

Explaining metal concentrations in sympatric *Chironomus* species

Sylvain Martin¹, Isabelle Proulx, and Landis Hare²

INRS-ETE, Université du Québec, 490 de la Couronne, Quebec City, Quebec G1K 9A9, Canada

Abstract

We compared metal concentrations in larvae of two *Chironomus* species (*Chironomus staegeri* and *Chironomus tigris*) living in the same lake and at the same depth and time. Concentrations of the nonessential metal cadmium (Cd) differed greatly (>8×) between the two species, whereas those of two essential metals differed either much less (zinc [Zn], 2×) or not at all (copper [Cu]). These trends were constant in all seasons. On the one hand, differences in Cd and Zn concentrations between the species were not explained by differences in either their size or their life cycle. Likewise, differential exposure to dissolved metals did not explain larval Cd and Zn concentrations because vertical gradients in dissolved metals did not correlate with depths of larval feeding. On the other hand, the species differed in the type of sediment that they consumed, and measurements of sulfur stable isotopes in larvae confirmed that whereas *C. staegeri* consumes mostly surface oxic sediment, *C. tigris* eats mainly deeper anoxic sediment. Because total metal concentrations in gut contents were not correlated with those in larvae, it is likely that metal bioavailability differs between the two sediment types. Overall, our results show that because metal concentrations can differ widely between sympatric congeners, extrapolation from one *Chironomus* species to another may not be justifiable. Furthermore, because larvae exposed to Zn in the laboratory did not accumulate this metal as they would in the field, we suggest that care is warranted when extrapolating from results obtained in laboratory tests to animals living in the field.

Measurements of metals in animals can be an important component of ecological risk assessments because they provide the link between metal exposure and metal toxicity (Chapman and Wang 2000). Metal concentrations in animals are also used to estimate the contamination level of whole ecosystems (Phillips and Rainbow 1993; Hare et al. in press). Animals used in this way should be identified to the lowest possible taxonomic level, so that behavioral and physiological differences among taxa will not confound trends in contaminant concentrations among sites or over time (Rainbow 2002; Skubala and Kafel 2004; Buchwalter and Luoma 2005). However, identifying freshwater invertebrates to the species level is often problematic, and thus necessity frequently dictates that species be grouped for contaminant analyses.

A case in point is the insect *Chironomus* (Diptera, Chironomidae, Chironominae, Chironomini); this genus has a worldwide distribution, and larvae are tolerant to and accumulate contaminants in lakes and rivers (Armitage et al. 1995), which makes them good candidates for use as biomonitors in these ecosystems. However, the identification of *Chironomus* species is difficult without recourse to examining the giant salivary chromosomes of larvae (e.g., Butler et al. 1995), knowledge of which is confined to a small number of specialists. Since congeners can differ markedly in how they take up, store, and lose contaminants (Aoki et al. 1989; Lobel et al. 1990; Rainbow 2002), information is needed to determine if it is justifiable to pool *Chironomus* species for contaminant analyses. Such studies would also shed light on whether data from toxicity tests using various species of *Chironomus* are likely to be comparable (Jeyasingham and Ling 2000; Watts and Pascoe 2000; Péry et al. 2005) and if the results of such tests can be extrapolated to congeners in the field. Lastly, comparative studies would be useful for explaining any differences in contaminant concentrations between closely related species.

We set out to determine if the larvae of two *Chironomus* species (*Chironomus staegeri* and *Chironomus tigris*) that share the same habitat differ in their concentrations of several trace metals of environmental importance: cadmi-

¹ Present address: Environment Canada, Meteorological Service of Canada, 1141 route de l'Église, Quebec City, Québec G1V 4H5.

² Corresponding author (landis@ete.inrs.ca).

Acknowledgments

Funding was provided by the Metals in the Human Environment Research Network and the Natural Sciences and Engineering Research Council of Canada. We thank Mac Butler, Louis Croisetière, René Rodrigue, and André Tessier for their comments and assistance.

um (Cd), copper (Cu), and zinc (Zn). To explain differences between the two species, we compared the size and life cycle of larvae and evaluated their exposure to metals in lake water and food (sediment).

Methods

Identities of the *Chironomus* species were confirmed by M. Butler (University of North Dakota, Fargo) based on the structure and number of larval giant salivary chromosomes; both species are unusual for the genus in having fewer than four haploid chromosomes (*C. tigris* has two and *C. staegeri* has three; Butler et al. 1995). In practice, we were also able to separate these species on the basis of differences in their head capsule: the eyespots of *C. tigris* are about twice the diameter of those of *C. staegeri*, the color of the dorsal portion of the head capsule (the fronto-clypeal apotome) is dark brown in *C. tigris* but unpigmented in *C. staegeri*, and the proximal mandibular tooth of *C. tigris* is almost completely fused to the mola, whereas that of *C. staegeri* is distinct from the mola.

We collected *Chironomus* larvae on several dates (May–February) at a single station (9-m depth) near the outlet of Lake Saint-Joseph (46°53'N, 71°38'W), a large (~13-km²) Canadian Shield lake located ~40 km WNW of Quebec City, Quebec. The lake's drainage basin is mainly forested, and the major local human effect on the lake is likely from the cottages situated along its shore. We measured a summer vertical stratification of the water column at the sampling site only in July, and sediment temperatures varied from a winter low of 4°C to a high of 20°C in August. Throughout the open-water period, lake water at the station is slightly acidic (pH 6–7), well oxygenated (>65% saturation), quite dilute (conductivity of 20–30 µS), and fairly transparent (Secchi depth 3.5–5.0 m).

We collected lake water for metal analyses in Plexiglas diffusion samplers (Hare et al. 2001) placed by divers in sediment near our sampling station for *Chironomus*. These samplers consist of a series of 3-mL cells positioned vertically at 1-cm intervals and covered by a polysulfone membrane (0.2-µm nominal pore size, Gelman). Following a 2-week equilibration period, samplers were retrieved by divers, and water samples were removed immediately by piercing the membrane with the acid-washed tip of a pipette and placing the contents in a Teflon vial to which 30 µL of 1 mol L⁻¹ Ultrex HNO₃ had been previously added.

On each sampling date, we collected sediment using an Ekman grab and sieved it through a 0.3-mm mesh-aperture net. Larvae and remaining sediment were either preserved in 10% formalin for determining life cycles or transported live to the laboratory. Life cycles of the two *Chironomus* species were determined by noting changes in the numbers of each larval instar and the length of individual larvae collected in 10 grab samples on each of five dates spanning the open-water season (May–October). We separated larval instars by the mean width of their head capsule; that is, pooled values (±SD, *n* = 45) for the fourth, third, and second instars of the two species measured 0.400 ± 0.028, 0.230 ± 0.017, and 0.121 ± 0.015 mm, respectively (head

capsule width did not differ significantly between the species).

In the laboratory, live fourth-instar larvae were held in lake water for 1–2 d to purge their gut of sediment. On one sampling date (mid-June), additional unpurged larvae were dissected to extract sediment for measurement of metal concentrations in gut contents. On a second date (mid-February), larvae were dissected into constituent parts to measure internal metal distributions. Dissections were carried out under a microscope by severing the head capsule and last abdominal segment so that the digestive tract (gut) could be pulled from the insect's body. To isolate gut contents, an incision was made near the junction of the esophagus and the midgut so as to free the peritrophic membrane. This membrane, along with the enclosed gut contents, was then pulled from the posterior of the gut. This procedure permitted us to separate larvae into three parts: gut contents, gut tissues, and body (parts and hemolymph remaining after dissection).

For metal measurements, whole larvae, larval parts, and gut contents were placed on separate pieces of preweighed, acid-washed Teflon® sheeting held in Teflon® tubes in a Petri dish, then lyophilized until constant weight. Each dried sample was placed in an acid-washed Teflon® vial and digested in 100 µL of concentrated Aristar nitric acid mg⁻¹ of dry weight at 121°C in an autoclave for 4 h. Certified reference material (TORT-1; lobster hepatopancreas, Canadian National Research Council) was digested along with insect samples. The volume of each digested sample was adjusted to a final ratio of 16.6% HNO₃:H₂O (5:1, v:v) with ultrapure water. Cadmium and copper were measured using an atomic absorption spectrophotometer (AAS; Varian AA-1275) equipped with a graphite tube atomizer (GTA; Varian GTA-95). Zinc was measured with an acetylene flame spectrophotometer (Varian AA-575). Measurements of Cd, Cu, and Zn in reference samples were within 5–8% of the certified values for these metals. Metals in water samples were measured by AAS-GTA.

To compare feeding habits, we noted the color of sediment in the gut (removed by dissection, as described previously) of 25 freshly killed, final-instar larvae of each species (collected in mid-June). We measured the length of the gut that comprised sediment of each color and then fit these data to an arbitrary scale ranging from 0 to 5, where 0 indicates that all gut contents were gray in color and 5 indicates that all gut contents were orange-brown. We also measured the rate at which sediment moves along the gut by placing freshly collected (mid-October) fourth-instar larvae in aquaria at room temperature for a 1-week acclimation period. Sediment from the collection site that had been sieved to remove animals was mixed with colored particles (1 g Radian Fluorescent Pigment [JST-300] per liter sediment), and 0.8 liter was added to a 2-liter acrylic container. Lake water (1 liter) was added slowly so as to not resuspend sediment and continuously aerated thereafter. Five *C. tigris* and two *C. staegeri* were added to each container. Each hour thereafter, the contents of two containers were sieved to isolate larvae, the guts of which were extracted by dissection (as described previously) and observed under ultraviolet light to determine the distance

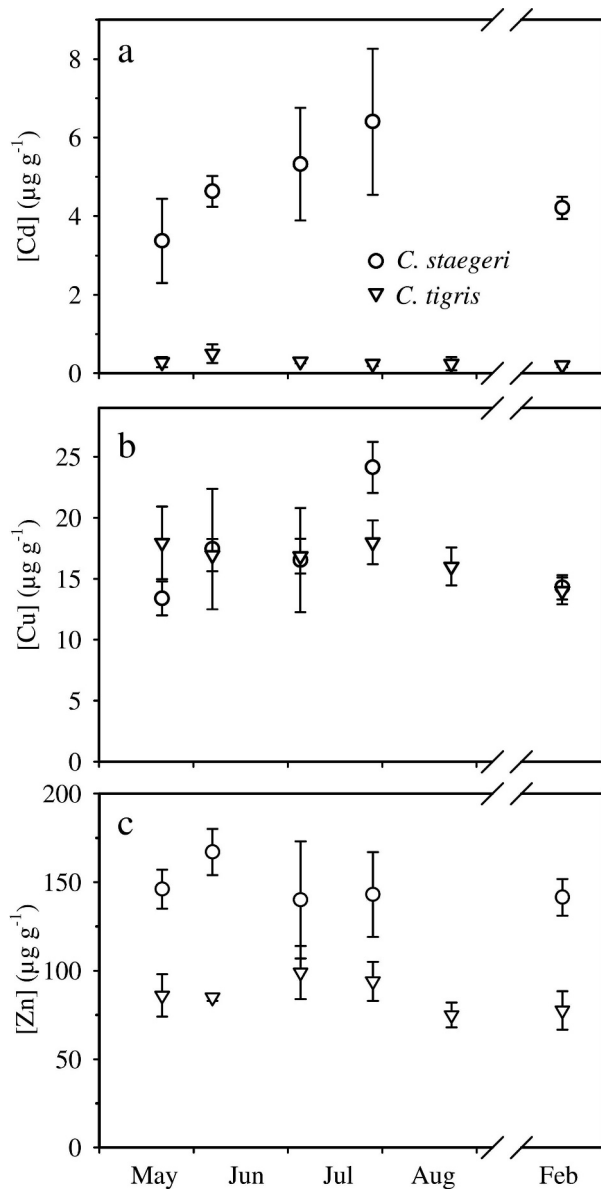


Fig. 1. Mean (\pm SD) trace metal concentrations in fourth-instar larvae of *C. staegeri* and *C. riparius* collected at the same station over a 10-month period: (a) Cd, (b) Cu, and (c) Zn.

traveled by the marked sediment. Because larvae differed somewhat in length, values were standardized to an individual 15 mm in length.

To compare Zn exchange rates of the *Chironomus* species, we added ⁶⁵Zn to unsieved sediments obtained from the larval collection site and mixed these sediments over a period of 2 weeks. We then placed 0.8 liters of this sediment, followed by 1.2 liters of lake water, in 2-liter round plastic containers to obtain a sediment depth of 7.5 cm. These containers were placed at 13°C in the dark for 1 week, and then larvae were added at field densities (10 *C. tigris* and four *C. staegeri* microcosm⁻¹). Four microcosms were sacrificed at each sampling time, and larvae were rinsed in EDTA to remove sorbed Zn, dissected to remove their gut contents, dried, weighed, and their ⁶⁵Zn content measured. After 66 d

in radioactive sediment, remaining larvae were transferred to uncontaminated microcosms (prepared as described previously but without added ⁶⁵Zn) to measure ⁶⁵Zn loss. Measurements of ⁶⁵Zn were made in an LKB Wallac 1282 Compugamma NaI(Tl) counter.

Stable sulfur isotopes were measured in pooled samples of larvae that had been freeze-dried and ground to a powder. Analyses were conducted by Iso-Analytical Ltd on an elemental analyzer–isotope ratio mass spectrometer (ANCA-GSL/20-20; Europa Scientific). Sulfur isotopic ratios are reported as $\delta^{34}\text{S} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where R is the ratio ³⁴S : ³²S and the standard used was sulfur from the Canyon Diablo troilite.

Results and discussion

Metal concentrations in the Chironomus species—Cadmium concentrations in *C. staegeri* were a minimum of eight times greater than those of *C. tigris* (Fig. 1a), and Zn concentrations in the former were consistently double those of the latter (Fig. 1c) throughout the year. In contrast, Cu concentrations did not differ significantly ($p > 0.05$, Kruskal–Wallis test) between the two species on the majority of sampling dates (Fig. 1b). For a given *Chironomus* species, metal concentrations in fourth-instar larvae did not vary significantly over time ($p > 0.05$, analysis of variance of log-transformed data) with the exception of Cu concentrations in *C. staegeri*, which showed small but significant ($p < 0.05$) temporal changes (Fig. 1a–c).

Cadmium concentrations in gut tissues of *C. staegeri* were much higher than those of *C. tigris* (Fig. 2a). Body Cd concentrations did not differ between the two species ($p > 0.05$, *t*-test of log-transformed data) and were lower than those in the gut (Fig. 2a). Thus, the excess Cd present in *C. staegeri* (Fig. 1a) is located largely in its gut tissues (Fig. 2d). Likewise, in the mayfly *Hexagenia limbata*, increases in its Cd burden are confined mainly to the gut (Hare and Campbell 1992; Michaud et al. 2005). Given the relatively high concentrations of Cd in the gut, this organ contained the majority of the Cd present in both *Chironomus* species (Fig. 2d) even though it represents only ~20% of the larval mass. The same is true for several types of aquatic insects (Seidman et al. 1986; Hare et al. 1991; Roy and Hare 1999). Much of the Cd in *Chironomus* is reportedly bound to metallothionein (Seidman et al. 1986).

Both the concentrations and the proportions of Cu in a given larval part were similar between the two *Chironomus* species ($p > 0.05$, *t*-tests of log-transformed data). Copper concentrations were higher in the guts than in the bodies of both species (Fig. 2b), but the quantities of Cu in these parts were similar (Fig. 2e). Zinc concentrations were similar in gut tissues of the two species but were higher in the body portion of *C. staegeri* (Fig. 2c); the bulk of the Zn was located in the body portion of both species (Fig. 2f), as it is in several other types of aquatic insects (Hare et al. 1991).

Life cycles of the Chironomus species—We compared the life cycles of the two *Chironomus* species to determine if

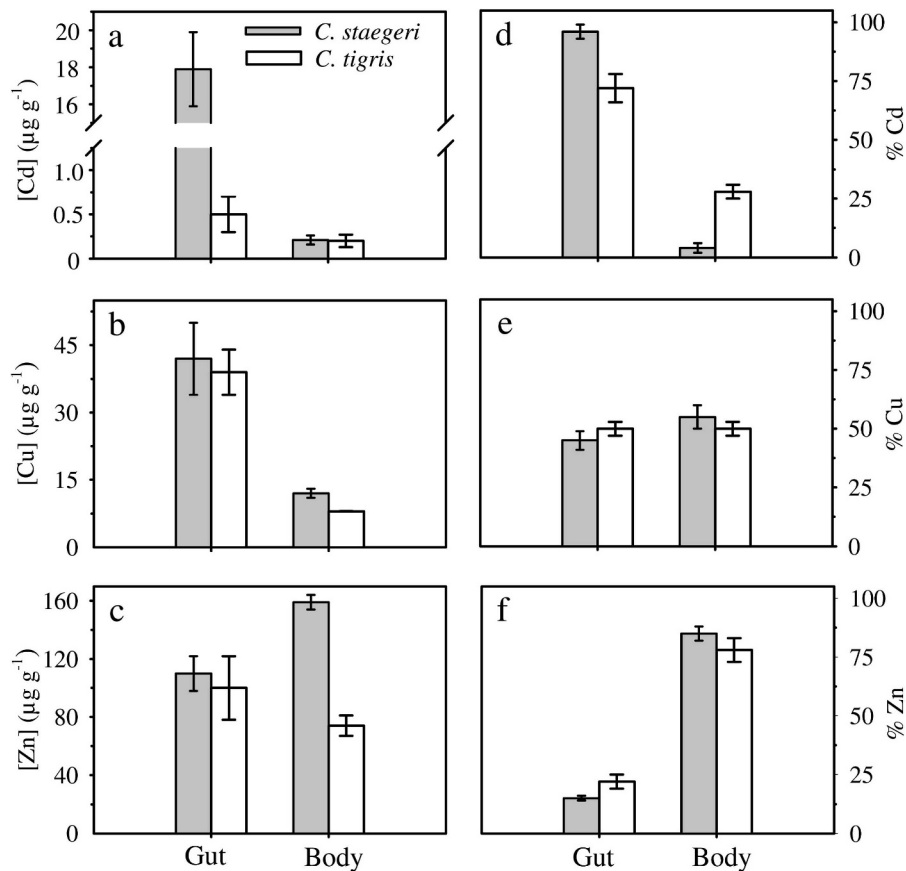


Fig. 2. Mean (\pm SD) trace metal (a–c) concentrations in parts (body and gut) of fourth-instar larvae of *C. staegeri* and *C. riparius* as well as the (d–f) percentage distribution of metals in these parts.

differences in their age or development rate could explain their metal concentrations (Péry et al. 2005). The maximum densities of both species (50 and 350 m⁻² for *C. staegeri* and *C. tigris*, respectively) were measured in September, when the largest numbers of young instars were present (Fig. 3d,i). By October, the majority of larvae had approximately doubled in length and were in the fourth instar (Fig. 3e,j). Between October and May, there was little change in the size structure of either population, suggesting that most individuals overwinter in the last larval instar (Fig. 3); indeed, in February, most larvae were in their fourth instar (data not shown), and their guts were empty on collection, which suggests that they do not feed in winter. In July, *C. staegeri* larvae were nearly absent from the sampling station (Fig. 3b), which indicates either that this species emerged in June or that larvae moved away from the station in July (low numbers were collected nearby) or both. In contrast, *C. tigris* larval abundances were similar in May and July but much lower in August (Fig. 3), which suggests that the emergence period of this species is centered on the month of August. Between mid-August and mid-September, the densities of both species increased dramatically (Fig. 3), which suggests a rapid rate of larval growth during this period. Thus, although the timing of their emergence differs somewhat, both species have a life cycle of the same duration (1 yr), which suggests

that fourth instars collected at a given time are of similar age and thus that differences in age between the species are not likely to explain their metal concentrations.

Larval size and metal concentrations of the Chironomus species—We compared the size of the two *Chironomus* species because metal concentrations in this genus can vary with larval size; concentrations of essential metals, such as Cu and Zn, are reported to remain constant as larvae grow, whereas those of nonessential metals, such as Cd, are reported to decrease with increasing larval size (Krantzberg 1990). Fourth-instar larvae of *C. staegeri* tended to be longer and heavier than those of *C. tigris*; for example, mean total lengths (\pm SD) were 20.3 \pm 3.3 ($n = 20$) mm and 13.5 \pm 2.4 ($n = 50$) mm, respectively (in mid-October; Fig. 3), whereas mean individual dry masses (\pm SD, $n = 60$) were 1.37 \pm 0.15 mg and 0.96 \pm 0.11 mg, respectively (in mid-February). As expected (Krantzberg 1990), Cu concentrations did not differ between the species, which suggests that larvae maintain their concentrations of this essential metal constant by controlling its influx or efflux or both (Rainbow 2002). The fact that Cd and Zn concentrations were higher in the larger-bodied species (*C. staegeri*) and thus were not diluted by its additional mass (Krantzberg 1990) suggests that differences in larval size do not explain the species differences in Cd and Zn concentrations.

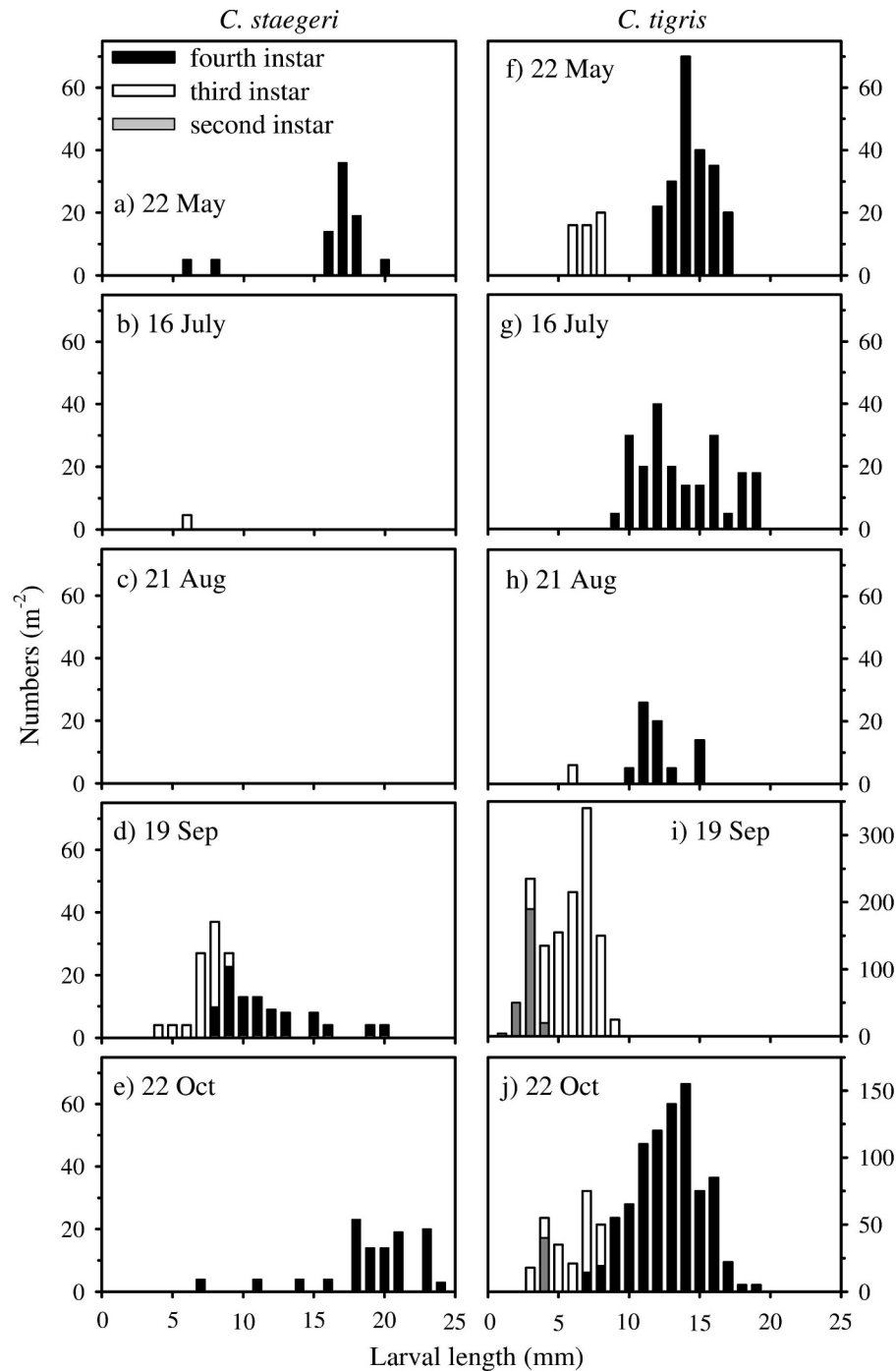


Fig. 3. Temporal changes in the population densities, length, and instar of (a–e) *C. staegeri* and (f–j) *C. riparius* larvae collected at a single station during the ice-free period on the following days: 22 May, 16 Jul, 21 Aug, 19 Sep, and 22 Oct. Note the changes in scale of the vertical axes for *C. tigris*.

Exposure to dissolved metals—Since neither the life cycles nor the sizes of the species explained their metal concentrations, we hypothesized that behavioral differences between the two influence their exposure to metals in either lake water, sediment, or both of these potential exposure routes. Dissolved Cd and Zn concentrations (Fig. 4a,c) were lower in oxic water overlying the sediment than in anoxic

interstitial water below the interface (oxygen generally disappears within a few millimeters below the interface; Frenzel 1990; Wang et al. 2001). In contrast, Cu concentrations were similar above and below the sediment–water interface (Fig. 4b). Thus, if these *Chironomus* species take up their metals mainly from the dissolved phase, our results suggest that *C. staegeri*, with its greater

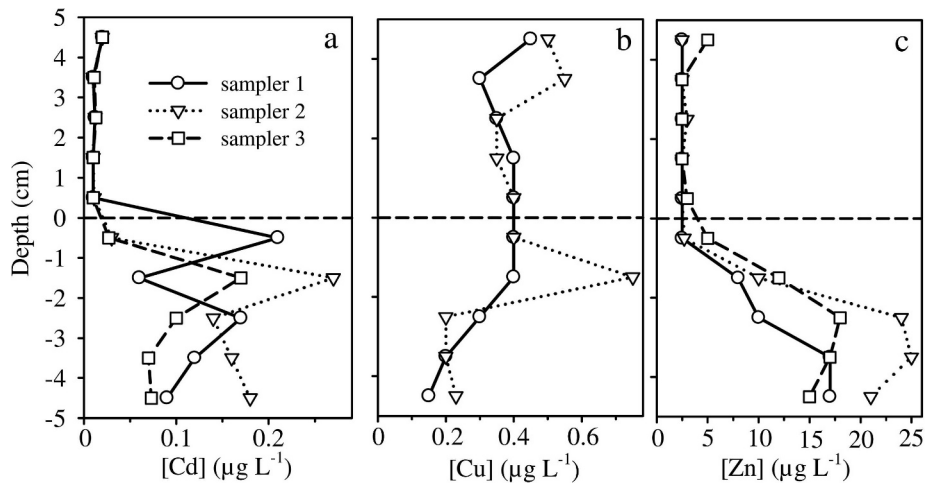


Fig. 4. Changes in dissolved concentrations of (a) Cd, (b) Cu, and (c) Zn across the sediment–water interface (dashed horizontal line) as measured in samples collected from three diffusion samplers.

Cd and Zn concentrations (Fig. 1a,c), is more exposed to metals in anoxic interstitial water, where Cd and Zn concentrations are higher, than is *C. tigris*. Coherent with this idea is that dissolved Cu concentrations did not differ with depth (Fig. 4b), and Cu concentrations in the two *Chironomus* species were similar (Fig. 1b).

Before accepting at face value these relationships between dissolved metals and metal concentrations in the two *Chironomus* species, we need to consider the assumptions involved. First, this comparison is based on total dissolved metal rather than on free metal ion concentrations, which, according to the free ion activity model, are more likely to be related to metal concentrations in aquatic invertebrates (Hare and Tessier 1996; Hare et al. in press). Second, although some *Chironomus* species can tolerate anoxia for up to 2 months in the field (Hamburger et al. 1995), the species we studied build U-shaped burrows (Charbonneau and Hare 1998) through which they likely pump the oxygenated water that overlies the sediment

(Jónasson 1972; Frenzel 1990). Thus, our study species are likely mainly exposed to oxygenated water containing low concentrations of Cd and Zn (Hare et al. 2001). Lastly, lake water may not be the main source of metals for these *Chironomus* species because for many aquatic insects, food is their major source of trace metals (Croisetièrre et al. 2006; Orvoine et al. 2006; Hare et al. in press).

Exposure to metal in food; gut passage time—Metal uptake from food is determined in part by ingestion rates (Orvoine et al. 2006), which in *Chironomus* are correlated with the velocity with which food moves along the digestive tract. In laboratory microcosms, *C. tigris* larvae began to feed within the first 1.5 h and had completely renewed their gut contents within ~4 h (Fig. 5). This value is similar to that of 2 h reported for *Chironomus plumosus* (Johnson et al. 1989). In contrast, *C. staegeri* did not consume sediment during its first 7 h in the microcosms and ate intermittently thereafter, with gut fullness varying widely among individuals (Fig. 5). Selck et al. (1999) have shown that the efficiency with which Cd is assimilated from sediment by a polychaete increases with increasing gut residence time. Thus, if we assume both that *Chironomus* larvae can be compared to polychaetes and that *Chironomus* held in the laboratory feed like those in the field, our data suggest that the shorter gut residence time of *C. tigris* would lead to less solubilization of Cd and Zn from sediment than would occur in the gut of *C. staegeri*. Such a difference between the *Chironomus* species would be consistent with the differences in their Cd and Zn concentrations measured in the field (Fig. 1).

Before we accept this difference in gut passage times at face value, we needed to determine if our results are artifacts caused by conditions in the laboratory. For example, the lag time in feeding and the wide differences among individuals of *C. staegeri* suggest that its behavior was altered by laboratory conditions. This supposition is supported by the fact that when the two *Chironomus* species were exposed to ^{65}Zn in sediment, *C. tigris* accumulated far

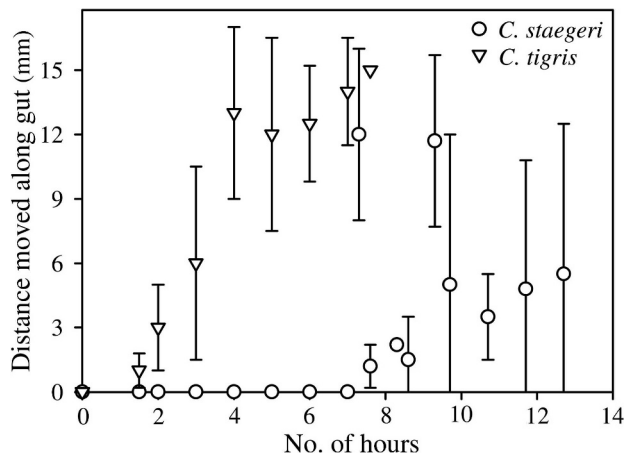


Fig. 5. Progression of colored sediments along the guts of *C. staegeri* and *C. tigris* (means \pm SD) held in laboratory microcosms.

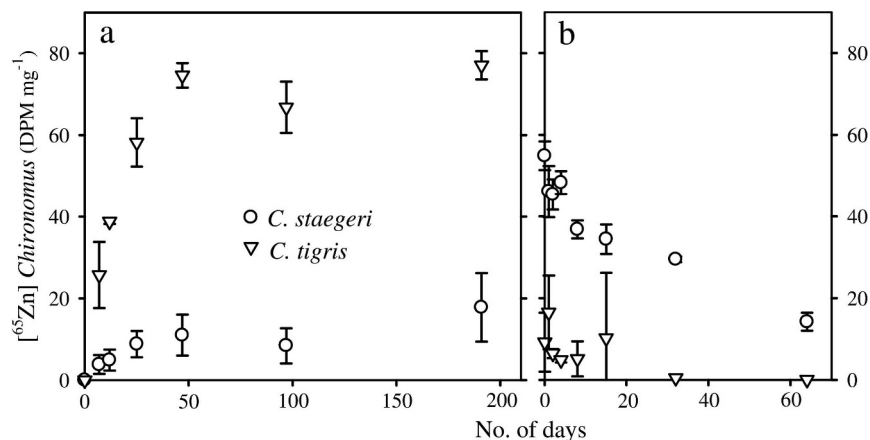


Fig. 6. Mean (\pm SD) ^{65}Zn concentrations (DPM mg^{-1} dry weight) in larvae of *C. staegeri* and *C. tigris* (a) exposed to this metal in laboratory microcosms containing sediment and lake water for 192 d and then (b) transferred to ^{65}Zn -free microcosms for a further 64 d.

more ^{65}Zn than did *C. staegeri* (Fig. 6a), contradicting the trend measured in the field for these species (Fig. 1). Furthermore, these laboratory results contradict those of Selck et al. (1999) because they suggest that by ingesting more sediment, *C. tigris* accumulated more Zn than did *C. staegeri*.

To determine which of the *Chironomus* species was not acting as it would in the field, we compared the ratio of Zn concentrations in larvae to those in sediment under laboratory and field conditions. These ratios were similar for *C. tigris*, at 0.28 and 0.22 (respectively), but for *C. staegeri* they were very much lower in the laboratory (0.02) than in the field (0.56), which suggests that the behavior of *C. staegeri* was indeed altered by confinement and that the rate at which it consumed sediment (and metals) in the laboratory is not representative of that occurring in nature. In a larger sense, our data indicate that the response of animals to contaminants in the laboratory does not necessarily mimic the manner in which they would respond in the field (Luoma 1995).

Having contaminated larvae with ^{65}Zn , we were able to measure Zn loss by holding larvae in ^{65}Zn -free sediments. The two species differed in that *C. tigris* retained measurable ^{65}Zn after 36 d, whereas *C. staegeri* did not (Fig. 6b). More importantly, the biological half-life of ^{65}Zn in *C. tigris* was somewhat longer than that of *C. staegeri* (13 and 5.5 d, respectively; calculated as in Michaud et al. 2005), which is the opposite trend that would be expected if Zn loss rates were to explain the lower Zn concentrations measured in *C. tigris* in the field.

Exposure to metal in food; gut contents—To estimate metal exposure via food, we measured metals in gut contents of the two *Chironomus* species. Cadmium concentrations (means \pm SD, $\mu\text{g g}^{-1}$) in gut contents did not differ significantly ($p > 0.05$) between *C. staegeri* (1.9 ± 0.5) and *C. tigris* (1.6 ± 0.2), whereas Cu concentrations were somewhat higher in the gut contents of the former (48 ± 11 vs. 27 ± 9 , respectively), and Zn concentrations were greater in the gut contents of the latter (240 ± 20 vs. 320 ± 40 , respectively). These trends do not fit those of metal

concentrations measured in the field in the two *Chironomus* species (Fig. 1). However, this does not mean that these insects do not take up metals from their food since chemical extractions do not necessarily release metals in quantities that are equivalent to those that would be released in the insect's gut (Chen and Meyer 1999). For example, metals are usually extracted from sediments in acid (as in our study), and yet the gut pH of many aquatic invertebrates, including *Chironomus* (Stief and Eller 2006), is circum-neutral (Hare 1992; Orvoine et al. 2006).

To determine if the quality of the gut contents differs between the two species, we removed the larval gut and noted the color of its contents under a microscope. Using an arbitrary scale from 0 (dark gray sediment only) to 5 (orange-brown sediment only), we found that sediment in the gut of *C. staegeri* was mainly orange-brown (4.4 ± 0.6), whereas sediment in *C. tigris* was mainly dark gray (1.2 ± 1.0) (means \pm SD). Our observations in the field and in laboratory microcosms indicate that surficial, mostly oxic, sediments are orange-brown (likely due to the presence of iron oxyhydroxides), whereas deeper, anoxic sediments are dark gray in color (likely due to the presence of metal sulfides). Thus, *C. staegeri* likely feeds mainly on surficial sediment, either in the walls of its burrow or at the sediment surface, whereas *C. tigris* feeds mostly on anoxic sediment. Jónasson (1972) reported that *Chironomus anthracinus* feeds on the lighter-colored surface oxic sediment around the mouth of its burrow, which leaves "the black subsurface mud exposed" in the form of a ring around the burrow opening. This type of behavior would explain the predominance of orange-brown sediment in the gut of *C. staegeri*. This species difference in feeding behavior indicates that larval metal concentrations are not controlled by exposure to dissolved metal since Cd and Zn concentrations are highest in anoxic interstitial waters (Fig. 4a,c) on which the least-contaminated species (*C. tigris*) feeds.

Sulfur isotopic values of the Chironomus species—The last piece of evidence that we have to explain metal concentrations in the two *Chironomus* species comes from measurements of their sulfur isotopic composition. Recent

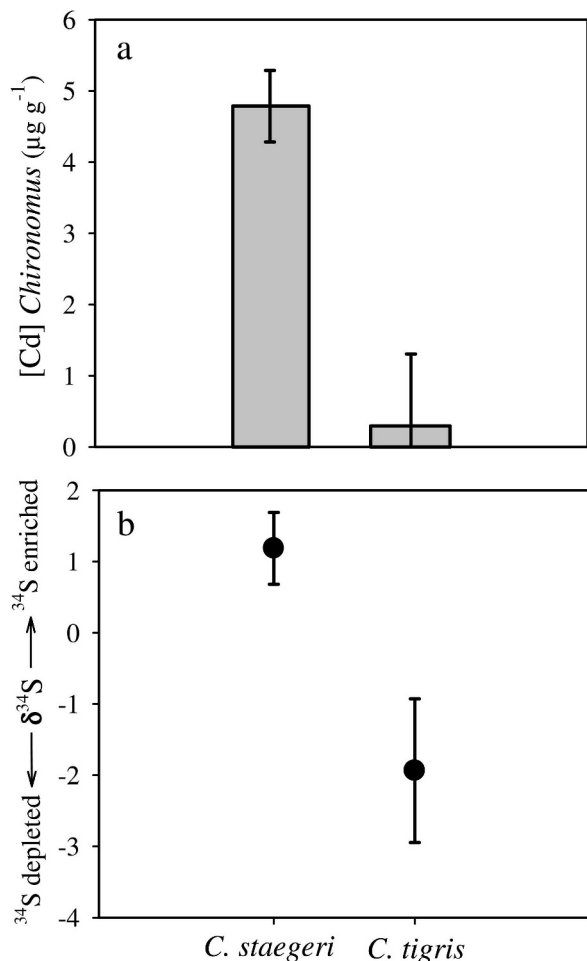


Fig. 7. Mean (\pm SD) (a) Cd concentrations in larvae of *C. staegeri* and *C. tigris* compared to (b) their $\delta^{34}\text{S}$ values.

research has shown that, in a given lake, animals feeding on particles in oxic water tend to have higher sulfur isotopic values ($\delta^{34}\text{S}$) than species feeding on anoxic sediment (Proulx and Hare, 2008). If our hypothesis that *C. staegeri* feeds mainly on surface oxic sediment and *C. tigris* feeds on deeper anoxic sediment is correct, then the $\delta^{34}\text{S}$ value of the former should be higher than that of the latter. This was indeed the case (Fig. 7), with the mean $\delta^{34}\text{S}$ value for *C. staegeri* being significantly greater ($p < 0.001$, t -test) than that for *C. tigris*. In addition, the mean $\delta^{34}\text{S}$ value for the water-column-feeding insect *Chaoborus punctipennis* (4.3 ± 0.2) was even higher than that of *C. staegeri* ($p < 0.001$, t -test), which suggests that the diet of this *Chironomus* species is not composed entirely of recently deposited particles from the water column but includes a minority of older sediment from below the oxic-anoxic interface (as evidenced by the color of its gut contents, as discussed previously).

Explaining differences between the Chironomus species—We conclude that *Chironomus* species collected in the same lake and at the same depth and time differed greatly in their concentrations of the nonessential metal Cd, whereas the concentrations of two essential metals differed either much

less (Zn) or not at all (Cu). Differences in Cd and Zn concentrations between the species were not explained by differences in either their size or their life cycle. Likewise, differential exposure to dissolved metals does not appear to explain differences in larval Cd and Zn concentrations because vertical gradients in dissolved metals did not correlate with presumed depth of larval feeding. The most likely explanation for the species differences in metal concentrations is that their uptake of metals from food differs. Although gut passage times differed between the two species, this result is likely an artifact due to laboratory conditions that also led to differences in Zn uptake that contradicted the pattern measured in the field.

In the field it is clear that the two *Chironomus* species feed on different types of sediment, as shown by differences in their S isotopic signatures and by the color of their gut contents; that is, *C. staegeri* feeds mainly on surface oxic sediment, whereas *C. tigris* feeds mostly on underlying anoxic sediment. Because the Cd and Zn concentrations in extracts of gut contents differed little between species, it follows that the availability of these metals in the sediment consumed by larvae is likely different. We speculate that Cd and Zn are either less readily dissociated from sulfides in anoxic sediment than from oxyhydroxides in oxic sediment (as is the case for Cu in marine sediments; Chen and Meyer 1999) or that these metals are associated with organic matter that is more labile (and thus more easily digested) in recently deposited surface sediments than in older, deeper anoxic sediments (Selck et al. 1999) or both. We further speculate that because these vertical gradients are usually destroyed when sediments are used in laboratory experiments, the results of such experiments may not mimic bioaccumulation patterns in nature.

Although we suggest that differences in metal concentrations between the *Chironomus* species are due to their feeding behaviors, we cannot rule out the influence of physiological differences between the species. For example, differences in Cd concentrations among species of the mayfly genus *Baetis* are thought to be a consequence of their abilities to synthesize the metal-binding protein metallothionein (Aoki et al. 1989). However, if physiological differences were the sole explanation for the differences between the two *Chironomus* species, then we would not have expected there to have been a difference in the ratio of larval to sediment metal concentrations between the laboratory and the field (as discussed previously). Clearly, further study is warranted on this and other invertebrates to determine the importance of food as a metal source and to elucidate how feeding behavior and gut physiology influence contaminant uptake. Such information would be valuable for underpinning the use of such animals as biomonitors and in risk assessments for contaminants.

References

- AOKI, Y., S. HATAKEYAMA, N. KOBAYASHI, Y. SUMI, T. SUZUKI, AND K. SUZUKI. 1989. Comparison of cadmium-binding protein induction among mayfly larvae of heavy metal resistant (*Baetis thermicus*) and susceptible species (*B. yoshinensis* and *B. sahoensis*). *Comp. Biochem. Physiol.* **93C**: 345–347.

- ARMITAGE, P., P. S. CRANSTON, AND L. C. V. PINDER. 1995. The Chironomidae: The biology and ecology of non-biting midges. Chapman and Hall.
- BUCHWALTER, D. B., AND S. N. LUOMA. 2005. Differences in dissolved cadmium and zinc uptake among stream insects: Mechanistic explanations. *Environ. Sci. Technol.* **39**: 498–504.
- BUTLER, M. G., I. I. KIKNADZE, J. K. COOPER, AND M. SHIRIN. 1995. Cytologically identified *Chironomus* species from lakes in North Dakota and Minnesota, USA, p. 498–504. *In* P. Cranston [ed.], Proceedings of the 12th International Symposium on Chironomidae. CSIRO.
- CHAPMAN, P. M., AND F. WANG. 2000. Issues in ecological risk assessment of inorganic metals and metalloids. *Hum. Ecol. Risk Assess.* **6**: 965–988.
- CHARBONNEAU, P., AND L. HARE. 1998. Burrowing behavior and biogenic structures of mud-dwelling insects. *J. N. Am. Benthol. Soc.* **17**: 239–249.
- CHEN, Z., AND L. M. MEYER. 1999. Assessment of sedimentary Cu availability: A comparison of biomimetic and AVS approaches. *Environ. Sci. Technol.* **33**: 650–652.
- CROISETIÈRE, L., L. HARE, AND A. TESSIER. 2006. A field experiment to determine the relative importance of prey and water as sources of As, Cd, Co, Cu, Pb and Zn for the alderfly *Sialis velata*. *Environ. Sci. Technol.* **40**: 873–879.
- FRENZEL, P. 1990. The influence of chironomid larvae on sediment oxygen microprofiles. *Arch. Hydrobiol.* **119**: 427–437.
- HAMBURGER, K., P. C. DALL, AND C. LINDEGAARD. 1995. Effects of oxygen deficiency on survival and glycogen content of *Chironomus anthracinus* (Diptera, Chironomidae) under laboratory and field conditions. *Hydrobiologia* **294**: 187–200.
- HARE, L. 1992. Aquatic insects and trace metals: Bioavailability, bioaccumulation and toxicity. *Crit. Rev. Toxicol.* **22**: 327–369.
- , AND P. G. C. CAMPBELL. 1992. Temporal variations of trace metals in aquatic insects. *Freshw. Biol.* **27**: 13–27.
- , ———, AND A. TESSIER. 1991. Trace element distributions in aquatic insects: Variations among genera, elements, and lakes. *Can. J. Fish. Aquat. Sci.* **48**: 1481–1491.
- , AND A. TESSIER. 1996. Predicting animal cadmium concentrations in lakes. *Nature* **380**: 430–432.
- , A. TESSIER, AND M. N. CROTEAU. In press. A biomonitor for tracking changes in the availability of lakewater cadmium over space and time. *Hum. Ecol. Risk Assess.*
- , ———, AND L. WARREN. 2001. Cadmium accumulation by invertebrates living at the sediment-water interface. *Environ. Toxicol. Chem.* **20**: 880–889.
- JEYASINGHAM, K., AND N. LING. 2000. Acute toxicity of arsenic to three species of New Zealand chironomids: *Chironomus zealandicus*, *Chironomus* sp. A and *Polypedilum pavidus* (Diptera, Chironomidae). *Bull. Environ. Contam. Toxicol.* **64**: 708–715.
- JOHNSON, R. K., B. BOSTRÖM, AND W. VAN DE BUND. 1989. Interactions between *Chironomus plumosus* (L.) and the microbial community in surficial sediments of a shallow, eutrophic lake. *Limnol. Oceanogr.* **34**: 992–1003.
- JÓNASSON, P. M. 1972. Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. *Oikos Suppl.* **14**: 1–148.
- KRANTZBERG, G. 1990. Metal accumulation by chironomid larvae: The effects of age and body weight on metal body burdens. *Hydrobiologia* **188/189**: 497–506.
- LOBEL, P. B., S. P. BELKHODE, S. E. JACKSON, AND H. P. LONGERICH. 1990. Recent taxonomic discoveries concerning the mussel *Mytilus*: Implications for biomonitoring. *Arch. Environ. Contam. Toxicol.* **19**: 508–512.
- LUOMA, S. N. 1995. Prediction of metal toxicity in nature from bioassays: Limitations and research needs, p. 609–659. *In* A. Tessier and D. R. Turner [eds.], Metal speciation and bioavailability in aquatic systems. Wiley.
- MICHAUD, A. L., L. HARE, AND P. G. C. CAMPBELL. 2005. Exchange rates of cadmium between a burrowing mayfly and its surroundings in nature. *Limnol. Oceanogr.* **50**: 1707–1717.
- ORVOINE, J., L. HARE, AND A. TESSIER. 2006. Competition between protons and cadmium ions in the planktonic food chain leading to the phantom midge *Chaoborus*. *Limnol. Oceanogr.* **51**: 1013–1020.
- PÉRY, A., R. MONS, AND J. GARRIC. 2005. Modelling of the life cycle of *Chironomus* species using an energy-based model. *Chemosphere* **59**: 247–253.
- PHILLIPS, D. J. H., AND P. S. RAINBOW. 1993. Biomonitoring of trace aquatic contaminants. Elsevier.
- PROULX, I., AND L. HARE. 2008. Why bother to identify animals used in contaminant monitoring? *Integr. Environ. Assess. Manag.* **4**: 125–126.
- RAINBOW, P. S. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environ. Pollut.* **120**: 497–507.
- ROY, I., AND L. HARE. 1999. Relative importance of water and food as Cd sources to the predatory insect *Sialis velata* (Megaloptera). *Can. J. Fish. Aquat. Sci.* **56**: 1143–1149.
- SEIDMAN, L. A., G. BERGTROM, D. J. GINGRICH, AND C. C. REMSEN. 1986. Accumulation of cadmium by the fourth instar larva of the fly *Chironomus thummi*. *Tissue Cell* **18**: 395–405.
- SELCK, H., A. W. DECHO, AND V. E. FORBES. 1999. Effects of chronic metal exposure and sediment organic matter on digestive absorption efficiency of cadmium by the deposit-feeding polychaete *Capitella* species I. *Environ. Toxicol. Chem.* **18**: 1289–1297.
- SKUBAIA, P., AND A. KAFEL. 2004. Oribatid mite communities and metal bioaccumulation in oribatid species (Acari, Oribatida) along the heavy metal gradient in forest ecosystems. *Environ. Pollut.* **132**: 51–60.
- STIEF, P., AND G. ELLER. 2006. The gut microenvironment of sediment-dwelling *Chironomus plumosus* larvae as characterised with O₂, pH, and redox microsensors. *J. Comp. Physiol. B* **176**: 673–683.
- WANG, F., A. TESSIER, AND L. HARE. 2001. Oxygen measurements in the burrows of freshwater insects. *Freshw. Biol.* **46**: 317–328.
- WATTS, M. M., AND D. PASCOE. 2000. A comparative study of *Chironomus riparius* Meigen and *Chironomus tentans* Fabricius (Diptera: Chironomidae) in aquatic toxicity tests. *Arch. Environ. Contam. Toxicol.* **39**: 299–306.

Received: 12 July 2007

Accepted: 2 October 2007

Amended: 9 October 2007