd	–160 to –151	–140 to –91	54	–464 to –315

Note: Abbreviations or symbols marked with an asterisk refer to the Cd loss experiment. Values followed by a different letter are significantly different (t test, $p < 0.05$). Also given are Cd exchange rates during our Cd loss experiment on the basis of parameter estimates at t = 0 and t = 10.

Fig. 3. Temporal changes in the dry weight (±SD) of (*a*) low-Cd *Chaoborus* species larvae that were exposed for 14 days to water and copepods (ad libidum) from a Cd-rich lake and (*b*) high-Cd *Chaoborus* species larvae that were exposed for 10 days to water and bulk zooplankton (ad libidum) from a low-Cd lake. Experimental data are represented by symbols, whereas lines represent model curves obtained with eq. 2 and the parameters W_0, W_0^*, k_g, and k_g^* given in Table 3. Triangles and circles indicate values for the small-bodied species (open for *C. punctipennis* and solid for *C. albatus*) and the large-bodied species (open for *C. americanus* and solid for *C. flavicans*), respectively.

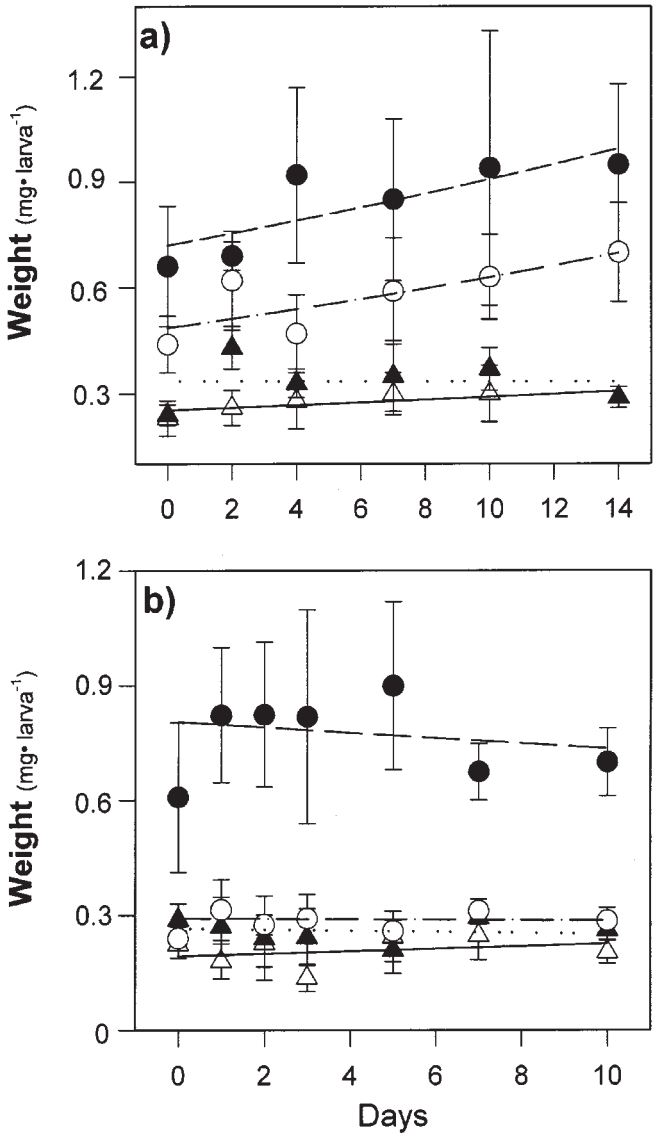
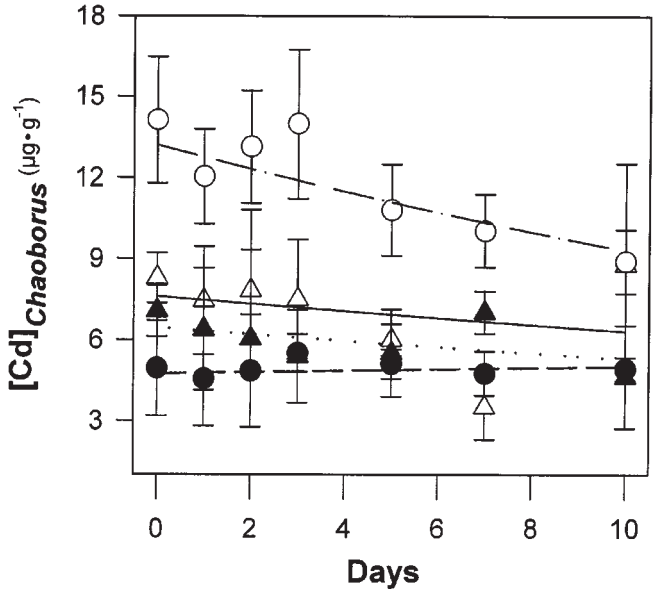


Fig. 4. Temporal changes in the Cd concentration (±SD) of high-Cd *Chaoborus* species larvae that were exposed for 10 days to water and bulk zooplankton (ad libidum) from a low-Cd lake. Experimental data are represented by symbols, whereas lines represent model curves obtained with eq. 3 and the parameters k_e, k_g^*, and $[Cd]^{0*}_{Chaoborus}$ given in Table 3. Triangles and circles indicate values for the small-bodied species (open for *C. punctipennis* and solid for *C. albatus*) and the large-bodied species (open for *C. americanus* and solid for *C. flavicans*), respectively.

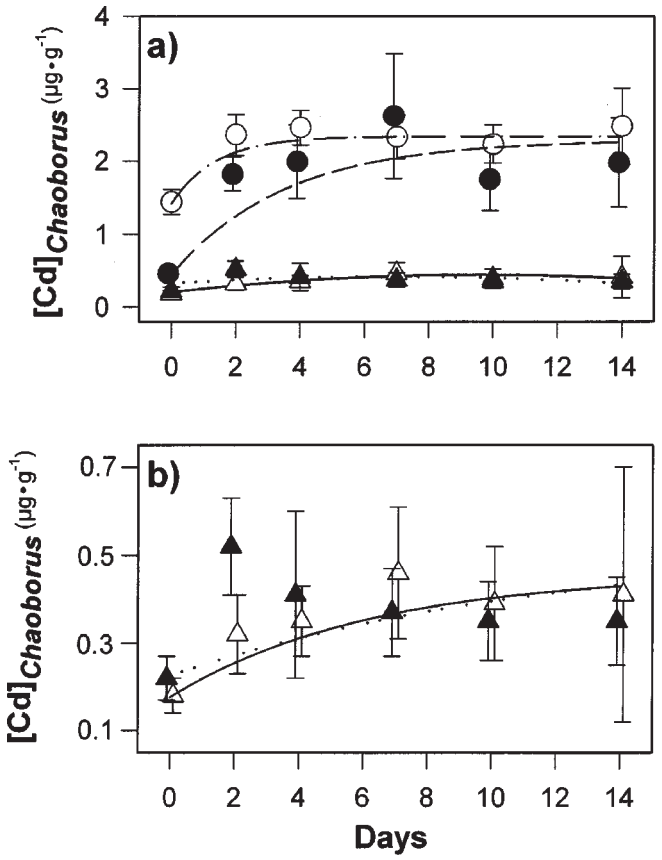


is reported to be common in nature (Hare 1992; C.Y. Chen et al. 2000).

Cadmium assimilation efficiencies differed substantially among species (Table 3), which suggests that it is mainly this variable that explains the species differences in Cd accumulation during our uptake experiment. Assimilation efficiencies were lowest for the small-bodied species *C. albatus* and *C. punctipennis* (6–7%) (Table 3); the value for the latter species (≈6%) is within the range reported for this taxon by Munger et al. (1999) in a field experiment (2–18%). The similarity of the Cd assimilation efficiency values for *C. albatus* and *C. punctipennis* is consistent with their similarity in body size, morphology, and migratory behaviour. Larvae of *C. flavicans*, although the largest in body size, had a somewhat lower assimilation efficiency (45%) than *C. americanus* (58%) (Table 3), the second largest of the four species. If species are ranked according to their gain in mass during our experiment, there is a statistically significant ($r^2 = 0.91$, $p = 0.045$) relationship with their Cd assimilation efficiency (Fig. 6). A similar correlation between the assimilation of C and that of Cd by larvae has been reported for a bivalve by Wang and Fisher (1996).

The explanation for species differences in Cd assimilation efficiencies likely resides in differences among the taxa in the manner in which they consume, digest, and assimilate prey. Species differences in Cd assimilation efficiency cannot be attributed to the degree to which they ingest prey either whole or in pieces (sloppy feeding) because (*i*) we usually found prey whole, albeit crushed, in the guts of all *Chaoborus* species, (*ii*) larval mouth parts are similar among species and do not appear to be designed to tear or dismember prey (Saether 1972), and (*iii*) *Chaoborus* are reported to be gape-limited predators, which suggests that they eat prey whole (Hare and Carter 1987). Following ingestion, prey are crushed in the pharynx, which is facilitated in some *Chaoborus* species by a pair of large teeth on the pharyngeal sphincter. Differences in teeth structure among species are reported to be related to the hardness of their prey (Hare and Carter 1987). All of the species that we studied possess a similar pair of simple spines on the pharyngeal sphincter

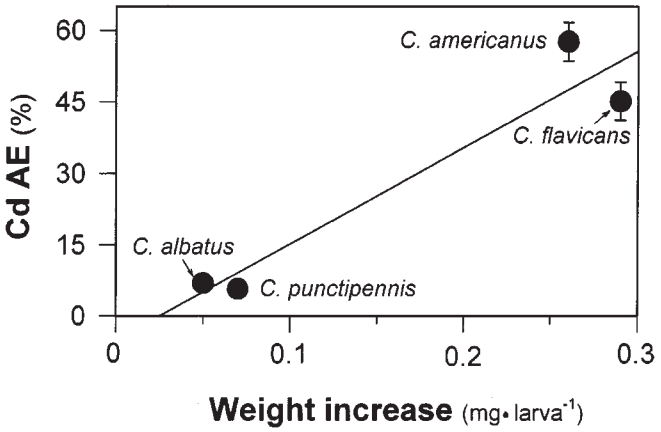
Fig. 5. Temporal changes in the Cd concentrations of *Chaoborus* species larvae from a Cd-poor lake that were exposed for 14 days to water and copepods (ad libidum) from a Cd-rich lake. In both panels, lines represent model curves (obtained with eq. 4 and the parameters assimilation efficiency, ingestion rate, W_0, k_g, k_e, and $[Cd]^0_{Chaoborus}$ given in Table 3) and symbols (±SD) represent our experimental data. The data for *C. punctipennis* and *C. albatus* larvae are repeated in Fig. 5*b* with the scale on the vertical axis expanded. Triangles and circles indicate values for the small-bodied species (open for *C. punctipennis* and solid for *C. albatus*) and the large-bodied species (open for *C. americanus* and solid for *C. flavicans*), respectively.



(similar to those illustrated for *Chaoborus ceratopogones* by Hare and Carter 1987), which suggests that differences in Cd assimilation efficiency among our *Chaoborus* species cannot be explained by this feature. After solid prey parts have been egested via the mouth, remaining liquids pass down the digestive tract where differences in residence time (Decho and Luoma 1991), enzyme efficiencies (Mayer et al. 1996; Z. Chen et al. 2000), and both the number and the nature of Cd uptake sites in the gut (Hare 1992) could lead to differences in C and Cd assimilation efficiencies. Because information on these subjects is lacking for *Chaoborus*, we are unable to speculate further about the cause of the large differences in Cd assimilation efficiency among our study species.

We assumed that Cd uptake by *Chaoborus* larvae during our Cd loss experiment was negligible. We can test this assumption by using eq. 1 and values found in both our Cd uptake experiment (assimilation efficiency, ingestion rate, $[Cd]_{Food}$, k_g (Table 3)) and our Cd loss experiment (k_e, k^*_g, $[Cd]^{0-10*}_{Chaoborus}$ (Table 3)) to estimate specific Cd uptake and loss rates during our Cd loss experiment. Excluding *C. flavicans* (which had a negative Cd loss rate), Cd uptake rates (calculated at both $t = 0$ and $t = 10$) were lower than Cd loss rates by a factor of from 12 to 55 times (Table 3), confirming that Cd uptake was negligible compared with Cd loss during our loss experiment. The negative Cd loss rate for *C. flavicans* is due to the combination of a very low k_e value and weight gain by this species during the Cd loss experiment (Fig. 3*b*). Because the Cd uptake rates given in Table 3 suggest that all *Chaoborus* species accumulated some Cd during our Cd loss experiment, Cd loss rates for all species are likely underestimates.

Fig. 6. Cadmium assimilation efficiency (±SE) (also see Table 3) of *Chaoborus* species larvae as a function of their increase in dry weight during a 14-day exposure to both Cd-contaminated food and water ($r^2 = 0.91$, $p = 0.045$).



Differences in Cd concentrations among *Chaoborus* species in the field

When Cd concentrations in *Chaoborus* larvae reach a steady state ($[Cd_{Chaoborus}]_{ss}$), i.e., when $d[Cd]_{Chaoborus}/dt$ equals zero, eq. 1 becomes

$$(5) \qquad [Cd_{Chaoborus}]_{ss} = \frac{AE \times IR \times [Cd]_{Food}}{k_e + k_g}.$$

Using the values of assimilation efficiency, ingestion rate, $[Cd]_{Food}$, and k_g from the Cd uptake experiment as well as those of k_e from the Cd loss experiment (Table 3), we estimated steady-state values of 10.1, 4.5, 1.9, and 1.1 µg Cd·g^{-1} for *C. flavicans*, *C. americanus*, *C. punctipennis*, and *C. albatus*, respectively. If we assume that in nature, coexisting species feed on the same prey type at the same rate, as in our experiments, we would expect to observe the following patterns among *Chaoborus* species in the field. Firstly, Cd concentrations in the two small-bodied species (*C. punctipennis* and *C. albatus*) should be similar, which was the case in the four lakes in which they coexisted (Table 2). This similarity in Cd accumulation is likely explained in part by the similar morphology of these species, which are placed apart from the large-bodied species in the subgenus *Sayomyia* (Borkent 1979). These small-bodied species could be treated as a unit for Cd monitoring purposes. Secondly, our laboratory experiments

would lead us to expect that Cd concentrations in *C. americanus* larvae would exceed those in the two small-bodied species, which was the case in the four lakes in which these species coexisted (Table 2). Because of these differences, grouping the small- and large-bodied species together for biomonitoring purposes would likely reduce the accuracy of predictions, as we observed in our earlier studies (Croteau et al. 1998; Hare and Tessier 1998). Lastly, we would expect that Cd concentrations in *C. flavicans* larvae should exceed those of the three other species. This prediction was borne out in only four of our 12 study lakes (Table 2). Because of this departure from our expectations, it is likely that our assumption of similar Cd concentrations in the diets of *C. flavicans* and the other species is not tenable. Major differences in diet have been reported between sympatric populations of *C. flavicans* and *C. punctipennis* (Sardella and Carter 1983), but comparative dietary data on other coexisting species is lacking. Other biological differences among species, such as their migratory behaviour, could also influence their accumulation of Cd in nature. Among our study species, larvae of *C. americanus* spend all of their time in the warm epilimnion during summer. In contrast, the other species spend the day in the colder waters or sediment of the hypolimnion (to avoid fish predation) and migrate into warmer surface waters only at night to feed on zooplankton. Differences in the temperatures to which larvae of the various *Chaoborus* species are exposed could alter their metabolic rate and their assimilation of Cd and C (Giguère and Dill 1980; Giguère 1981).

Overall, our experimental results suggest that even with the same food source, Cd accumulation can differ substantially among *Chaoborus* species due in the main to differences in the efficiency with which they assimilate Cd from their food.

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