Why bother to identify *Chironomus* species used for contaminant monitoring?

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Abstract

Chironomid larvae have great potential as contaminant biomonitors because they are present in a wide variety of freshwaters, including those that are highly contaminated. Since chironomid species are notoriously difficult to identify, closely related species are often pooled for contaminant analysis. However, in doing so, one presumes that contaminant concentrations do not differ markedly among the pooled species. We tested this assumption on the widespread midge *Chironomus* by collecting larvae of this genus from lakes located along a metal-contamination gradient and then identifying species using a combination of molecular and morphological techniques. By measuring trace metal concentrations in each *Chironomus* species we discovered that, within a given lake, the concentrations of some metals (Cd and Se) differed among sympatric species. Among lakes, metal concentrations in the various *Chironomus* species differed in a consistent manner such that certain species had consistently higher concentrations of some metals than did others. To determine why species sharing the same habitat differ in their metal concentrations, we measured larval sulfur isotopic ratios. These measurements revealed that *Chironomus* species sharing the same habitat tend to feed at different depths in sediment where metal concentration and/or bioavailability are likely to differ. Overall, our results suggest that a "one-size fits all" approach for *Chironomus* species may not be valid and that behavioral differences among these morphologically-similar larvae influence their exposure to contaminants.

Introduction

In principle, measurements of contaminants in burrowing chironomid larvae can be used to estimate bioavailable contaminant concentrations in sediments.

However, before measuring contaminants in chironomids, larvae need to be identified, but ...

species identification can be very challenging!!

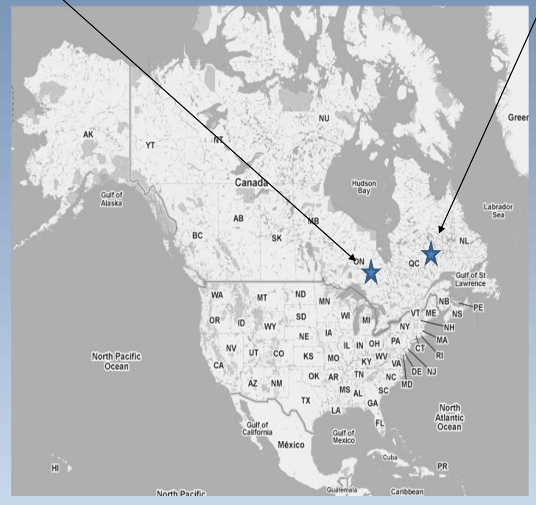
One way around this constraint is to pool species.

Is pooling chironomid species justifiable in contaminant studies?

We tested the validity of this practice by measuring trace elements in various species of *Chironomus* collected from lakes situated along a contamination gradient in northern Canada.

Collection of larvae

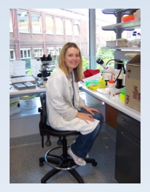
We collected fourth-instar *Chironomus* larvae from a single site in each of 28 lakes in Quebec and Ontario, Canada.

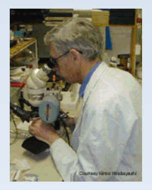


We used the following tools to sort and identify *Chironomus* larvae to species:

- morphology (mouthparts, tubuli, etc.),
- number and structural arrangement of giant salivary chromosomes,
- RFLP analyses,
- DNA barcoding using a mitochondrial gene: cytochrome C oxidase subunit I (*COI*).

Acknowledgements





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References

Croisetière L., Hare L., Tessier A. & Cabana G. (2009) Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshwater Biology* 54: 1006–1015.

Martin J. (2010, with updates) North American cytospecies of the genus *Chironomus* (includes *Camptochironomus*, *Chaetolabis*, *Lobochironomus* and some *Einfeldia*). Available from: http://www.genetics.unimelb.edu.au /Martin/NACytfiles/NAChiron.html (9 March 2010).

Martin S., Proulx I. & Hare L. (2008) Explaining metal concentrations in sympatric *Chironomus* species. *Limnology and Oceanography* 53: 411–419.

Results

Do trace element concentrations differ between sympatric *Chironomus* species?

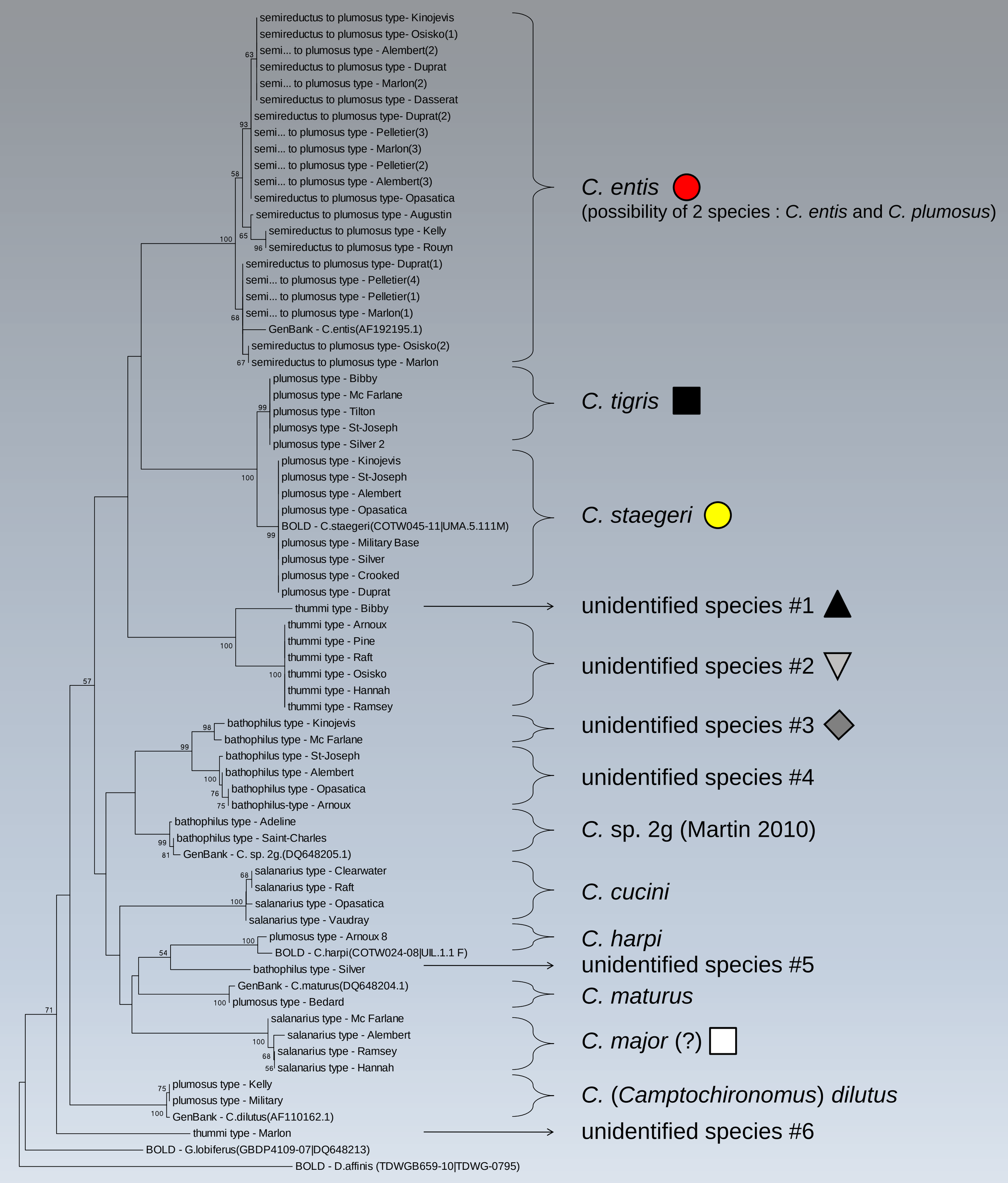


Figure 1. Neighbour-joining tree based on *COI* sequences of *Chironomus* species collected from 28 lakes (larval morpho-type and lake names are indicated) and of species sequences obtained from either the Barcode of Life Data Systems (labelled as BOLD with the species name and the accession number) or from GenBank (labelled as GenBank with the species name and the accession number). C. = *Chironomus*, G. = *Glyptotendipes*, D. = *Drosophila*

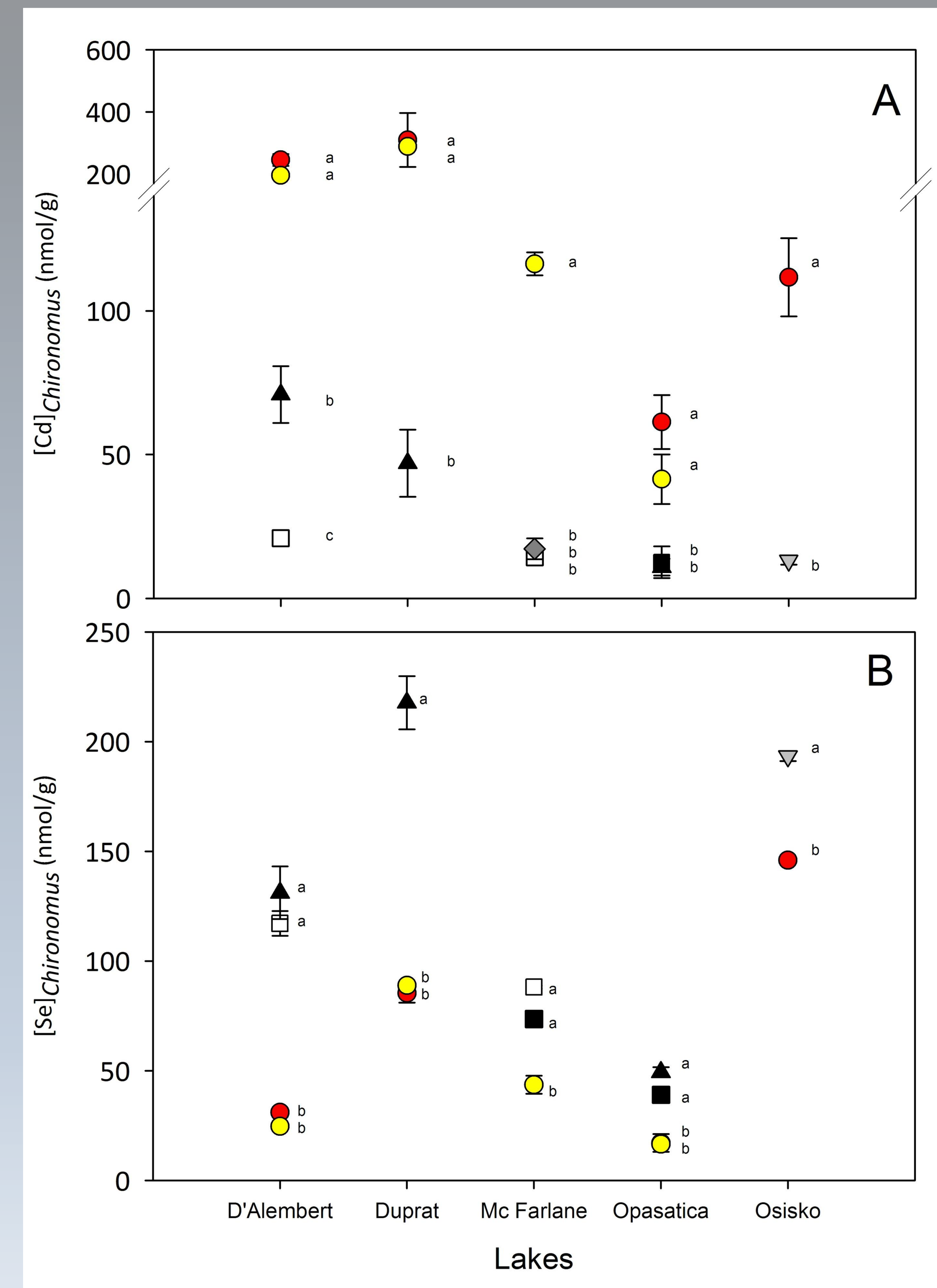


Figure 2. Mean (± SD) cadmium (Cd) (A) and selenium (Se) (B) concentrations in various *Chironomus* species (see symbols in figure 1) collected from 5 lakes (as measured by ICP-MS). In a given lake, values that do not differ significantly ($p > 0.05$) are followed by the same letter.

-[Cd] and [Se] can differ widely among *Chironomus* species.

-Within a given lake, some species always have higher concentrations than do others.

-For Cd, the species represented by the circles (●●) always have higher concentrations than do the other *Chironomus* species (■▲▽◆□).

-For Se, the reverse is true.

-The same pattern was observed in all 28 lakes!

Why do *Chironomus* species sharing the same habitat differ in their trace element concentrations?

Differences among *Chironomus* species in the colour of the gut contents led us to believe that their feeding habits differed. To test this idea, we measured stable sulphur isotopic ratios ($\delta^{34}S$) in *Chironomus* larvae, where

$$\delta^{34}S = [(^{34}S_{sample}/^{32}S_{sample})/(^{34}S_{standard}/^{32}S_{standard})-1] * 1000$$

This approach is based on the fact that animals feeding on oxic particles tend to have higher $\delta^{34}S$ values than do those feeding on anoxic particles (Martin *et al.* 2008; Croisetière *et al.* 2009)

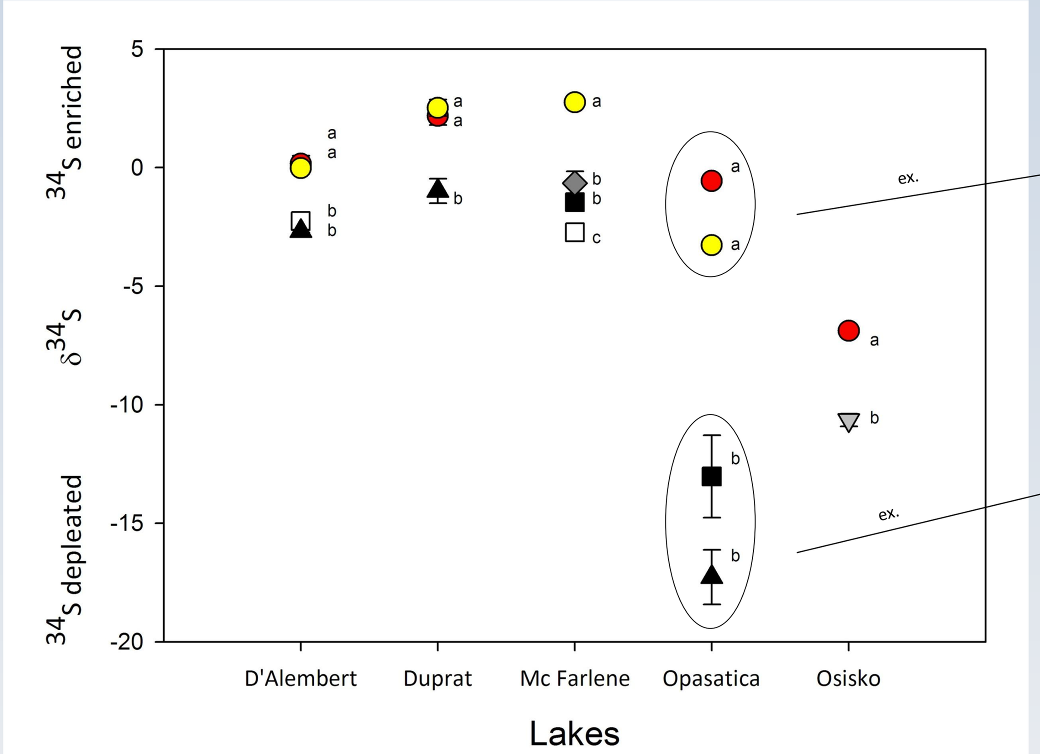
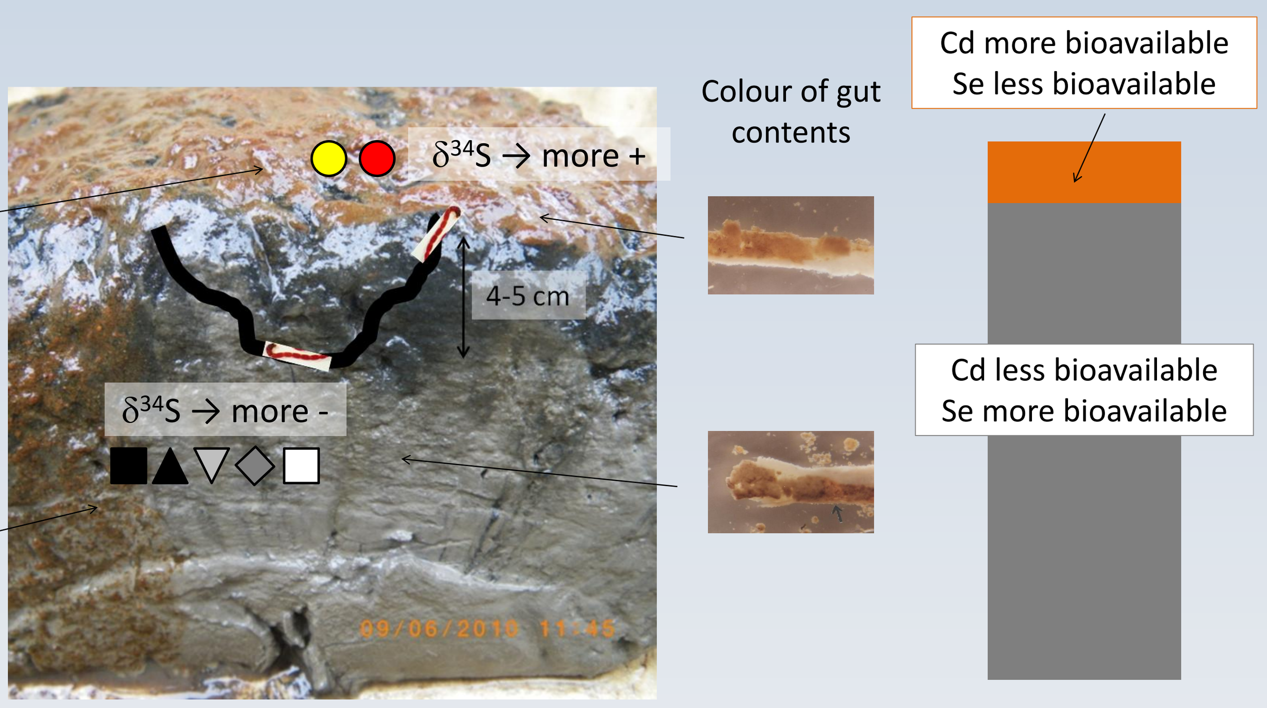


Figure 3. Mean $\delta^{34}S$ values (‰, ±SD) for various *Chironomus* species (see symbols in figure 1) collected from 5 lakes. In a given lake, values that do not differ significantly ($p > 0.05$) are followed by the same letter.



-Within a given lake, some species (●●) consistently have more positive $\delta^{34}S$ values than do others (■▲▽◆□).

-Species having higher $\delta^{34}S$ values (●●) feed on oxic sediments, where Cd is more bioavailable and Se is less bioavailable. The reverse is the case for species having lower $\delta^{34}S$ values (■▲▽◆□).

-The trend is the same in all 28 lakes!

Table 1. Feeding behaviours of *Chironomus* species as deduced from $\delta^{34}S$ measurements in larvae.

Chironomus species feeding on oxic sediments	*Chironomus* species feeding on anoxic sediments	to be determined…
C. entis	*C. cucini*	*C.* sp. 2g (Martin 2010)
C. staegeri	*C. harpi*	*C. maturus*
C. (Camptochironomus) dilutus	*C. major* (?)	
	C. tigris	
	unidentified species #1	
	unidentified species #2	
	unidentified species #3	
	unidentified species #4	
	unidentified species #5	
	unidentified species #6	

Conclusions

- Feeding behaviours can differ among sympatric *Chironomus* species.
- Behavioural differences among species influence their exposure to contaminants.
- A "one-size fits all" approach may not be valid for taxa such as *Chironomus*.
- Species differences should not be disregarded in risk assessments.