Environmental Toxicology and Chemistry

Original Article

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Environ Toxicol Chem., **Accepted Article** • DOI: 10.1002/etc.4294

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RUNNING HEAD: Pooling *Chironomus* species in contaminant studies

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This article contains online-only Supplemental Data

Submitted 20 April 2018; Returned for Revisions 5 October 2018; Accepted 6 October 2018

ABSTRACT

Larvae of the insect *Chironomus* (Chironomidae, Diptera) have great potential for estimating the bioavailability of sedimentary trace elements since they are common in fine sediments and tolerate high concentrations of these contaminants. Their use as biomonitors is limited by the fact that they are difficult to identify to species, and species can differ in their trace element concentrations. To determine if pooling species would compromise their use as trace element biomonitors, we identified species of *Chironomus* larvae collected from 22 lakes and measured their concentrations of 9 trace elements. We found that the concentrations of As, Ba, Co, Cu, Mn and Ni did not generally differ between sympatric *Chironomus* species, which indicates that they could be pooled for analyses of these trace elements. In contrast, we found that Cd, Se and Zn concentrations differed between species living at the same site according to their feeding behavior, that is, *Chironomus* species feeding on oxic sediments tended to have higher Cd and Zn concentrations, whereas those feeding on deeper anoxic sediments had higher Se concentrations. Since Se and Zn concentrations in sympatric *Chironomus* species usually differed by only a factor of two, separating species based on their feeding behavior might not be as crucial as for Cd if larval Se and Zn concentrations vary greatly from site to site.

KEYWORDS: Bioaccumulation, bioavailability, *Chironomus*, metals, sediment assessment

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INTRODUCTION

In lakes contaminated by trace elements, the concentrations of these contaminants tend to be high in sediments and in the benthic animals that feed on them (Michaud et al. 2005; Martin et al. 2008), which can lead to toxic effects (Borgmann and Norwood 2002). Consequently, assessing the bioavailability of sedimentary trace elements is important in ecological risk assessments designed to protect benthic communities and the predators that feed on them (Chapman et al. 2003; Väänänen et al. 2018). Although total trace element concentrations in sediments can be useful indicators of past contamination history (Couillard et al. 2004; Gallon et al. 2006; Couillard et al. 2008), they are not usually related to trace element concentrations in benthic animals because the majority of sedimentary trace elements are strongly bound to particles and thus unavailable for uptake by these animals (Luoma and Rainbow 2008). Approaches that consider the speciation and bioavailability of trace elements have been used to improve predictions of trace element bioaccumulation and toxicity (Väänänen et al. 2018). However such approaches cannot consider all of the important processes that affect sediment trace element bioavailability and thus their predictions tend to be approximate at best (Luoma and Rainbow 2008). A more direct means of evaluating contaminant exposure is to measure trace elements in the benthic animals themselves; organisms used in this way are referred to as biomonitors (Luoma and Rainbow 2008) or sentinels (Beeby 2001).

In the fine sediment of lakes and slow-flowing rivers, a good candidate for monitoring sedimentary trace elements is larvae of the insect *Chironomus* (Chironomidae, Diptera) since most *Chironomus* species burrow into and eat sediment. Furthermore, the genus is distributed worldwide, and larvae are tolerant to and accumulate trace elements (Armitage et al. 1995). Although cultured *Chironomus* larvae of a given species are routinely used to assess trace

element availability and sediment toxicity in the laboratory (e.g. Galluba et al. 2012; Marinković et al. 2012; Mogren et al. 2012), their use in the field has lagged behind in part because they are notoriously difficult to identify to species. Thus, *Chironomus* species are often pooled in environmental studies, which could confound trends if there are important behavioral and physiological differences among species that influence their accumulation of trace elements. For example, studies on annelids (Bryan and Gibbs 1987), arthropods (Moore and Rainbow 1987; Rainbow et al. 1993; Croteau et al. 2001; Cain et al. 2011; Cresswell et al. 2014) and molluscs (Lobel et al. 1990) have demonstrated that species belonging to the same genus and living in the same habitat (sympatric species) can differ widely in their trace element concentrations. In the case of *Chironomus*, significant differences have been reported in cadmium (Cd) concentrations between sympatric species (Martin et al. 2008; Proulx and Hare 2008, 2014).

Thus, we investigated whether or not it is justifiable to pool *Chironomus* species in contaminant studies (1) by determining if arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn) concentrations differ between species living at the same site, and if so, (2) by determining why some sympatric *Chironomus* species differ in their trace element concentrations.

MATERIAL AND METHODS

Collection of samples

We collected *Chironomus* larvae, lake water and sediments in late spring to early summer (May-July 2006, 2007, 2009, 2010 and 2011) from lakes located on the Precambrian Shield in the mining areas of Rouyn-Noranda (Quebec) and Sudbury (Ontario), Canada (Table 1). Some of the selected lakes are located close to and downwind of metal smelters, whereas others are located

far and/or upwind from them (Figure 1). Some of the selected lakes were also contaminated by acidic mine drainage (Arnoux, Dufault, Osisko and Kelly) and hospital or municipal untreated sewage discharges (Osisko and Kelly, respectively). Overall, Cd and Cu have been determined as the most likely to cause toxicity in Rouyn-Noranda lakes (Borgmann et al. 2004) whereas Ni have been determined as the most likely cause of toxicity in Sudbury lakes (Borgmann et al. 2001).

Chironomus larvae, feces and fecal matter. *Chironomus* larvae were collected from a single location in each lake using an Ekman grab, the contents of which were sieved through a 0.5 mm mesh-aperture net. Five to 20 grab samples were collected at each site depending on larval densities. Larvae were held in lake water at field temperatures for transport to the laboratory where they were separated into morphological groups according to the presence/absence and morphology of their abdominal tubules (Table 2) as well as the coloration (pale or dark) of their frontoclypeus (Proulx et al. 2013). Fourth (final) instars were chosen for study as determined from the head-capsule widths of pre-pupal larvae (Proulx et al. 2013).

Ten individuals from each morphological group were decapitated and their head capsules were mounted on microscope slides for detailed morphological study whereas their bodies were preserved in 94% ethanol for cytological and/or DNA analyses. Morphological identifications were based on the presence and shape of the ventral and lateral tubules, the coloration of the frontoclypeus and the gula, the teeth of the mentum, mandible and pecten epipharyngis as well as the shape of the anterior margin of the ventromental plates (Proulx et al. 2013). For cytological analyses, giant polytene chromosomes were removed from the salivary glands and stained so as to determine the number of chromosomes, their structural arrangement and their banding patterns (Proulx et al. 2013). DNA analysis was conducted through DNA barcoding of the *cox1* and *gb2B*

genes. For more details on the identification techniques used and the species collected see Proulx et al. (2013).

Chironomus larvae to be used for trace-element measurements were held at 4°C, for a mean of 4 (± 1, SE) days, in water from their collection site to allow them to empty their gut contents. Feces were removed daily to prevent coprophagy and those from larvae collected in Crooked Lake, Hannah Lake, Pine Lake and Raft Lake were retained for Cd measurements. Furthermore, additional larvae from those lakes and McFarlane Lake were dissected to remove their gut contents for Cd, Se or Zn analyses. To verify that differences in depuration time did not influence larval trace element concentrations, we measured trace elements in three *Chironomus* species collected from three lakes and held in water for 1 to 14 days. There were generally no statistical differences ($p>0.05$) in the concentrations of trace elements (As, Ba, Cd, Co, Cu, Mn, Ni, Se, Tl and Zn) among individuals that were depurated for various lengths of time (results are presented in Supplemental Data S1). Larvae with empty guts were stored at -20^{o}C until analysis.

Lake water. At each collection site, we measured vertical profiles of dissolved oxygen and temperature (YSI Model 50B). At the time of collection, the water column of all lakes was well mixed. At most sites, triplicate water samples were collected in the water column using *in situ* Plexiglas diffusion samplers (216 mm x 72 mm x 12 mm) suspended about 1 m below the lake surface where they were left to equilibrate for 3 days (Croteau et al. 1998). These samplers contain eight 4 mL compartments separated from lake water by a 0.2 µm nominal pore-size polysulfone membrane (Gelman Ht-200). Ultrapure water (Milli-Q system water; > 18 Mohm cm^{-1}) was used to fill the diffusion samplers and they were then sealed individually in clean plastic bags prior to their transport to the lakes. Details of the protocol used to collect water subsamples from the diffusion samplers are described in Ponton and Hare (2009). Briefly,

subsamples of 4 mL, 1 mL, 3 mL and 4 mL were collected from the water samplers for the determination of dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), pH and anions (Cl, NO_3 and SO_4), respectively. The remaining water was used for the analysis of major cations (Al, Ca, Fe, K, Mg and Na) and trace elements (As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn). All samples were stored at 4^{o}C prior to analysis.

Sediments. We collected sediment at each site using an Ekman grab from which the overlying water was allowed to drain passively so as not to disturb the sediment surface (verified visually). Using a plastic spatula, we removed three samples of surface oxic sediment (uppermost several mm; identifiable by its light, orange-brown, color in Sudbury lakes or by its greenish color in the Rouyn-Noranda lakes) followed by three samples of darker, subsurface, anoxic sediment. All sediment samples were placed in Whirl-Pak bags and kept cool until analysis.

Analyses

Trace elements were measured in larvae, water and sediments from locations where more than one species were collected.

To prevent inadvertent contamination, all labware used for trace element analyses was soaked in 15% (v/v) nitric acid for at least 1 day, rinsed 7 times with ultrapure water and dried under a laminar flow hood.

Larvae. Chironomus species collected for trace element analysis were placed on a weighed piece of Teflon sheeting in a plastic microcentrifuge tube before being stored at -20^{o}C. Our objective was to obtain 3 to 5 pooled samples of each species at each site. The number of individuals in a pooled sample varied from 1 to 7 depending on the number of individuals available at a given

site. At a few sites, larval numbers of some uncommon species were insufficient to make more than a single pooled sample.

Chironomus larvae were freeze-dried (FTS Systems) and weighed using a microbalance (Sartorius M2P PRO 11). Dried larvae were placed in acid-washed High Density Polyethylene (HDPE) bottles and digested for 5 days in nitric acid (Omnitrace grade, Fisher Scientific; 100 µL per mg dry weight), followed by 3 days in concentrated hydrogen peroxide (trace-select ultra for trace analysis, Fluka analytical; 40 µL per mg dry weight); digest volume was completed to 1 mL per mg dry weight using ultra-pure water. Digestions were done at room temperature. Similar masses of the following certified biological reference materials were digested in the same manner to verify the efficacy of the digestion method: bovine liver (reference material 1577a, National Institute of Standards and Technology reference material, Washington, DC, USA) and lobster hepatopancreas (reference material TORT-2, National Research Council, Ottawa, ON, Canada).

Trace element concentrations (As, Ba, Cd, Co, Cu, Mn, Ni, Zn) concentrations were measured by inductively coupled plasma-mass spectrometry (ICP-MS, Thermo Elemental X Series, Winsford, England) using external calibration standards and rhodium as an internal standard. The quality of the digestion method was assessed through the analysis of digestion blanks and the certified reference materials (bovine liver and lobster hepatopancreas). The quality of the analytical process was controlled by the analysis of certified standards (900Q30, PT89-7, PT 89-9, PT89-10 and PT91-10; Inter-laboratory study, Environment Canada) and calibration standards and through the use of blanks, regular sample spiking and duplicate determination. Data were corrected for signal drift when needed. For As and Se analyses, collision cell technology (with a 93:7 mixture of helium and hydrogen) was used to remove the polyatomic interferences that

occur at their mass-to-charge ratios. Overall, we did not encounter problems with interferences from other elements with the exception of Se, for which high concentrations of bromide (Br) in some *Chironomus* samples gave erroneously high Se concentrations. This problem was overcome by appropriate sample dilutions to lower Br concentrations below a level that interfered with Se measurements. Lead (Pb), which can be found in high concentration in sediments (Gallon et al. 2006), was not analyzed in this study, because our digestion method was not sufficient to completely solubilize all Pb in our samples. To completely solubilize Pb in samples, acid digestions need to be heated to 120°C (Hare and Tessier 1998).

Trace element concentrations in both analytical and digestion blanks were below detection limits and analytical certified standards were within 10% of the certified values. All measured values were above the detection limit. The As, Cd, Co, Mn, Ni, Se and Zn concentrations of certified biological reference materials were within the 95% confidence limits of the certified means, whereas Cu concentrations were within 85% to 90% of the certified concentrations. To our knowledge, no certified biological reference material is available for Ba determination. Most samples were measured on more than one occasion and, for all trace elements, the relative standard deviations among measurements were generally <10%.

Lake water. Trace element concentrations (As, Ba, Cd, Co, Cu, Mn, Ni, Zn) in lake water were measured by ICP-MS. The quality of the analytical process was assessed as described in the subsection Larvae (see above). We were not able to measure Se concentrations using ICP-MS because of interferences with Br. For this element, we used the values published by Ponton and Hare (2013) who used an alternative method (atomic fluorescence spectrometry; Millennium Excalifur System, PS Analytical) to measure Se in our water samples.

Free Ca^{2+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} concentrations were estimated using the Windermere Humic Aqueous Model (WHAM), version 7.1 (Tipping et al. 2011). Measured WHAM input parameters included pH, total dissolved concentrations of metals (Ba, Cd, Co, Cu, Ni and Zn), major cations (Al, Ca, Fe, K, Mg, Mn and Na), anions (SO_4^{2-}, NO_3^{2-} and Cl^-), inorganic carbon as well as fulvic and humic acids. Major cations were measured using an inductively coupled plasma - atomic emission spectrometer (ICP-AES, Vista AX CCD, Varian, Mississauga, Ontario, Canada). Anions were determined by ion chromatography (AS-18 column, System ICS-2000, Dionex, Bannockburn, IL, USA). Dissolved inorganic carbon was measured by gas chromatography (CombiPal injection and CP-Porabond U column, 3800 Varian, Mississauga, Ontario, Canada). The concentrations of humic and fulvic acids were estimated from measurements of dissolved organic carbon (DOC) by its combustion and transformation into CO_2 (TOC-VCPH, Shimadzu, Columbia, MD, USA). To estimate the concentrations of humic and fulvic acids, we assumed that the DOM (dissolved organic matter) to DOC ratio was 2 (Buffle 1988), that 65% of DOM was active in the complexation of metals (Bryan et al. 2002) and that this active fraction was composed of fulvic acids only (Mueller et al. 2012). Thus, we considered that the concentration of humic acids was 0 g/L. For all quality measurements, blanks and appropriate standard reference materials were analyzed in accordance with the Institut national de la recherche scientifique – Centre Eau Terre Environnement quality assurance and quality control protocols. For variables that were below the method limit of detection, half of the detection limit was used in the calculation of free ion concentrations.

Sediments, fecal matter and gut contents. Sediments in all the study lakes were composed of clay and silt particles. Oxic and anoxic sediments were homogenized and dried at 60°C for 12 hours whereas gut contents and fecal matter were freeze-dried (FTS Systems) and weighed using a

microbalance (Sartorius M2P PRO 11). Ten to 15 mg of dried sediment and 0.5 to 5 mg of fecal matter or gut contents were digested in polypropylene graduated tubes, using the same method as described for *Chironomus* larvae (see subsection Larvae), and centrifuged at 7,000 rpm for 3-5 minutes. The supernatant was transferred to another propylene tube for trace element analyses. We used this partial digestion method rather than a total digestion method because it does not include highly bound trace elements that would likely be unavailable for uptake by *Chironomus* larvae. Cd and Se concentrations were measured by ICP-MS, whereas Zn concentrations were measured by ICP-AES. The quality of the analytical process was controlled as described above for the trace element analyses in larvae.

Statistical analysis

All numerical data are presented as the mean ± standard deviation (SD). Statistical analyses were conducted using SigmaPlot® version 11.0 (Systat® Software Inc., San Jose, California, USA).

Differences in trace element concentrations were assessed as follows. When comparing two means we used either the t-test, when data satisfied the criteria for a parametric test ($p>0.05$), or the non-parametric Mann-Whitney Rank Sum test when this was not the case ($p\leq0.05$). Likewise, when comparing more than two means, we used a one-way analysis of variance (ANOVA), when data satisfied the criteria for a parametric test ($p>0.05$), or the Kruskal-Wallis non-parametric test, followed by Dunn's test, if this was not the case ($p\leq0.05$). We note that for all of these tests the sample number should ideally exceed 15, whereas our sample sizes were fewer than 8. Mean values obtained from 2 samples or less were not included in the statistical tests.

Relationships between trace element concentrations in larvae (dependent variable) and water or sediments (independent variables) were assessed through linear regressions. First, relationships were examined through bivariate scatter plots. We then checked that the data were normally distributed around the regression line (normality test (Shapiro-Wilk test)) ($p>0,05$) and that the variance of the dependent variable was constant regardless of the value of the independent variable and the P value calculated by the test (constant variance test) ($p>0.05$). When these assumptions were not met, data were $\log_{10}$-transformed.

Relationship between Se concentrations in oxic sediments and water was evaluated with the Pearson Product Moment Correlation. This correlation test was chosen because it does not require the variables to be assigned as independent and dependent. The data respected the assumption of a parametric test.

RESULTS/DISCUSSION

Trace element concentrations in Chironomus species

Overall, 15 *Chironomus* species were collected, of which 12 have been identified (Table 1) and the status of 3 others remains uncertain (*C*. sp. NAI-III; Table 1). All species are described in detail in Proulx et al. (2013). At 73% of our collecting sites, 2 to 5 *Chironomus* species were collected (Table 1) suggesting that, in the field, multiple *Chironomus* species at a given site is the norm. Trace element concentrations in larvae from sites harboring more than one *Chironomus* species are presented in Figures 2 to 4. Note that some Cd values in Figure 2 were published previously in Proulx and Hare (2014) (Lakes D'Alembert, Duprat, Opasatica (09-8.5 m), Osisko, Pelletier, Hannah, Kasten, Kelly, McFarlane, Raft, Ramsey, Silver (07), Silver (11) and Tilton (11)).

Enormous differences in Cd concentrations were measured among sympatric *Chironomus* species (Figure 2A). For example, in Lake Marlon, the range in Cd concentrations among *Chironomus* species (3 to 520 nmol/g) is similar to the range in Cd concentrations that we measured among species from various lakes (1 to 520 nmol/g). To simplify comparisons among the various *Chironomus* species in Figures 2 and 3, colored (brown, orange, red, or yellow) circles represent species that did not differ significantly in their Cd concentrations but whose values for these species were always significantly higher than those measured for species indicated by greyscale (white, grey, or black) symbols (see Table 3 for species names). Previously published data for 2 of the species are consistent with this trend, that is, Cd concentrations in *C. staegeri* (red circles) were significantly higher than those in *C. 'tigris'* (white circles) collected from Lake St. Joseph (QC, Canada) (Martin et al. (2008).

Within a given lake, Zn concentrations often differed significantly among sympatric *Chironomus* species (Figure 2B). In such lakes (Duprat, Marlon, Osisko and McFarlane), species represented by the colored circles always had higher Zn concentrations than those represented by the greyscale symbols, as was the case with larval Cd concentrations. Martin et al. (2008) also reported higher Zn concentrations in *C. staegeri* (red circle) than in *C. 'tigris'* (white circle).

Significant differences in Se concentrations were measured among some sympatric *Chironomus* species with Se concentrations in species represented by the greyscale symbols being ~2x higher than those represented by the colored circles (Figure 2C). This trend is the opposite of that for Cd and Zn, the concentrations of which were higher in species represented by the colored circles than in species represented by the greyscale symbols. In general, within each group, Se concentrations did not differ significantly among species (Figure 2C).

In most cases, the concentrations of As, Ba, Co, Cu, Mn and Ni did not significantly differ between sympatric *Chironomus* species (Figures 3 and 4). Although there were significant differences between some species in some lakes, no species had consistently higher concentrations of these elements than did others. Likewise, Cu concentrations are reported to not significantly differ between *C. staegeri* (red circle) and *C. 'tigris'* (white circle) (Martin et al. 2008).

Explaining differences among species – feeding behavior

Measurements of sulfur stable isotopes in the *Chironomus* species we collected indicate that the larvae of some species feed on oxic particles whereas other species feed on anoxic particles (Table 3; Proulx and Hare 2014). In our study, species feeding on oxic particles (represented by the colored circles in Figures 2 to 4) had higher Cd and usually higher Zn concentrations but lower Se concentrations than species feeding on anoxic particles (represented by the greyscale symbols in Figures 2 to 4). These results suggest that differences in Cd, Se and Zn concentrations among species are related to their feeding behavior.

The type of particle that *Chironomus* larvae ingest will determine in part the trace element concentrations to which they are exposed. Additionally, the quality/composition of the food particles will influence the efficiency with which larvae are able to assimilate trace elements from them, since it will determine how tightly bound the trace elements are and thus their availability for release into the gut environment (Luoma and Rainbow 2008).

Type of particles ingested by larvae. Chironomus species feeding on anoxic particles must clearly be ingesting deposited anoxic sediment. However, *Chironomus* species feeding on oxic particles could be feeding on particles that are either suspended in the water column (e.g.

phytoplankton) or deposited at the sediment-water interface. Studies on the gut contents of *Chironomus* larvae have shown that some species feed largely on algae that they filter from the water column (Walshe 1951), whereas other species feed on deposited sediment particles (Jónasson 1972). To evaluate the likelihood that the larvae of *Chironomus* species feeding on oxic particles ingest mainly suspended phytoplankton, we compared larval concentrations of all the trace elements measured in this study (Figures 2 to 4) to those in lake water (Table 4). We also compared the concentrations of trace elements in larvae feeding on anoxic particles (Figures 2 to 4) to those in lake water (Table 4) to further confirm that these larvae do not feed on phytoplankton. Our premise for making these comparisons is that if the *Chironomus* species that we studied feed mainly on phytoplankton, then their trace element concentrations should be related to those in water, albeit indirectly. The precedent for such comparisons is that strong correlations have been reported between Cd, Se and Ni concentrations in lake water and those in the predatory insect *Chaoborus* (Munger and Hare 1997; Croteau et al. 1998; Ponton and Hare 2009, 2013) even though this insect takes up these trace elements mainly from its zooplankton food (Orvoine et al. 2006; Ponton and Hare 2013). For these comparisons, we grouped species according to their feeding behavior (Table 3). Data from Lake Arnoux, Lake D'Alembert, Lake Opasatica, Silver Lake in 2011 and Tilton Lake in 2011 were not included in these comparisons because we did not measure trace elements in water, either at a given site (Lake Arnoux, Lake D'Alembert, Lake Opasatica and Tilton Lake (11)) or in a given year (Silver Lake (11)).

The concentrations of As, Ba, Cd, Co, Cu, Mn and Zn in *Chironomus* larvae feeding on oxic particles and in those feeding on anoxic particles were not significantly ($p>0.05$) related to the total dissolved concentrations of these elements in the water column (bivariate plots are presented in Supplemental Data S2). Since the concentrations of cationic trace metals (Ba, Cd,

Co, Cu, Mn, Ni and Zn) in organisms is generally better predicted by the concentration of their free ions rather than their total dissolved concentration (Campbell 1995), we also calculated free ion concentrations in lake water for comparison with those in *Chironomus* larvae. Considering the free ion concentrations of Ba, Cd, Co, Cu, Mn and Zn, rather than their total dissolved concentrations, did not result in significant correlations ($p>0.05$) with the concentrations of these metals in *Chironomus* larvae (bivariate plots are shown in Supplemental Data S3). Since we collected *Chironomus* larvae from lakes having a wide range in pH (5.9-8.5) and Ca concentrations (Ca=69-4600 mmol/L) (Table 4), we also considered the likelihood that the competitive influence of other cations could have obscured relationships between the concentrations of cationic trace elements in *Chironomus* larvae and in lake water using the approach described in Hare and Tessier (1996, 1998). Thus H^+ ions are known to compete with cationic trace elements at uptake sites on organisms and thereby decrease the uptake rate of metals such as Cd and Ni into planktonic food chains (Craig et al. 1999; Orvoine et al. 2006; Hare et al. 2008; Ponton and Hare 2009). Likewise, high Ca^{2+} concentrations are reported to decrease Cd accumulation by larvae of *C. staegeri* (Craig et al. 1999). However, considering H^+ and Ca^{2+} as competitors with the free ions of Ba, Cd, Co, Cu, Mn and Zn for uptake sites on organisms did not strengthen correlations between the concentrations of these metals in lake water and in *Chironomus* larvae.

In contrast, Ni concentrations in *Chironomus* larvae feeding on oxic particles and in those feeding on anoxic particles were correlated with those in lake water (total dissolved and free ion) ($r^2=0.40$ to 0.76, $p<0.05$) (bivariate plots are shown in Supplemental Data S2 and S3). This is likely a consequence of the fact that Sudbury lakes have received high loadings of Ni compared to those in the Rouyn-Noranda area such that mean Ni concentrations in sediments, water and

larvae from the former region were, respectively, 25 (720 nmol/g in Rouyn-Noranda and 17,700 in Sudbury), 43 (Table 4) and 10 times (Figure 4C) higher than in those of the latter region. However, within a given region there were no significant ($p>0.05$) relationships between the concentrations of Ni in *Chironomus* larvae and in lake water.

There was a significant linear relationship between the logarithm of Se concentrations in *Chironomus* larvae feeding on oxic sediments and the logarithm of total dissolved Se concentrations ($r^2=0.67$, $p=0.05$) (bivariate plot is presented in Supplemental Data S2). However, this significant relationship could be a consequence of Se concentrations in water being significantly correlated with those in oxic sediments ($r^2=0.77$, $p=0.04$) (bivariate plot is shown in Supplemental Data S4).

The overall lack of relationships between trace element concentrations in lake water and in larvae confirms that *Chironomus* species feeding on anoxic particles do not ingest particles directly from the water column. It also suggests that *Chironomus* species feeding on oxic particles take up these particles from deposited sediment rather than from phytoplankton that they filter from the water column. This conclusion is supported by the fact that visual examination of the gut contents that we collected showed them to be sediments rather than algae. Thus, species feeding on oxic particles ingest oxic sediments at the sediment-water interface, whether these interfaces are with the overlying water column or in the walls of burrows through which larvae pump oxygenated overlying water (Gallon et al. 2008). Species feeding on anoxic particles consume particles below these thin oxic layers. It follows then that differences in Cd, Se and Zn concentrations between some sympatric *Chironomus* species are likely to be explained by differences in either the concentrations or the bioavailability of these elements in oxic and anoxic sediments.

Concentration and bioavailability of Cd, Se and Zn in food particles. Differences in Cd, Se and Zn concentrations between *Chironomus* species feeding on oxic and anoxic particles could be related to differences in the concentrations of these trace elements in the two sediment types on which they feed. If this was the case, then the concentrations of Cd and Zn would have been be higher in oxic sediments than in anoxic sediments, which was not the case (Table 5). Cadmium and Zn concentrations did not always significantly differ, and where there were differences these were contrary to the trend for larvae in that they were higher in anoxic sediments than in oxic sediments (Table 5). Likewise, in most lakes, the concentrations of Se did not differ significantly ($p>0.05$) between oxic and anoxic sediments (Table 5) in spite of the fact that Se concentrations were higher in larvae feeding on anoxic sediments. The lack of trends between trace element concentrations in sediments and in larvae could be obscured if larvae select the particles on which they feed such that trace element concentrations in bulk sediments do not represent those that larvae actually ingest. Thus, we compared Cd, Se and Zn concentrations in gut contents of larvae feeding on oxic sediments and in those feeding on anoxic sediments from McFarlane Lake (Table 6). Selenium and Zn concentrations in gut contents did not differ significantly (Table 6; $p<0.05$) indicating that differences in larval Se and Zn are more likely a consequence of differences in the bioavailability rather than the concentrations of those trace elements in oxic and anoxic sediments ingested by larvae. As for Cd, concentrations in gut contents of larvae feeding on oxic sediments were higher than in those feeding on anoxic sediments (Table 6; $p>0.05$) suggesting that larval differences in Cd concentrations can be in part attributable to higher Cd concentrations in oxic than anoxic sediments particles selected by larvae.

To determine if the bioavailability of Cd also differs between the oxic and anoxic sediments particles ingested by larvae, we compared Cd concentrations in gut contents and feces of larvae

feeding on oxic and anoxic sediments (Figure 5). Since most of the gut contents that we removed from larvae came from the anterior midgut and because Cd is assimilated in the posterior midgut (Craig et al. 1998), the considerably lower Cd concentrations in feces compared to those in gut contents suggests that Cd was efficiently assimilated from sediments in the posterior midgut of *Chironomus* larvae and thus was highly bioavailable. Cadmium concentrations in feces of larvae feeding on oxic sediments were lower than in their gut contents, whereas Cd concentrations in feces and gut contents of larvae feeding on anoxic sediments differed little. These results suggest that in our lakes, Cd is highly bioavailable in oxic sediments but much less so from anoxic sediments, which also explains why Cd concentrations in larvae feeding on oxic particles are significantly higher than in those feeding on anoxic particles.

Overall, our results suggest that differences in larval Cd, Se and Zn concentrations between sympatric *Chironomus* species are related to their feeding behavior and that the bioavailabilities of Cd, Se and sometimes Zn differ between oxic and anoxic sediments in our study lakes. Data from McFarlane Lake also indicate that Cd concentrations can differ between oxic and anoxic sediments ingested by larvae (Table 6).

Explaining differences among species – other factors

Several factors can be responsible for differences in trace elements among sympatric species. In the following section, we evaluate the possibility that factors, other than the type of particle larvae ingest, could also explain the differences that we measured in the concentrations of some trace elements among sympatric *Chironomus* species. We have structured our discussion of these factors around the terms of the biodynamic model presented in the equation (2).

Concentration of a given trace element can be described as the difference between the rate at which the trace element enters larvae (from water and food) and the rate at which it leaves larvae (through efflux from larvae or via dilution due to larval growth), that is

$$\frac{d[TE]_{Chironomus}}{dt} = k_{uw}[TE]_{water} + AE \times IR \times [TE]_{food} - k_e[TE]_{Chironomus} - k_g[TE]_{Chironomus} \quad (1)$$

where $[TE]_{Chironomus}$ is the trace element (TE) concentration in *Chironomus* larvae; k_{uw} is a rate constant for TE uptake from water; $[TE]_{water}$ (nmol/L) is the concentration of the TE dissolved in water; AE is the efficiency with which *Chironomus* assimilate the TE from food; IR is the rate at which *Chironomus* ingest food, $[TE]_{food}$ is the TE concentration in food; k_e is the rate constant for physiological TE loss and k_g is the growth rate constant for apparent TE loss due to larval growth.

Assuming that trace element concentrations in *Chironomus* larvae are at steady state (ss), equation 1 can be rewritten as follows

$$[TE]^{ss}_{Chironomus} = \frac{k_{uw}[TE]_{water} + AE \times IR \times [TE]_{food}}{k_e + k_g} \quad (2)$$

Trace element influx from food ($AE \times IR \times [TE]_{food}$). In addition to the trace element concentration in food ($[TE_{food}]$) and the quality/composition of the food particles, the uptake rate of a trace element from food is determined by the ingestion rate of food (IR), the chemistry of the gut and the propensity of the gut membrane to assimilate a trace element. We cannot evaluate the potential influence of the quantity of food particles ingested (IR) on larval trace element concentrations because there are no published comparisons of IRs among sympatric *Chironomus* species.

We hypothesize that the propensity of the gut membrane to assimilate an atom of a given trace element is unlikely to vary among *Chironomus* species given the similarity in the physiology of membranes in the region of the gut where trace elements are likely to be assimilated (the midgut; Hare et al. 1991; Craig et al. 1998; Leonard et al. 2009). Likewise, we assume that the chemistry of the gut (e.g., enzymes, surfactants, pH), which determines the proportion of a trace element that will be liberated from the food in the gut and thus made available for assimilation (Mayer et al. 1996), is not likely to vary among congeners. By assuming no variation in these factors among species, we are assuming that these factors do not influence the efficiency with which various *Chironomus* species assimilate trace elements (AE). We acknowledge that biochemical and physiological measurements of these processes in *Chironomus* larvae are needed to support these assumptions, and that differences in AE's have been reported among sympatric species of the same genus of predatory dipteran (Croteau et al. 2001).

Trace element uptake from water ($k_{uw}[TE]_{water}$). The uptake of a trace element by an animal from water is dictated by the physiology of its uptake membranes (which influences k_{uw}) and the concentration of the trace element in water ($[TE_{water}]$).

For a given trace element, differences in membrane physiology (e.g., permeability, number of uptake sites) between taxa can potentially influence the rate constant for trace element uptake from water (k_{uw}). However, given the great similarities in physiology, morphology and size among *Chironomus* species, differences in k_{uw} among them seem unlikely. Cain et al. (2011) and Buchwalter and Luoma (2005) found that rates of dissolved metal uptake were similar among species of the same insect genus, but differed among insects from different genera or orders. Although there are no published comparisons of uptake rates of dissolved trace elements between *Chironomus* species, these facts suggest that, for a given trace element, there should be

little difference in the rate at which sympatric *Chironomus* species take up trace elements from water.

All *Chironomus* species burrow in sediments and irrigate their tubes with overlying water to keep them oxygenated (Jónasson 1972; Charbonneau and Hare 1998; Stief and De Beer 2002; Huryn et al. 2008). Although sympatric insects from different orders can differ widely in the rates at which they irrigate their tubes, due to differences in their tolerance to hypoxia (Wang et al. 2001), this is unlikely for *Chironomus* species given the physiological similarities among them. This is not to say that temperature and oxygen concentrations do not influence the irrigation activity of *Chironomus* larvae (Roskosch et al. 2012), however, they should not vary for sympatric species. Thus, *Chironomus* species living at the same site should be exposed to the same dissolved trace element concentrations ($[TE]_{water}$). With these facts in mind, we suggest that $[TE]_{water}$ cannot explain the differences that we measured in Cd, Zn and Se concentrations among sympatric *Chironomus* species.

Rate constant for trace element physiological loss (k_e). Although rate constants of trace element loss (k_e) can differ between related genera of bivalves (Ke and Wang 2001), studies on insects in the same order as *Chironomus* (the dipteran *Chaoborus*) have shown that they do not differ among congeners (Croteau et al. 2001), which suggests that, for a given trace element, loss rate constants are likely to be similar among *Chironomus* species.

Rate constant for trace element "loss" via growth dilution (k_g). Growth can dilute trace element concentrations if larval tissue is added faster than trace elements are taken up (Luoma and Rainbow 2008). If *Chironomus* species differ in their growth rate and if differences in growth rates are a major key factor in governing differences in trace element concentrations between

sympatric species, we would expect the concentrations of all trace elements to be higher in some species relative to others, which was not the case.

Overall, our analysis suggests that no major factors, other than the concentrations or bioavailabilities of Cd, Se and Zn in food particles can explain the differences in larval concentrations of these trace elements between sympatric *Chironomus* species. However, we acknowledge that biodynamic studies are needed to confirm some our hypotheses.

Assessing trace element bioavailability in sediments

Given the variety of geochemical processes that can influence the bioavailability of trace elements in oxic and anoxic sediments, it is difficult to estimate trace element bioavailability in these two sediment types without using a biomonitor. Our data suggest that *Chironomus* larvae can serve this purpose. In fact, we found no published information suggesting that other types of freshwater benthic invertebrates have shown such promise in this regard.

On the one hand, our results suggest that, in the lakes we studied, the bioavailable concentrations of As, Ba, Co, Cu, Mn and Ni do not differ between oxic and anoxic sediments. On the other hand, Se was more bioavailable in anoxic sediments than in oxic sediments. In contrast, Cd, and in some lakes Zn, were more bioavailable in oxic sediments than in anoxic sediments. These results for Cd and Zn are consistent with those for a polychaete (Wang et al. 1999) and two species of bivalves (Chong and Wang 2000) that assimilated a greater proportion of these elements when they ingested oxic as opposed to anoxic sediments.

Pooling Chironomus species for contaminant analyses

The concentrations of As, Ba, Co, Cu, Mn and Ni did not differ between sympatric *Chironomus* species, which suggests that in our study lakes species could be pooled for use as biomonitors of these elements. In contrast, concentrations of Cd, Se and Zn differed among some sympatric *Chironomus* species indicating that larvae should not be pooled in contaminant analyses. At a given site, species feeding on oxic particles had Cd concentrations that were 3 to 108 times higher than species feeding on anoxic particles. Thus, comparing larval Cd concentrations to evaluate sediment bioavailability among lakes without identifying species could compromise such comparisons. However, since Se and Zn concentrations in sympatric *Chironomus* species usually differed by only a factor of two, identifying species might not be as crucial if larval Se and Zn concentrations vary greatly from site to site. The fact that sympatric *Chironomus* species can differ in their trace element concentrations suggests that caution should be used when comparing the results of laboratory studies using different species (e.g., *C. riparius*, *C. tentans* or *C. dilutus*), or when the results of laboratory studies are extrapolated to the field. Lastly, the results of field studies that do not discriminate among chironomid genera should be interpreted with even greater caution.

Not being able to pool *Chironomus* species in contaminant studies could be viewed as a drawback for using these larvae as biomonitors. Until recently, their definitive identification depended greatly on the study of salivary-gland chromosomes, which was the specialty of only a few experts worldwide. However, recent advances in genetic techniques, have rendered the identification of *Chironomus* species more accessible to non-experts (Proulx et al. 2013). Furthermore, our data suggest that not all *Chironomus* larvae need to be identified to species, since Cd, Se and Zn concentrations differed only between species that feed on oxic or anoxic particles and some larvae from these two feeding groups can be separated morphologically based

on the presence/absence, shape and length of their ventral and lateral abdominal tubules (Table 2; Proulx et al. 2013). Thus larvae that lack lateral tubules on the posterior margin of the tenth abdominal segment (salinarius-, thummi-, bathophilus-, fluviatilis-type) feed on anoxic sediments, whereas larvae with lateral tubules on the posterior margin of the tenth abdominal segment feed on either oxic or anoxic particles (Table 2). Below is a provisional key, based on the morphology of our study species from eastern Canada, that can be used to determine if a given type of *Chironomus* larva is likely to feed on oxic or anoxic particles (Proulx and Hare 2014). We hope that this tentative key can be expanded in the future to include larvae from different regions and for other *Chironomus* species.

Morphological key to *Chironomus* larval feeding behavior in our study lakes

1. Lacking lateral tubules … larvae feed on anoxic particles

- Lateral tubules present … 2

2. Short pair of ventral tubules (≤ the width of the 11th segment) (semireductus type) ... larvae feed on oxic particles

- Long pair of ventral tubules (> the width of the 11th segment) … 3

3. Straight to slightly curved pair of ventral tubules (melanotus type) … larvae feed on anoxic particles

- Anterior pair of ventral tubules shaped like an elbow and posterior pair of ventral tubules are coiled (plumosus type) … larvae can either feed on oxic or anoxic particles

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002#etc.xxxx.

Acknowledgement—Funding was provided by the Metals In the Human Environment – Strategic Network and the Natural Sciences and Engineering Research Council of Canada. We thank D. Ponton, M. Rosabal-Rodriguez, V. Thomasson and J. Lacharité for their assistance in collecting samples and L.-P. Lavoie for producing Figure 1.

Data availability—Data are available by request from the corresponding author.

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FIGURE CAPTIONS

FIGURE 1: Location of lakes and metal smelters

FIGURE 2: Mean (± SD; n=3-8) (A) Cd, (B) Zn and (C) Se concentrations (nmol/g dry weight) in *Chironomus* species (see Table 3 for species names) collected from various lakes (see Table 1 for lake abbreviations). Note the differences in the vertical scales for Cd and Zn concentrations. For a given lake, values that do not differ significantly ($p>0.05$) are followed by the same letter (for values where $n \geq 3$).

FIGURE 3: Mean (± SD; n=3-8) (A) As, (B) Ba and (C) Co concentrations in *Chironomus* species (see Table 3 for species names) collected from our study lakes (see Table 1 for lake abbreviations). For a given lake, values that do not differ significantly ($p>0.05$) are followed by the same letter (for values where $n \geq 3$).

FIGURE 4: Mean (± SD; n=3-8) (A) Cu, (B) Mn and (C) Ni concentrations in *Chironomus* species (see Table 3 for species names) collected from our study lakes (see Table 1 for lake abbreviations). Note the differences in the vertical scales for Cu and Ni. For a given lake, values that do not differ significantly ($p>0.05$) are followed by the same letter (for values where $n \geq 3$).

FIGURE 5: Mean Cd concentrations (mean ± SD, nmol/g) in gut contents (□, n=1-2) and feces (▨ , n=2-4) of *Chironomus* larvae feeding on oxic (■) or anoxic (■) sediments from Crooked Lake (CR), Hannah Lake (HA), Pine Lake (PI) and Raft Lake (RA). Differences in Cd concentrations in gut contents and feces of larvae from each lake could not be assessed through a statistical test because values in gut contents were obtained from only 1 to 2 samples.

TABLE 1: Location, year and depth of the collection sites, as well as the *Chironomus* species encountered

Lake	Lake code	Year of collection	Depth (m)	Location of lakes	*Chironomus* species collected
ROUYN-NORANDA (QC)					
Lake Arnoux	AR (1.5m)	2010	1.5	48° 14.968'N, 79° 20.146'O	*C. bifurcatus*, *C. harpi*
Lake Arnoux	AR (4.5m)	2010	4.5	48° 14.968'N, 79° 20.146'O	*C. anthracinus*
Lake Bousquet	BO	2006	14	48° 12.934'N, 78° 38.069'O	*C. cucini*
Lake D'Alembert	DA	2006	5	48° 22.870'N, 79° 0.508'O	*C. bifurcatus*, *C. entis*, *C. plumosus*, C. sp. NAIII, *C. staegeri*
Lake Dufault	DF	2006	4	48° 16.930'N, 79° 0.293'O	*C. decorus*-group sp. 2
Lake Duprat	DP	2010	6.5	48° 20.069'N, 79° 7.516'O	*C. bifurcatus*, *C. plumosus*, *C. staegeri*
Lake Fortune	FO	2006	5-6	48° 11.388'N, 79° 18.458'O	*C. plumosus*
Lake Marlon	MN	2010	1-2	48° 15.892'N, 79° 3.868'O	*C. entis*, *C.* nr. *atroviridis* (sp. 2i), *C. plumosus*
Lake Opasatica	OP (07)	2007	9	48° 9.767'N, 79° 19.629'O	*C. bifurcatus*, *C. plumosus*, *C. staegeri*, *C. 'tigris'*

Lake Opasatica	OP (09 - 3m)	2009	3	48° 9.767'N, 79° 19.629'O	*C. decorus*-group sp. 2, *C. ochreatus*
Lake Opasatica	OP (09 - 8.5m)	2009	8.5	48° 9.767'N, 79° 19.629'O	*C. bifurcatus*, *C. staegeri*, *C. 'tigris'*
Lake Osisko	OS	2010	6	48° 14.530'N, 78° 59.743'O	*C. anthracinus*, *C. plumosus*
Lake Pelletier	PE	2010	5	48° 12.852'N, 79° 3.194'O	*C. entis*, *C. plumosus*
Lake Rouyn	RO	2010	4	48° 14.545'N, 78° 56.598'O	*C. plumosus*, *C. staegeri*
SUDBURY (ON)					
Clearwater Lake	CL	2007	19	46° 22.221'N, 81° 3.046'O	*C. cucini*
Crooked Lake	CR	2010	5-6	46° 25.167'N, 81° 2.087'O	*C. staegeri*
Hannah Lake	HA	2007	7	46° 26.590'N, 81° 2.255'O	*C. anthracinus*, *C.* sp. NAIII
Kasten (Bibby) Lake	KA	2007	7.5	46° 22.022'N, 80° 58.171'O	*C.* sp. NAI, *C. 'tigris'*
Kelly Lake	KE	2010	5	46° 27.104'N, 81° 3.041'O	*C. dilutus*, *C. plumosus*
McFarlane Lake	MC	2007	10	46° 24.971'N, 80° 57.509'O	*C. bifurcatus*, *C.* sp. NAIII, *C. staegeri*, *C. 'tigris'*
Pine Lake	PI	2010	4-6	46° 22.586'N, 81° 1.457'O	*C. anthracinus*

Raft Lake	RA	2010	10	46° 24.542'N, 80° 56.756'O	*C. anthracinus*, *C.* sp. NAIII
Ramsey Lake	RM	2007	12	46° 28.399'N, 80° 56.829'O	*C. anthracinus*, *C.* sp. NAIII
Silver Lake	SI (07)	2007	4	46° 25.823'N, 81° 0.764'O	*C.* sp. NAII, *C. staegeri*, *C. 'tigris'*
Silver Lake	SI (11)	2011	4	46° 25.823'N, 81° 0.764'O	*C. anthracinus*, *C. decorus*-group sp. 2
Tilton Lake	TI (07)	2007	4	46° 21.403'N, 81° 4.348'O	*C. staegeri*, *C. 'tigris'*
Tilton Lake	TI (11)	2011	4	46° 21.403'N, 81° 4.348'O	*C. bifurcatus*, C. *staegeri*, *C. 'tigris'*

TABLE 2: Classification of *Chironomus* larval types (taken from Proulx et al. 2013)

Larval type	Pair of lateral tubules on 10th segment	Two pairs of ventral tubules on 11th segment[a]	
		Anterior pair	Posterior pair
salinarius	absent	absent	absent
halophilus	absent	absent or short	short
bathophilus	absent	straight; long	straight; long
fluviatilis[b]	absent	slightly curved, coming to a point at ends; long	slightly curved, coming to a point at ends; long
thummi	absent	with elbow, long	coiled; long
reductus	present	absent	absent
semireductus	present	straight; short	straight or may be curved; short
melanotus	present	straight or slightly curved; long	straight or slightly curved; long
plumosus	present	with elbow; long	coiled; long

[a] long: ventral tubules > the width of 11th segment

short: ventral tubules < the width of 11th segment

[b] Often hard to distinguish from bathophilus-type

TABLE 3: Larval type and feeding behavior of collected *Chironomus* species

Chironomus species	Symbols in Figures 1 and 2	Larval type (Proulx et al. 2013)	Inferred feeding particles (Proulx and Hare 2014)
C. entis Shobanov, 1989		semireductus	oxic particles
C. plumosus (Linnaeus 1758)		semi-reductus to plumosus	oxic particles
C. dilutus Shobanov, Kiknadze & Butler, 1999		plumosus	oxic particles
C. staegeri Lundbeck, 1898		plumosus	oxic particles
C. harpi Wulker, Sublette & Martin, 1991		plumosus	anoxic particles
C. 'tigris' *nomen nudum* in Martin *et al*. (2008) for *C*. sp. Am1 of Kiknadze *et al*. (1993)		plumosus	anoxic particles
C. decorus-group sp. 2 Butler *et al.*, 1995		bathophilus, fluviatilis or	anoxic particles

		melanotus	
C. bifurcatus Wuelker *et al*., 2009	▲	bathophilus	anoxic particles
C. anthracinus Zetterstedt, 1860	□	thummi	anoxic particles
C. atroviridis (described as *C.* nr. *atroviridis* (sp. 2i) in Proulx et al., 2013; Proulx and Hare 2014) (Martin 2014)	■	thummi	anoxic particles
C. ochreatus (Townes 1945)	■	thummi	anoxic particles
C. sp. NAI of Proulx *et al*. (2013)	□	thummi	anoxic particles
C. cucini (Webb 1969)	Not shown in Figures 2 to 4	salinarius	anoxic particles
C. sp. NAII of Proulx *et al*. (2013)	☆	salinarius	anoxic particles
C. sp. NAIII of Proulx *et al*. (2013)	☆	salinarius	anoxic particles

TABLE 4: Total dissolved As, Ba, Cd, Co, Cu, Mn, Ni, Se, Zn and Ca concentrations (n=3) and pH (n=3) in lake water from which *Chironomus* larvae were collected

Lake[a]	As (nmol/L)		Ba (nmol/L)		Cd (nmol/L)		Co (nmol/L)		Cu (nmol/L)		Mn (nmol/L)		Ni (nmol/L)		Se (nmol/L)		Zn (nmol/L)		pH	Ca (nmol/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	Mean	SD
ROUYN-NORANDA																					
DP	10.8	0.8	18.6	1.0	0.30	0.11	0.38	0.03	42.2	7.8	34	3	12.3	8.3	1.2	0.4	59	12	7.6	140.0	8.5
MN	43.2	2.3	34.0	2.6	1.36	0.32	0.98	0.39	177.0	16.7	107	67	13.5	2.9			54	21	7.7	168.3	3.8
OP (07)	6.2	0.3	55.7	2.1	0.16	0.06	1.22	0.45	44.7	1.5	141	9	19.3	1.1			8	6	8.2	215.7	1.5
OS	17.6	0.3	174.0	2.6	0.48	0.01	1.48	0.06	52.5	1.7	22	2	32.8	1.3	6.8	1.2	65	2	8.5	690.0	6.9
PE	14.1	1.1	131.3	8.1	0.15	0.04	4.02	0.72	38.8	10.0	1,193	129	37.1	10.8	7.6	1.6	13	4	8.3	825.7	41.2
RO	24.5	1.3	147.0	7.8	3.91	0.15	10.69	1.36	103.0	13.7	916	81	103.3	5.8	20.6	1.8	337	23	8.0	2060.0	314.8
SUDBURY																					
HA	14.1	0.1			1.43	0.07	2.84	0.80	290.0	4.0	101	24	2,110.0	36.1			60	6	7.4	265.5	0.8
KA	9.7	0.2			0.49	0.02	3.72	0.10	137.7	1.5	377	3	986.0	22.6			115	24	6.4	69.0	0.2

KE	21.5	0.5	225.0	5.3	10.27	1.86	86.23	3.76	185.0	8.9	1,317	21	4,873.3	30.6	38.8	2.5	197	15	7.5	4596.7	160.4
MC	13.6	0.5			0.32	0.03	1.88	0.22	108.0	5.6	236	58	890.0	11.3	2.8	0.2	96	15	7.3	400.6	0.7
RA	8.0	0.0	78.4	0.3	0.97	0.02	0.42	0.02	113.0	1.0	106	10	1,043.3	5.8	4.3	0.1	72	1	7.3	78.5	2.1
RM	15.3	0.7			0.33	0.01	1.56	0.17	163.0	6.6	105	29	869.0	15.9			53	31	7.1	381.2	0.5
SI (07)	15.1	0.2			2.17	0.15	13.00	0.70	161.7	7.4	734	318	1,590.0	17.3			244	45	5.9	194.4	0.2
TI (07)	8.3	0.3			0.54	0.05	1.12	0.05	100.1	2.5	169	6	675.7	0.6			95	11	6.5	88.5	0.9

Lake names are given in Table 1.

SD = Standard deviation

TABLE 5: Cadmium, Se and Zn concentrations (n=3) in oxic and anoxic sediments from which *Chironomus* larvae were collected

Water body	Cd (nmol/g)[a]				Se (nmol/g)[a]				Zn (nmol/g)[a]			
	Oxic sediments		Anoxic sediments		Oxic sediments		Anoxic sediments		Oxic sediments		Anoxic sediments	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ROUYN-NORANDA												
DA	51.4B	1.0	56.4A	2.0	24.5A	1.2	24.8A	0.3	3,600B	70	3,840A	40
DP	52.2B	1.1	65.3A	2.7	25.1A	0.9	26.2A	0.6	9,200B	30	10,430A	380
MN	98.6A	0.2	95.0A	12.8	43.3A	1.2	45.9A	2.1	6,210A	130	6,350A	550
OP (07)	7.7B	0.4	9.7A	0.4	14.0A	0.2	14.6A	0.3	2,220B	80	2,420A	90
OS	943.1A	172.4	924.4A	218.1	419.9A	30.2	435.3A	42.7	123,240A	6,030	130,720A	8,100
PE	76.2A	6.6	85.0A	2.0	63.3A	7.5	72.4A	3.3	15,800A	1,410	16,430A	250
RO	679.8B	47.6	888.2A	75.9	363.8B	22.2	444.6A	37.9	50,820B	6,540	64,190A	2,540
SUDBURY												
HA	38.2B	0.3	49.4A	1.6	289.5B	8.4	403.4A	33.7	2,420B	30	2,580A	30

KA	27.5A	0.4	27.5A	2.0	85.8A	4.3	83.5A	2.4	2,560A	50	2,780A	130
KE	23.0A	0.3	18.7A	3.9	477.0A	4.3	292.1B	87.4	4,210A	300	3,620A	440
MC	48.3A	2.7	51.4A	0.7	84.3A	4.6	83.9A	0.7	6,740B	100	7,110A	100
RA	19.7A	2.5	22.4A	1.9	98.8A	3.5	100.9A	7.2	1,460A	250	1,570A	170
RM	43.0B	1.8	49.2A	0.8	174.2B	4.3	197.8A	3.5	4,520B	170	4,960A	120
SI (07)	15.3A	0.8	9.5A	0.5	173.2A	6.8	157.5B	2.9	1,650A	90	1,090B	40
TI (07)	16.1B	1.3	29.5A	0.4	165.4A	5.9	156.9B	3.1	1,470B	100	2,320A	10

[a] For each lake and each trace element, sediment data followed by the same letter do not differ significantly ($p<0.05$)

Lake names given in Table 1.

SD = Standard deviation

TABLE 6: Cadmium, Se and Zn concentrations in the gut contents of *Chironomus* species feeding on either oxic sediments (n=3) or on anoxic sediments (n=6) from McFarlane Lake

Trace element	Species feeding on oxic sediments (nmol/g)[a]		Species feeding on anoxic sediments (nmol/g)[a]	
	Mean	SD	Mean	SD
Cd	535A	98	90B	46
Se	98A	16	71A	6
Zn	4,155A	310	3,862A	418

[a] For each trace element, values followed by the same letter do not differ significantly ($p<0.05$).

SD = Standard deviation

FIGURE 1

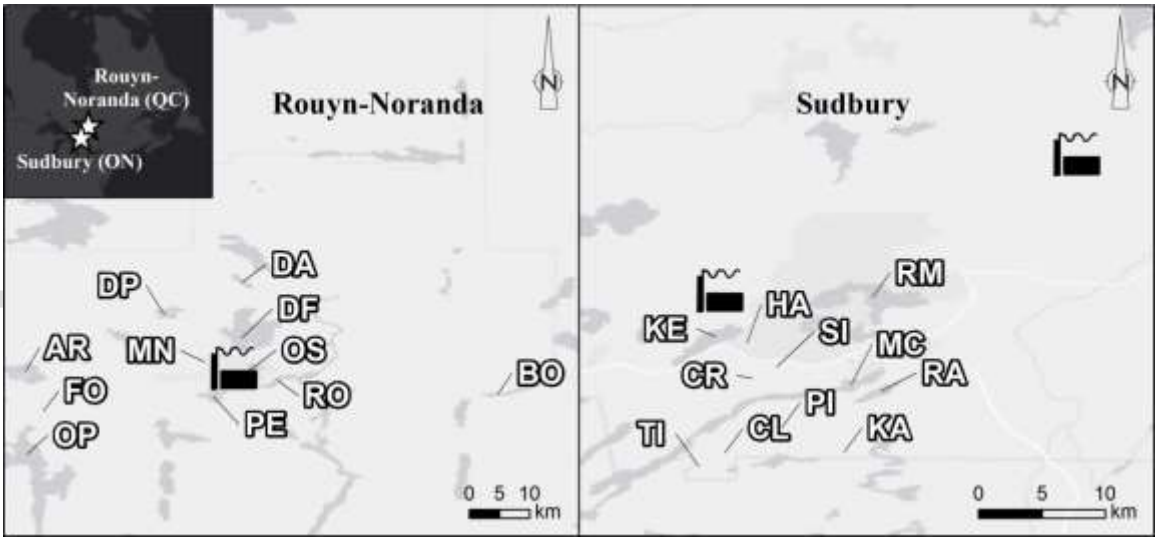

FIGURE 2

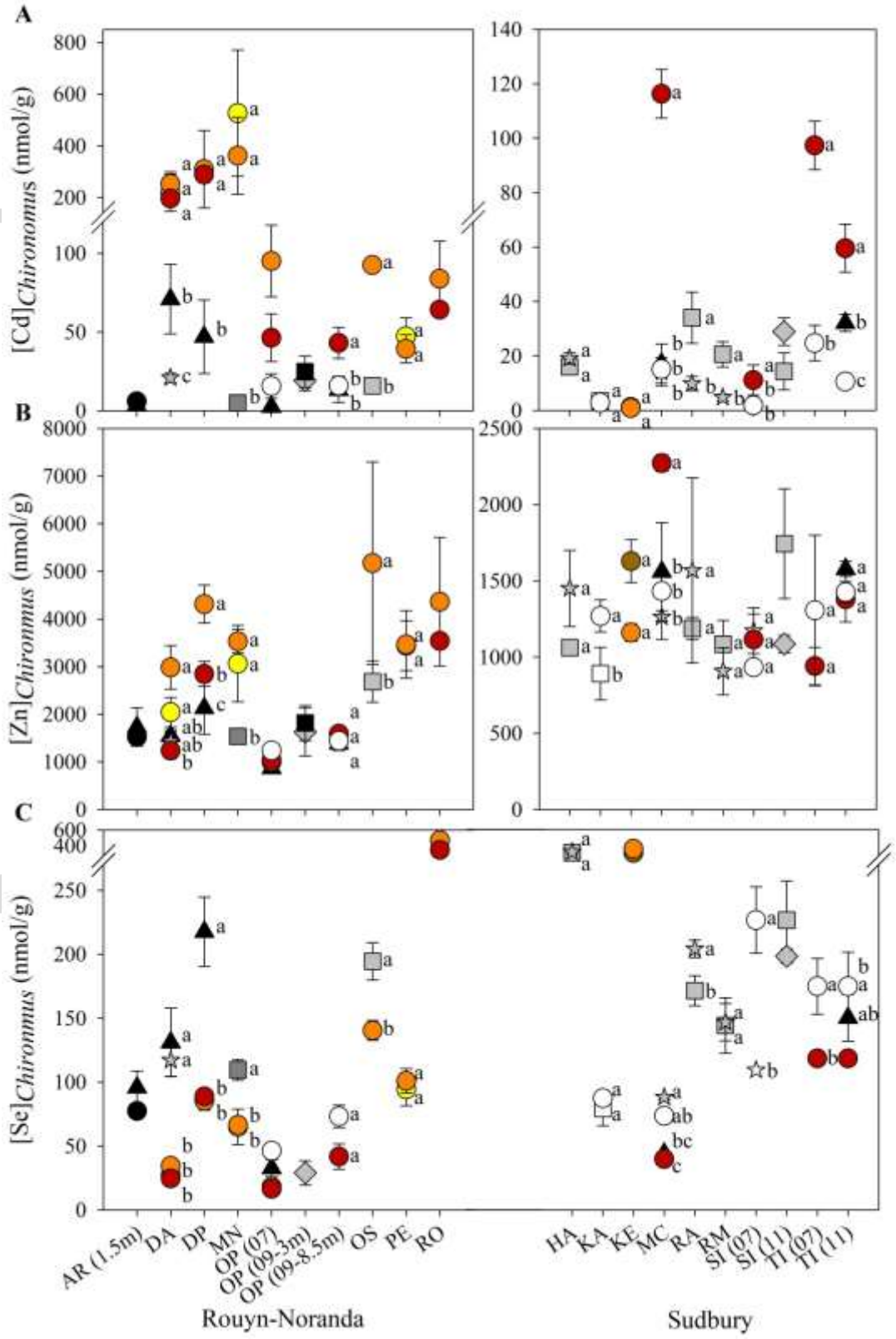

FIGURE 3

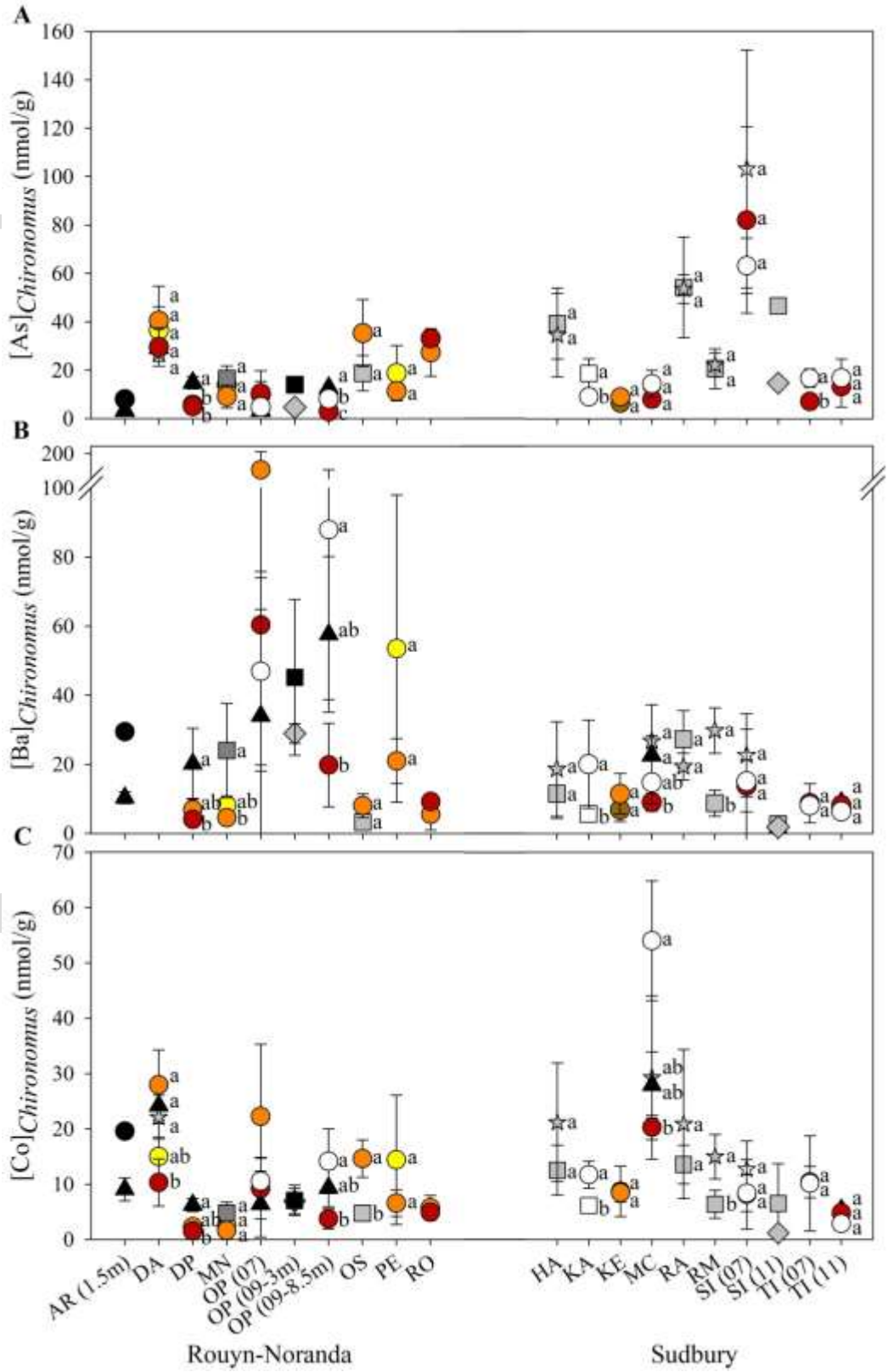

FIGURE 4

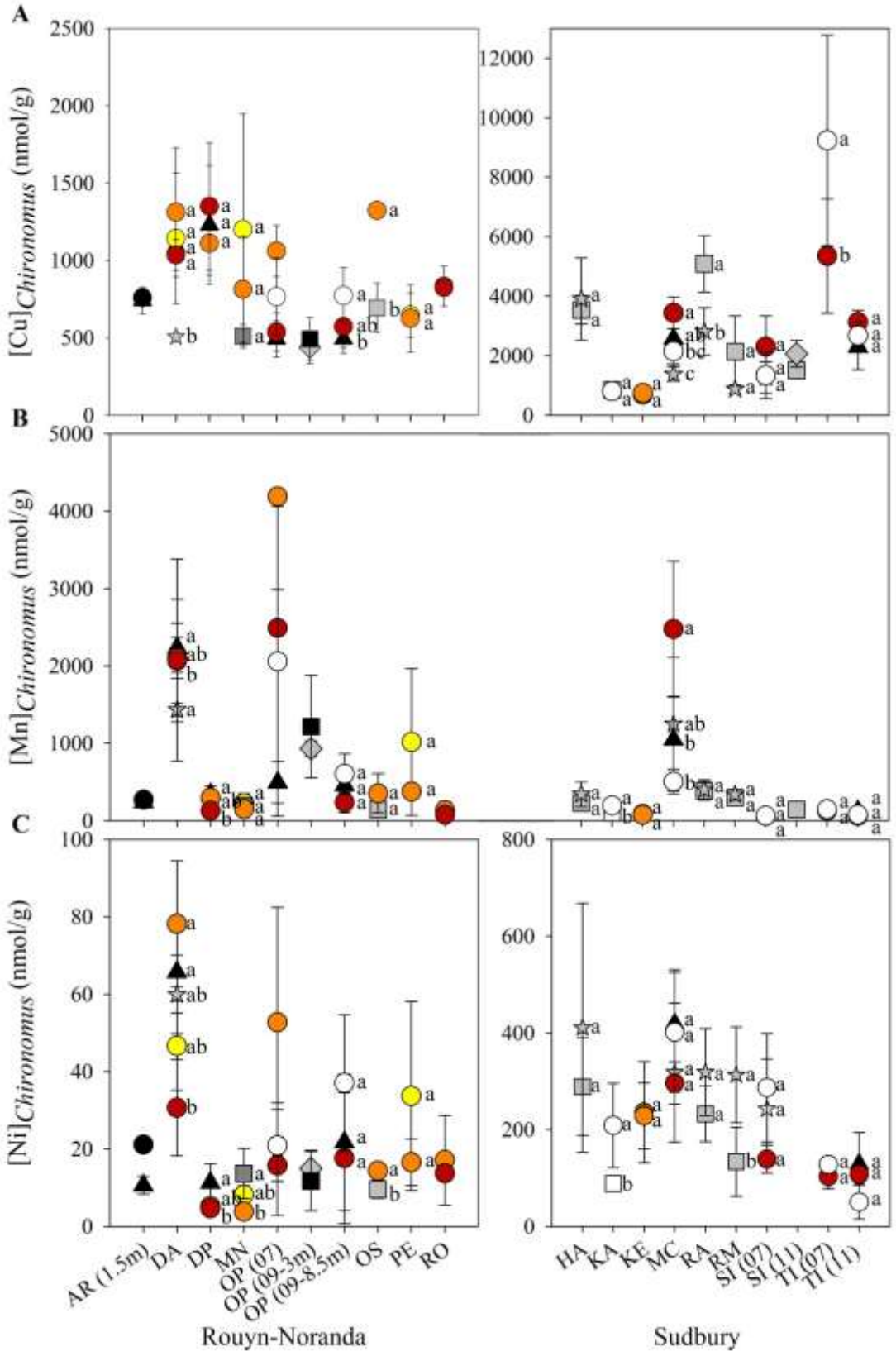

FIGURE 5