Université du Québec
Institut National de la Recherche Scientifique
Centre Eau Terre Environnement

ÉVALUATION DU POTENTIEL D'UTILISER LES LARVES DE *CHIRONOMUS* (DIPTERA, CHIRONOMIDAE) COMME BIOMONITEURS DE LA BIODISPONIBILITÉ DES ÉLÉMENTS TRACES DANS LES SÉDIMENTS

Par

Isabelle Proulx

Thèse présentée pour l'obtention du grade de
Philosophiae doctor (Ph.D.)
en sciences de l'eau

Jury d'évaluation

Examinatrice externe	D[re] Karen Kidd University of New Brunswick
Examinatrice externe	D[re] Elisabeth Stur Norwegian University of Science and Technology
Examinatrice interne	D[re] Mélanie Desrosiers MDDELCC
Directeur de recherche	D[r] Landis Hare INRS – Centre Eau Terre Environnement

À mes parents et à mes amours

REMERCIEMENTS

Je n'aurais pas pu compléter cette thèse sans l'aide et le soutien de plusieurs personnes. Je tiens tout d'abord à remercier mon directeur de thèse Landis Hare. J'ai eu le privilège d'avoir un directeur de thèse pédagogue et dévoué. Merci Landis pour ta disponibilité, ton respect, ta patience, ta compréhension et ton encouragement durant toutes ces années d'étude.

Cette thèse repose en grande partie sur l'identification des larves de *Chironomus* au niveau de l'espèce. L'identification des espèces de *Chironomus* s'est avérée beaucoup plus ardue qu'anticipée et a suscité des collaborations. Je tiens à remercier Melissa Carew de l'université de Melbourne (Australie) qui m'a accueillie dans son laboratoire de génétique. Merci aussi à Veronika Golygina de l'*Institute of Cytology and Genetics* à Novosibirsk (Russie) qui, à l'aide d'analyses de chromosomes, a identifié mes larves de *C. entis* et *C. plumosus*. Finalement, je tiens particulièrement à remercier Jon Martin de l'université de Melbourne (Australie) qui a généreusement accepté de m'aider dans l'identification de mes espèces de *Chironomus*. Ses 40 années d'expérience en taxonomie des *Chironomus* ont indéniablement contribué à la qualité de nos résultats.

Je tiens à remercier les membres du jury d'évaluation d'avoir pris le temps de lire ma thèse. Leurs commentaires ont permis d'améliorer la version définitive de ce document.

Durant mes études, j'ai bénéficié du soutien du personnel professionnel et technique de l'INRS-Centre Eau Terre Environnement. Je tiens à souligner leur compétence, leur disponibilité et leur gentillesse. Merci à Anissa Bensadoune, Michelle G. Bordeleau, Sébastien Duval, Pauline Fournier, Sandra Jobidon, Julie Perreault, Stéfane Prémont, Lise Rancourt et René Rodrigue. Je remercie aussi les stagiaires Anaïs Nanou Clercq, Nicolas Fabien-Ouellet, Hugo Lavoie et Victor Thomasson ainsi que mon collègue Julien Lacharité qui ont été d'une très grande aide sur le terrain. Je tiens à saluer mes consoeurs de course, mes collègues de bureau, mon groupe à la maîtrise, mes collègues du groupe de biogéochimie et toutes autres personnes que j'ai côtoyées à l'INRS-Centre Eau Terre Environnement. Je salue aussi mes collègues Dominic Ponton et Maikel Rosabal-Rodriguez avec qui j'ai passé de beaux moments sur le terrain et tout au long de mon doctorat. Je les remercie pour leur aide sur le terrain, en laboratoire et lors d'analyses de données.

Cette recherche a été rendue possible grâce à l'aide financière du réseau de recherche *Metals In the Human Environment - Strategic Network* et du Conseil de recherches en sciences naturelles et en génie du Canada (CRSNG).

Je tiens à remercier mes parents qui tout au long de mon parcours m'ont soutenue, écoutée et encouragée. Merci pour tout ce que vous faites pour moi. Je n'y serais pas arrivée sans votre aide et votre appui. Finalement, je tiens à remercier mon conjoint qui par son soutien inestimable m'a permis d'atteindre mes objectifs et terminer cette thèse. Merci aussi à mon petit rayon de soleil pour toute la joie que tu apportes à ta maman tous les jours.

AVANT-PROPOS

Cette thèse est composée de deux sections distinctes. La première section comprend une synthèse de l'ensemble des travaux effectués lors du doctorat. Tandis que la deuxième section contient les articles scientifiques qui ont été publiés ou écrits suite à ces travaux. Les articles sont :

1. Proulx I & Hare L (2008) Why bother to identify animals used for contaminant monitoring? *Integrated Environmental Assessment and Management* 4(1):124-132.
2. Proulx I, Martin J, Carew M & Hare L (2013) Using various lines of evidence to identify *Chironomus* species in eastern Canadian lakes. *Zootaxa* 3741(4):401-458.
3. Proulx I & Hare L (2013) Differences in feeding behaviour among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae. *Freshwater Biology* 59(1):73-86.
4. Proulx I & Hare L (2014) Using *Chironomus* larvae to assess the bioavailability of trace elements in sediments (à soumettre).

La contribution des auteurs des articles aux projets de recherche s'établit comme suit :

Isabelle Proulx : Conception et réalisation des expériences, échantillonnage, analyses de laboratoire, traitement des données et rédaction des articles.

Landis Hare : Conceptions des projets, contribution à l'interprétation des données et à la rédaction finale des articles.

Jon Martin : Contribution à la création et à l'interprétation de données et à la rédaction finale de l'article 2.

Melissa Carew : Contribution à la création de données et à la rédaction finale de l'article 2.

RÉSUMÉ

Au cours des derniers siècles, l'industrie minière a grandement contribué à contaminer les sédiments de nombreuses étendues d'eau par le relargage d'importantes quantités d'éléments traces dans l'environnement. Comme les éléments traces ont le potentiel d'être assimilés par les animaux benthiques et d'être remobilisés dans l'environnement aquatique, il y a intérêt à ce que leur biodisponibilité soit évaluée dans les sédiments. La meilleure façon d'estimer la biodisponibilité de ces contaminants est de mesurer leurs concentrations chez des organismes qui consomment et qui habitent les sédiments. Les larves de chironomidés du genre *Chironomus* ont le potentiel d'être utilisées comme biomoniteurs, car ils souvent présents dans les sédiments fins d'étendues d'eau propres et contaminées. Cependant, leur utilisation est limitée par le fait qu'il est difficile, voire impossible, de séparer morphologiquement les larves au niveau de l'espèce, car beaucoup d'entre elles sont identiques. En fait, jusqu'à récemment, la séparation des larves de *Chironomus* en Amérique du Nord au niveau de l'espèce requérait l'examen de leurs chromosomes polytènes, une technique d'identification qui ne compte que quelques experts dans le monde.

L'objectif global de la thèse était d'évaluer le potentiel et la faisabilité d'utiliser les larves de *Chironomus* comme biomoniteur de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments. Pour ce faire, nous avons récolté des larves de *Chironomus* dans les sédiments de lacs situés principalement dans les régions minières de Rouyn-Noranda et Sudbury. Les larves ont été séparées selon leur morphologie, leur ADN et leurs chromosomes polytènes. Nos résultats démontrent qu'il est maintenant possible pour des non-experts en cytologie de séparer et d'identifier correctement des larves de *Chironomus* au niveau de l'espèce selon leur morphologie et leurs séquences d'ADN (*cox1* et *gb2β*). Nos recherches ont permis de publier des outils morphologiques et moléculaires simples à utiliser qui faciliteront à l'avenir la séparation et l'identification d'espèces de *Chironomus* en Amérique du Nord.

La comparaison entre les concentrations d'éléments traces chez les larves et les concentrations d'éléments traces dans l'eau et les sédiments d'où les larves ont été récoltées suggèrent que *Chironomus* est un bon biomoniteur de la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments. Cependant, nos résultats démontrent qu'il n'est pas toujours justifiable de regrouper les espèces de *Chironomus* lors d'analyses de contaminants, car, selon le comportement alimentaire des larves, les concentrations de certains éléments traces diffèrent entre espèces de *Chironomus* vivant au même endroit. En fait, nous avons établi que les larves de *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri* assimilent principalement les éléments traces des sédiments oxiques tandis que les larves de *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C*. sp. NAI, *C*. sp. NAII, *C*. sp. NAIII et *C*. « *tigris* » assimilent principalement les éléments traces des sédiments anoxiques. D'un lac à l'autre, les espèces qui se nourrissent de sédiments oxiques avaient des concentrations de Cd et parfois de Zn plus élevées et des concentrations de Se plus basses que celles qui ingèrent des sédiments anoxiques. Pour ce qui est des autres éléments, il n'y avait pas de différences. Les concentrations larvaires indiquent que la biodisponibilité du Cd, du Se et parfois du Zn diffère entre les sédiments oxiques et anoxiques.

Bien qu'il n'est pas toujours justifiable de regrouper les larves de *Chironomus* au niveau du genre, elles peuvent tout de même être utilisées de façon pratico-pratique comme biomoniteurs. Nos résultats démontrent que les larves n'ont pas besoin d'être séparées au niveau de l'espèce, mais seulement en fonction de leur comportement alimentaire; ce qui peut être facilement fait en examinant la morphologie des larves alors qu'elles sont toujours vivantes. Nos

résultats démontrent que les larves de *Chironomus* sont de très bons outils pour comparer, à un site donné, la biodisponibilité des éléments traces dans les sédiments oxiques et anoxiques.

ABSTRACT

Over the past centuries, mining activities have greatly increased trace element loadings into sediments. Since trace elements in sediments have the potential to be assimilated by benthic animals and thus, remobilized into the aquatic environment, there is a need to assess their bioavailability. The best way to estimate the bioavailability of trace elements in sediments is to measure contaminant concentrations in organisms that live and feed in sediments. Potential candidates for this type of measurement are non-bitting midges of the genus *Chironomus* because of there frequent presence in sediments of clean and contaminated bodies of water. However, a major drawback of using *Chironomus* larvae in studies is that they cannot be separated and identified morphologically to species, as many of them are identical. Until recently, the identification of *Chironomus* species in North America was restricted to a few specialists, who identified larvae through the analysis of their polytene chromosomes.

The overall aim of the thesis was to evaluate the potential and feasibility of using *Chironomus* larvae as biomonitors of As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn in sediments. To do this, we collected *Chironomus* larvae from lakes located mostly in the mining areas of Rouyn-Noranda and Sudbury. Larvae were separated according to their morphology, their DNA and their polytene chromosomes. Our study demonstrates that it is now possible for non-cytological experts to correctly separate and identify *Chironomus* larvae to species through their morphology and using DNA (*cox1* and *gb2β*) analyses. Our research has led to the publication of easy to use morphological and molecular tools that will facilitate the separation and identification of North American *Chironomus* species in the future.

By comparing the concentrations of trace elements in larvae to those in water and sediments from which larvae were collected, we conclude that *Chironomus* larvae can be used as biomonitors to assess the bioavailability of As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn in sediments. However, our results demonstrate that it is not always justifiable to pool *Chironomus* species in contaminant analyses, because, according to their feeding behaviour, species living at the same site differ in their trace element concentrations. In fact, we found that *C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri* mainly assimilate trace elements from oxic sediments; whereas, *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C*. sp. NAI, *C*. sp. NAII, *C*. sp. NAIII and *C*. '*tigris*' larvae mainly assimilate traces elements anoxic sediments. From one lake to another, species that feed in oxic sediment had higher Cd and sometimes Zn concentrations and lower Se concentration than those that feed in anoxic sediments. As for the other trace elements, there were no differences between species living at the same site. Larval concentrations indicate that the bioavailability of Cd, Se and sometimes Zn differs between oxic and anoxic sediments.

Not being able to pool *Chironomus* species in contaminant studies could be viewed as a drawback for using these larvae as biomonitors. However, our data suggest that not all *Chironomus* larvae need to be identified to species, since trace element concentrations generally only differed between species that feed in oxic and anoxic sediments and living larvae from these two feeding groups can be separated morphologically. Overall, our results demonstrate that *Chironomus* larvae are very useful for comparing the bioavailability of trace elements in oxic and anoxic sediments at a given site.

TABLE DES MATIÈRES

LISTE DES TABLEAUX

LISTE DES FIGURES

LISTE DES ENCADRÉS

LISTE DES ABRÉVIATIONS

ADN	acide désoxyribonucléique
Ag	argent
ARN	acide ribonucléique
As	arsenic
AVS	sigle du terme anglais, *acid-volatile sulfides*
Ba	baryum
Bi	bismuth
BOLD	*Barcoding of Life Data*
Cd	cadmium
Co	cobalt
cox1	*cytochrome oxydase sous-unité 1*
Cr	chrome
Cu	cuivre
gb2β	*globine sous-unité 2β*
Mn	manganèse
Ni	nickel
Pb	plomb
PCR	réaction en chaîne par polymérase (sigle du terme anglais, *polymerase chain reaction*)
PCR-RFLP	réaction en chaîne par polymérase – polymorphisme de longueur des fragments de restriction (sigle du terme anglais, *polymerase chain reaction – restriction fragment length polymorphism*)
S	soufre
Sb	antimoine
Se	sélénium

SEM	sigle du terme anglais, *simultaneously extracted metals*
Tl	thallium
Zn	zinc

SECTION 1 - Synthèse

1 INTRODUCTION

Les métaux sont des composés intégrants de la planète Terre. Cependant, durant les dernières décennies, voire même des siècles, l'extraction et le raffinage des métaux ont entraîné une mobilisation sans précédent d'éléments traces de la croûte terrestre vers les sols, l'eau et l'air. Cette mobilisation a eu des impacts majeurs sur le cycle naturel de certains de ces éléments à l'échelle globale et régionale (Campbell *et al.*, 2004, Nriagu, 1989). Les éléments traces libérés dans l'environnement atteignent les systèmes lacustres par voie aérienne, ruissellement ou déversement direct (Bonham-Carter *et al.*, 2006, Luoma *et al.*, 2008). Ainsi dans les lacs situés en régions minières comme Sudbury (Ontario) et Rouyn-Noranda (Québec), on retrouve des concentrations de certains éléments traces qui sont beaucoup plus élevées que celles retrouvées dans les lacs en régions peu contaminées (Nriagu *et al.*, 1982, Pyle *et al.*, 2005, Shuhaimi-Othman *et al.*, 2006). À pH neutre, les éléments traces en solution se lient fortement aux particules en suspension et finissent pas sédimenter (Elbaz-Poulichet *et al.*, 1996, Luoma *et al.*, 2008). Par conséquent, les sédiments dans les lacs contaminés constituent de véritables réservoirs de ces contaminants. Dans les sédiments, ces contaminants ont le potentiel d'être assimilés chez les animaux qui y vivent, d'être retransférés chez les prédateurs de ces animaux et ainsi d'être remobilisés dans le milieu aquatique (p. ex. Dubois *et al.*, 2009a, Dumas *et al.*, 2008). Même avec l'arrêt des apports externes de ces éléments traces dans un écosystème aquatique, les concentrations de ces contaminants peuvent rester élevées chez les animaux dans la colonne d'eau, et ce, pour plusieurs années, suite à la remobilisation des éléments traces provenant des sédiments contaminés (Nriagu *et al.*, 1998, Rainbow *et al.*, 2011). Au-delà d'une certaine concentration, un élément trace chez un animal peut être toxique. Ce seuil de concentration diffère d'un élément et d'une espèce à l'autre. La contamination d'un écosystème aquatique par les éléments traces peut aussi grandement altérer la structure des communautés et des écosystèmes aquatiques (Borgmann *et al.*, 2004a, Keller *et al.*, 1991, Rasmussen *et al.*, 2008). La biodisponibilité d'éléments traces dans les sédiments est donc d'intérêt lors d'évaluations des risques environnementaux.

1.1 **Distribution et biodisponibilité des éléments traces dans les sédiments**

Dans les sédiments, les éléments traces peuvent être liés aux particules sédimentaires ou dissous dans l'eau interstitielle (figure 1-1). Les concentrations totales des éléments traces dans les sédiments sont d'une utilité limitée pour prédire l'occurrence d'impacts environnementaux, car ces contaminants étant présents sous différentes formes (p. ex. associés aux oxydes de fer, à la matière organique ou aux sulfures) ne sont pas toujours disponibles pour les êtres vivants (Luoma, 1989). En raison de la complexité et l'hétérogénéité des sédiments, il est beaucoup plus difficile de déterminer la spéciation des éléments traces et leur distribution entre les phases aqueuses et solides des sédiments que d'établir la spéciation des éléments traces dans l'eau.

Dans la colonne d'eau, les éléments traces peuvent être liés à des ligands inorganiques dissous[1], à des ligands organiques dissous ou même être adsorbés à la matière en suspension. La spéciation des cations traces (métaux) dissous peut être calculée à l'aide de logiciels de spéciation chimique (p. ex. WHAM). Pour ce qui est des anions d'arsenic (As) et de sélénium (Se), les concentrations des diverses espèces dissoutes peuvent être déterminées analytiquement (Ma *et al.*, 2014, Ponton *et al.*, 2013). Dans la colonne d'eau, il a été démontré que les concentrations des métaux chez les animaux étaient généralement reliées aux concentrations de l'ion libre dans l'eau (Campbell, 1995). Quant aux anions, leur biodisponibilité est généralement dictée par leur état d'oxydation (ex. Cr(VI) est plus biodisponible que Cr(III), Se(IV) plus biodisponible que Se(VI) et As (III) et plus biodisponible que As (IV)) (Luoma *et al.*, 2008, Ponton *et al.*, 2009).

Dans les sédiments, la présence ou l'absence d'oxygène influencent fortement la spéciation des éléments traces. Dans les lacs où les sédiments contiennent de la matière organique en décomposition, l'oxygène n'est présent que dans les premiers millimètres de sédiments à partir de l'interface eau-sédiments (Luoma *et al.*, 2008). Cette interface se situe soit à la surface des sédiments ou dans le tube des animaux benthiques qui irriguent leur tube (Charbonneau *et al.*, 1998). Quant au reste des sédiments, il y a absence d'oxygène.

Dans la couche oxique des sédiments, les oxyhydroxides de fer et de manganèse sont des ligands inorganiques sur lesquels les éléments traces peuvent se lier (Davies-Colley *et al.*, 1984, Tessier *et al.*, 1993, Tessier *et al.*, 1996). La distribution des particules d'éléments traces

[1] Matière dont le diamètre <0.45 µm.

dans les sédiments oxiques (c.-à-d. adsorbées, liées aux carbonates, liées aux oxydes de fer et de manganèse, liées à la matière organique et liées aux minéraux) peut être évaluée à l'aide de la méthode chimique d'extraction séquentielle proposée par Tessier *et al.* (1979). Dans les sédiments anoxiques, les ligands majeurs sont les sulfures (Ankley, 1996). La matière organique dissoute et particulaire lie aussi les éléments traces, mais contrairement aux oxydes de fer, aux oxydes de manganèse et aux sulfures, sa présence n'est pas influencée par l'état d'oxydoréduction des sédiments (Mahony *et al.*, 1996, Tessier *et al.*, 1993, Tessier *et al.*, 1996, Wang *et al.*, 1997). Dans l'eau interstitielle oxique, les éléments traces se lient aux mêmes types de ligands que dans la colonne d'eau oxygénée. D'un autre côté, dans l'eau interstitielle anoxique, les ligands dominants sembleraient être des espèces réduites comme des sulfures et des polysulfures (Wang *et al.*, 2009).

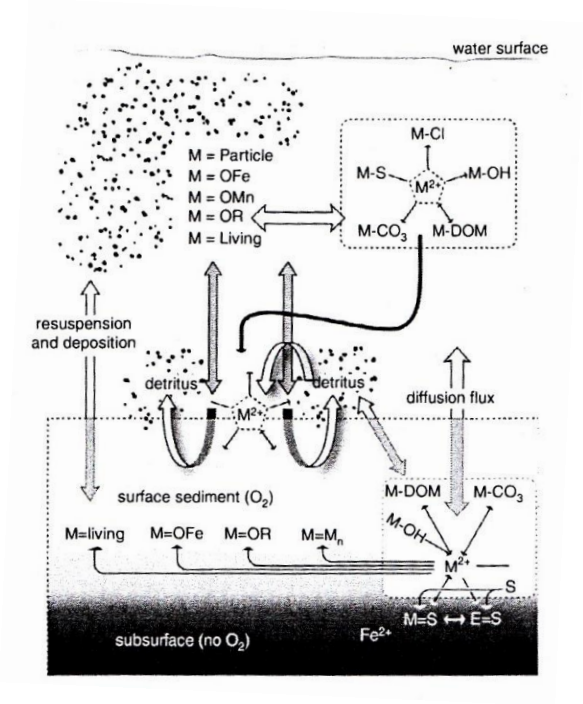


Figure 1-1 **Ensemble des processus qui affectent le partitionnement et la spéciation des métaux dans un environnement aquatique (figure tirée de Luoma *et al*., 2008).**
Cl=chlorure, CO_3=carbonates, DOM=matière organique dissoute, E=élément, M=métal, M^{2+}=ion libre du métal, OFe=oxydes de fer, OH=hydroxyde, OMn=oxydes de manganèse, OR=organique et S=sulfure.

Présentement, aucune approche chimique ne permet de bien évaluer la biodisponibilité des éléments traces dans les sédiments. Certaines études ont démontré que normaliser les concentrations d'éléments traces dans les sédiments par la fraction de la matière organique permettait de mieux prédire les concentrations de certains éléments traces chez les animaux

benthiques (ex. Langston, 1982). Pour ce qui est plus particulièrement des sédiments oxiques, il a aussi été démontré que corriger les concentrations d'un élément trace par les concentrations d'oxydes de Fe améliorait la relation entre les concentrations de cet élément chez les animaux et les sédiments (ex. Langston, 1980, Luoma *et al.*, 1978). Dans les sédiments anoxiques, une approche nommée SEM-AVS (sigles des termes anglais, « simultaneously extracted metals » et « acid-volatile sulfides ») a été suggérée afin d'évaluer la toxicité des métaux chez les animaux benthiques et par le fait même leur biodisponibilité. Cette approche a pour principe de base que ce sont les concentrations de métaux dans les eaux anoxiques interstitielles qui prédisent le mieux la toxicité des métaux chez les animaux benthiques (Ankley *et al.*, 1996, Di Toro *et al.*, 2005, Swartz *et al.*, 1986). Certains métaux traces d'importance environnementale (Ag, Cd, Cu, Ni, Pb et Zn) ont une forte affinité pour le soufre et, par conséquent, leurs concentrations dans les eaux interstitielles sont contrôlées par des réactions de précipitation avec ce composé. L'argent (Ag), le cadmium (Cd), le cuivre (Cu), le nickel (Ni), le plomb (Pb) et le zinc (Zn) ayant des produits de solubilité plus faibles que celui du sulfure de fer (FeS) amorphe, ont tendance à déplacer le Fe dans le FeS pour ainsi former des sulfures insolubles (Ankley *et al.*, 1996). D'après le modèle, si les concentrations des SEM n'excèdent pas celle de l'AVS, les concentrations de métaux dans l'eau interstitielle anoxique devraient être faibles et l'on ne devrait pas observer de toxicité chez les animaux benthiques. Cependant, si la concentration des SEM excède celle de l'AVS, les effets toxiques deviennent alors possibles. Bien que cette approche ait été largement utilisée lors d'évaluations du risque environnemental (surtout aux États-Unis) et que de nombreuses études aient démontré une relation entre la [SEM]-[AVS] et la toxicité chez les animaux aquatiques, celle-ci reste contestée (Campbell *et al.*, 2006). C'est que le modèle ne prédit pas bien l'accumulation de métaux traces chez les invertébrés benthiques (ex. Hare *et al.*, 2001). De Jonge *et al.* (2009) ont même démontré que les animaux benthiques pouvaient accumulés des métaux malgré des concentrations d'AVS en excès par rapport à celles de SEM.

1.2 **Utilisation de biomoniteurs pour évaluer la biodisponibilité des éléments traces dans les sédiments**

Une autre approche pour évaluer la biodisponibilité des éléments traces dans les sédiments est de tout simplement mesurer les concentrations d'éléments traces dans les organismes qui y vivent. Les organismes utilisés pour évaluer la biodisponibilité des éléments traces dans un milieu sont communément appelés biomoniteurs (Luoma *et al.*, 2008) ou organismes sentinelles

(Beeby, 2001). La concentration d'un contaminant chez un biomoniteur est une mesure relative de la biodisponibilité de ce contaminant provenant de toutes les voies d'exposition au cours du temps (encadré 1-1). Les mesures d'éléments traces dans les tissus des organismes sont donc des mesures de ce qui est réellement biodisponibles pour les organismes benthiques (encadré 1).

Encadré 1-1 Facteurs dictant les concentrations d'un élément trace chez un organisme

La concentration d'un élément trace chez un organisme est le résultat de ce qui a été assimilé moins ce qui a été éliminé. Selon le modèle biodynamique (Luoma *et al.*, 2005), la concentration d'un élément trace chez un organisme à l'état stationnaire est définie comme suit :

$$[\mathrm{M}]^{\mathrm{es}} = \frac{\mathrm{k_u}[\mathrm{M}]_{\mathrm{eau}} + (\mathrm{EA} \times \mathrm{TI} \times [\mathrm{M}]_{\mathrm{nourriture}})}{\mathrm{k_e} + \mathrm{k_g}} \quad (1)$$

où k_u est la constante de vitesse pour la prise en charge à partir de l'eau, k_e est la constante de vitesse pour l'efflux, k_g est la constante de croissance, $[M]_{eau}$ est la concentration du métal dissous, $[M]_{nourriture}$ est la concentration du métal dans la nourriture, EA est l'efficacité d'assimilation et TI est le taux d'ingestion de la nourriture.

L'assimilation d'un élément trace par un organisme peut se faire via son exposition à l'eau et/ou via sa diète. Il est à noter que ce ne sont pas tous les animaux benthiques fouisseurs qui sont exposés à l'eau interstitielle des sédiments. Beaucoup d'entre eux sont aussi exposés à l'eau de la colonne d'eau puisqu'ils irriguent leurs tunnels pour le garder oxygéner (ex. *Sialis*, *Hexagenia* et *Chironomus*).

Tout organisme qui vit dans les sédiments a le potentiel d'être utilisé comme biomoniteur de contaminants dans les sédiments (ex. Proulx *et al.*, 2012). Cependant, pour être de bons biomoniteurs de la contamination des sédiments, les organismes doivent accumuler les contaminants provenant des sédiments dans leurs tissus. Un organisme dont les concentrations internes d'un contaminant restent constantes n'est pas un bon biomoniteur de ce contaminant. Une corrélation entre les concentrations d'un contaminant chez un organisme benthique et celles dans les sédiments permet de démontrer l'efficacité de l'organisme en tant que biomoniteur des sédiments.

À des fins pratico-pratiques, un taxon utilisé comme biomoniteur d'un élément trace doit aussi :

1. avoir une large distribution géographique;
2. être abondant, facile à échantillonner, de bonne taille (pour pouvoir mesurer les contaminants) et robuste (pour survivre à l'échantillonnage);
3. être sédentaire et donc représentatif du site d'échantillonnage;
4. être tolérant à une grande gamme de conditions physico-chimiques;
5. être tolérant aux contaminants;
6. être facile à identifier.

En milieu marin, des organismes, dont des polychètes (ex. *Nereis diversicolor* et des espèces des familles des nephtyidés, des ampharetidé et des térébellidés) et des mollusques bivalves (ex. *Macoma balthica* et *Scrobicularia plana*) ont déjà été utilisés pour évaluer la contamination des sédiments par les éléments traces (Luoma *et al.*, 2008). D'un autre côté, en eau douce, le crustacé *Hyalella azteca* a largement été utilisé comme animal de laboratoire lors de tests de toxicité des sédiments (Borgmann *et al.*, 2004a, Norwood *et al.*, 2007). Toutefois, le désavantage d'utiliser *H. azteca* comme biomoniteur sur le terrain est qu'il n'est pas présent en milieu hautement acide. De plus, l'idée d'utiliser *H. azteca* comme biomoniteur de la contamination des sédiments a été mise en doute par le fait que dans certaines études, les concentrations de certains métaux étaient mieux corrélées à celles dans l'eau que celles dans les sédiments (Borgmann *et al.*, 2005, Wang *et al.*, 2004). Des éphémères dont *Baetis rhodani* et *Hexagenia limbata* ont aussi été utilisés comme biomoniteurs de la biodisponibilité des éléments traces dans les sédiments d'eau douce (Fialkowski *et al.*, 2003, Lacharité, 2011). Cependant, le désavantage de l'utilisation des éphémères (et aussi des trichoptères et des plécoptères) est qu'un grand nombre d'espèces ne tolèrent pas une large gamme de concentrations de métaux (Cain *et al.*, 2004) et d'acidité (Petrin *et al.*, 2007). Dans les sédiments contaminés, on retrouve généralement des oligochètes. Cependant, le désavantage d'utiliser ces organismes est qu'il est très difficile de les séparer au niveau de l'espèce et même voir à des niveaux taxonomiques supérieurs.

1.3 Utilisation des larves de *Chironomus* en tant que biomoniteurs

Un autre animal qui a le potentiel d'être utilisé comme biomoniteur de la contamination des sédiments en milieu d'eau douce est *Chironomus*. Les larves de ce genre d'insecte vivent et se nourrissent dans les sédiments fins. Elles sont distribuées mondialement et on les retrouve

autant dans les étendues d'eau contaminées que non contaminées (Armitage *et al.*, 1995). Les espèces de *Chironomus* sont souvent abondantes, faciles à échantillonner et de bonnes tailles. En étant d'importantes sources de nourriture pour d'autres invertébrés et des poissons, elles peuvent être aussi des sources de contamination pour leurs prédateurs (Dubois *et al.*, 2009a, Dumas *et al.*, 2008). Des espèces de *Chironomus* (plus particulièrement *C. tentans*, *C. dilutus* et *C. riparius*) ont déjà fait l'objet de nombreux tests de toxicité des sédiments (Environnement Canada, 1997, Péry *et al.*, 2005). De plus, diverses données sur les larves *Chironomus* et les éléments traces ont été publiées. En voici quelques exemples :

1. mesures de la distribution interne d'éléments traces chez les larves de *Chironomus* (Craig *et al.*, 1998, S. Martin *et al.*, 2008);
2. mesures de la distribution subcellulaire d'éléments traces chez les larves de *Chironomus* (Béchard *et al.*, 2008, Dubois *et al.*, 2009a, Dubois *et al.*, 2009b, Dumas *et al.*, 2008);
3. mesures de concentrations de métaux dans le cytosol des larves de *Chironomus* (Péry *et al.*, 2008);
4. mesures de concentrations de métallothionéine dans les larves de *Chironomus* par rapport aux concentrations de métaux dans leurs tissus ou dans l'environnement (Gillis *et al.*, 2002, Gillis *et al.*, 2006);
5. données de prise en charge du Cd par des canaux de calcium chez les larves de *Chironomus* (Craig *et al.*, 1999, Gillis *et al.*, 2008);
6. données sur l'excrétion et la séquestration du Cd par le tube digestif et les tubules de Malpighi des larves de *Chironomus* (Leonard *et al.*, 2009);
7. influence de la salinité (Bidwell *et al.*, 2006), de la température (Bervoets *et al.*, 1996) et du pH (Bervoets *et al.*, 2000) sur la prise en charge de métaux par les larves de *Chironomus.*

Cependant, le désavantage d'utiliser des *Chironomus* récoltés sur le terrain est que l'identification morphologique des espèces est difficile et parfois même impossible. Afin de contrer ce problème, certaines études sur les métaux regroupent les espèces de *Chironomus* récoltées au même endroit soit au niveau du genre ou même avec d'autres genres au niveau de la famille (p. ex. Bervoets *et al.*, 1998, Chételat *et al.*, 2008, Desrosiers *et al.*, 2008, Filion *et al.*, 2000) et de l'ordre (p. ex. Muscatello *et al.*, 2009). Cependant, il y a lieu de se demander si cette pratique est justifiable. En somme, elle ne peut être valide que si les espèces regroupées accumulent les métaux de façon similaire. Il est à noter que des

différences de concentrations de métaux entre espèces de même genre ont été mesurées chez l'annélide *Tharyx* (Bryan *et al.*, 1987), le mollusque *Mytilus* (Lobel *et al.*, 1990) et les arthropodes *Chaoborus* (Croteau *et al.*, 2001), *Orchestia* (Moore *et al.*, 1987) et *Balanus* (Rainbow *et al.*, 1993). Chez *Chironomus*, des différences de concentrations de Cd et Zn ont été mesurées chez les larves de deux espèces vivant au même endroit (S. Martin *et al.*, 2008).

1.4 Écologie des *Chironomus*

Les larves de *Chironomus* sont de couleur rouge et ont un corps cylindrique dont la tête est séparée du reste du corps (larves eucéphales). Elles font partie de la famille des Chironomidae (encadré 1-2), la famille de diptères la plus importante en milieu aquatique comptant plus de 10 000 espèces (Armitage *et al.*, 1995).

Encadré 1-2 Classification de *Chironomus*

Embranchement	→	Arthropode
Classe	→	Insecte
Ordre	→	Diptère
Sous-ordre	→	Nematocère
Famille	→	Chironomidae
Sous-famille	→	Chironominae
Tribu	→	Chironomini
Genre	→	*Chironomus*

Les larves de *Chironomus* ont des pattes ventouses (*prolegs* en anglais) antérieures et postérieurs et de tubules anaux (encadré 1-3). Certaines espèces ont aussi des tubules ventraux et/ou latéraux[2] sur leurs 10^{e} et 11^{e} segments respectivement.

Les espèces de *Chironomus* ont un cycle de vie constitué de quatre stades larvaires, un stade de pupe et un stade adulte (Armitage *et al.*, 1995). Après l'émergence, le stade adulte de *Chironomus* ne dure que quelques jours. Durant ce stade, la fonction principale des

[2] La fonction des tubules ventraux et latéraux reste encore à être déterminée. Dans certains ouvrages (ex. Tachet *et al.*, 2002), il est mentionné que ces extensions sont des branchies. Cependant, ce n'est pas logique que ces extensions soient des branchies, car certaines espèces ne sont pas dotées de ces tubules.

Chironomus et de se disperser et de se reproduire (Tachet *et al.*, 2002). Le développement larvaire des espèces de *Chironomus* étant grandement influencé par la température, la quantité et la qualité de la nourriture et les périodes de quiescence lorsque le milieu n'est plus favorable au développement, le cycle de vie des espèces de *Chironomus* peut être d'une durée de 6 mois allant jusqu'à 7 ans (Merritt *et al.*, 2008). Au lac St-Joseph (situé près de la ville de Québec, Québec, Canada), le cycle de vie de *C. staegeri* et *C. « tigris »* est de 1 an (S. Martin *et al.*, 2008).

Encadré 1-3 Mosaïque de photos de larves, pupe et adultes de *Chironomus*

Photos 1-4 : Photos de larve (1), pupe (2) et adultes (3 et 4) qui ont été prises par Klaus Peter Brodersen (https://picasaweb.google.com/110992905327203171768/MidgesChironomidae).
Photos 5-6 : À noter que lorsqu'elles sont préservées dans l'éthanol ou le formaldéhyde, les larves de *Chironomus* se décolorent.

Tous les stades préadultes sont benthiques sauf le premier stade larvaire qui est planctonique (Jónasson, 1972). Durant leur période benthique, les *Chironomus* habitent dans les sédiments où ils construisent des tunnels qui s'interconnectent (Charbonneau *et al.*, 1998). Ces tunnels sont généralement sous forme de U (Charbonneau *et al.*, 1998) et sont tapissés de soie sécrétée par les glandes salivaires des larves (Jónasson, 1972). Des études ont rapporté que les galeries atteignaient des profondeurs allant de 4 à 40 cm dans les sédiments (K Berg, 1938, Charbonneau *et al.*, 1998, Hilsenhoff, 1966). Donc, il va sans dire qu'une grande partie de ces galeries se retrouvent dans les sédiments anoxiques, puisqu'en milieu lacustre, la couche oxique des sédiments n'est généralement que de quelques millimètres d'épaisseur. Pour

maintenir leurs tunnels oxygénés, les larves de *Chironomus* ondulent leur corps pour faire circuler l'eau oxygénée provenant de la colonne d'eau (Jónasson, 2003). Cette circulation de l'eau permet aussi d'expulser les métabolites et le dioxyde de carbone accumulés.

La respiration des larves de *Chironomus* est cutanée (Merritt *et al.*, 2008). Sous la cuticule, la présence d'un système de trachées se divisant en trachéoles permet de distribuer l'oxygène dans le corps (Merritt *et al.*, 2008). Contrairement à la plupart des autres insectes, les *Chironomus* sont aussi dotés d'hémoglobine dans l'hémolymphe. C'est cette molécule, qui confère la couleur rouge aux larves. La synthèse d'hémoglobine débute à partir de la fin du 1er stade larvaire (Osmulski *et al.*, 1986). Contrairement aux vertébrés, les molécules d'hémoglobine des *Chironomus* ont une forte affinité pour l'oxygène. Cette hémoglobine permet aux larves d'entreposer de l'oxygène lorsqu'il est abondant dans le milieu et de le relâcher lorsque les concentrations deviennent faibles (Merritt *et al.*, 2008). Walshe (1950) a démontré que cet entreposage d'oxygène par l'hémoglobine permet de fournir de l'oxygène aux tissus des larves pendant 9 minutes en situation d'hypoxie. Si l'absence d'oxygène persiste, les larves de *Chironomus* peuvent passer en mode de respiration anaérobique. Par exemple, *C. thummi* et *C.* gr. *plumosus* ont été décrits comme pouvant fermenter de l'éthanol en l'absence d'oxygène (Redecker *et al.*, 1988, Scholz *et al.*, 1998).

Les espèces de *Chironomus* se nourrissent de phytoplancton, de macrophytes ou de détritus (M. B. Berg, 1995). Les larves de *Chironomus* sont décrites comme étant des détritivores (Jónasson, 1972) ou des filtreurs (Walshe, 1951). Les larves filtreurs construisent des cônes de soie à travers desquels elles filtrent des particules de la colonne d'eau grâce à des ondulations de leur abdomen. Lorsque le filtre est plein, elles l'ingèrent et en tissent un autre (Tachet *et al.*, 2002). Les larves ayant un comportement détritivore ingèrent des particules oxiques ou anoxiques de sédiments à la surface ou à l'intérieur de leurs tubes.

2 OBJECTIFS

L'objectif global de la thèse était d'évaluer le potentiel et la faisabilité d'utiliser des larves de *Chironomus* en tant que biomoniteurs des éléments traces dans les sédiments. Pour ce faire, nous avons subdivisé la thèse en 5 sous-objectifs suivants :

2.1 Séparer et identifier les larves de *Chironomus* au niveau de l'espèce

Comme mentionné ci-dessus, la séparation et l'identification des larves de *Chironomus* au niveau de l'espèce sont problématiques, car les larves de certaines espèces sont identiques morphologiquement (espèces cryptiques). Étant donné que le 2^{e} objectif de la thèse était de déterminer si les concentrations d'éléments traces diffèrent entre espèces vivant au même endroit, nous avons donc axé une partie importante de nos travaux sur la séparation et l'identification des larves.

En plus d'être basée sur la morphologie des larves, des prépupes et des adultes, la taxonomie des *Chironomus* repose tout particulièrement sur la composition et la structure de leurs chromosomes polytènes. Chez les larves de diptères, les chromosomes polytènes sont principalement retrouvés dans les cellules des glandes salivaires[3] (J. Martin, 1979). Ces chromosomes géants résultent de la réplication de l'ADN du noyau d'une cellule sans qu'il y ait division de la cellule elle-même. Bref, les chromosomes polytènes sont donc formés d'environ mille copies de chromatides (Wuelker, 2010) restées soudées entre elles. Grâce à leur grande taille pouvant atteindre ¼ de mm de longueur (Wuelker, 2010), il est possible d'observer ces chromosomes sous un microscope à faible grossissement. Il suffit de disséquer les glandes salivaires, de séparer les cellules et d'ajouter un colorant (voir dans Proulx *et al.*, 2013). Les chromosomes polytènes des diverses espèces de *Chironomus* diffèrent de par leur nombre[4], leur configuration[5], leurs profils de bandes[6] et la présence de nucléoles ou des *Balbiani rings*[7].

[3] Chez les chironomidés, les chromosomes polytènes sont aussi présents dans d'autres tissus dont les tubes de Malpighi et les tubules anaux. Cependant, avec ces chromosomes, il est plus difficile de faire de bons montages sur lame (J. Martin, 1979).

[4] La plupart des espèces de *Chironomus* ont généralement 4 chromosomes, mais certaines en possèdent que 2 tandis que d'autres en ont 3.

[5] Les chromosomes polytènes chez *C. cucini* sont regroupés par un chromocentre tandis que chez les autres espèces, ils sont séparés.

Les études caryosystématiques consistent donc à analyser toutes ces caractéristiques afin d'identifier les espèces. Malheureusement, il n'y a qu'une dizaine de personnes dans le monde qui ont l'expertise nécessaire pour séparer et identifier les larves de *Chironomus* selon ces structures.

Il n'existe aucune clé taxonomique complète permettant d'identifier les espèces de *Chironomus* en Amérique du Nord. Le document le plus complet sur la taxonomie des espèces de *Chironomus* récoltées en Amérique du Nord a été écrit par Jon Martin. Ce document, disponible sur internet, est mise à jour régulièrement, mais n'a jamais été publié officiellement. La structure des chromosomes polytènes et la morphologie des larves, des prépupes et des adultes des espèces répertoriées y sont décrites. Ce document comprend aussi une liste d'individus dont l'identification reste à être confirmée.

Durant les dernières décennies, le séquençage de brins d'ADN spécifiques, un nouvel outil en taxonomie, a permis la séparation et l'identification d'espèces. Le fragment d'ADN le plus communément utilisé pour l'identification d'espèces animales est situé dans la partie 5' du génome mitochondrial codant pour la première sous-unité de la *cytochrome oxydase* (*cox1* ou *COI*) (Hebert *et al.*, 2003). Le terme barcoding moléculaire (ou DNA barcoding ou code-barres génétique) réfère plus particulièrement à l'utilisation de cette partie du gène *cox1* pour l'identification d'espèces. Cependant, dans cette thèse, nous utilisons le terme barcoding moléculaire pour désigner tout fragment d'ADN utilisé pour identifier des espèces. La séquence du gène *cox1* a été prônée par Hebert *et al.* (2003) qui a démontré que les différences entre les séquences de ce gène sont généralement faibles entre les individus d'une même espèce et élevées entre des individus d'espèces différentes. Cette séquence d'ADN a déjà été utilisée pour identifier des espèces d'insectes provenant d'une variété de groupe taxonomique, dont les collemboles (Hogg *et al.*, 2004), les *Ephemerella* (Alexander *et al.*, 2009), les éphéméroptères (Ball *et al.*, 2005, Elderkin *et al.*, 2012), les coléoptères (Davis *et al.*, 2011) et les chironomidés (Carew *et al.*, 2011, Carew *et al.*, 2007, Ekrem *et al.*, 2007, Ekrem *et al.*, 2010, Pfenninger *et al.*, 2007, Sinclair *et al.*, 2008, Stur *et al.*, 2011). Son utilisation est facilitée par le fait que des amorces universelles sont capables d'amplifier cette séquence chez une variété d'invertébrés (Folmer *et al.*, 1994). Bien que les séquences *cox1* aient aussi été utilisées avec succès pour

[6] Sur les chromosomes, il est possible d'observer un profil de bandes qui est en somme l'aboutissement de modifications chromosomiques telles que des duplications, des délétions, des inversions et des mutations (Voet *et al*., 1995). La configuration des bandes chez un chromosome polytène représente donc une carte cytologique parallèle à sa carte génétique (Voet *et al*., 1995).

[7] C'est une région déroulée du chromosome où il y a des sites de transcription d'ARN.

séparer certaines espèces de *Chironomus* (Carew *et al.*, 2003, Pfenninger *et al.*, 2007, Sharley *et al.*, 2004), J. Martin *et al.* (2002) ont démontré que *C. entis* et *C. plumosus* ne pouvaient être distinguées à partir de ces séquences. Ils ont démontré que ces espèces pouvaient être toutefois séparées à partir d'une séquence du gène nucléaire *globine 2B* (***gb2β***). Cette séquence a aussi déjà été amplifiée avec succès chez d'autres espèces de *Chironomus* (V. Guryev *et al.*, 2001, V. P. Guryev *et al.*, 2002, Hankeln *et al.*, 1997, Kao *et al.*, 1994, J. Martin *et al.*, 2002).

Grâce à la technique PCR-RFLP, il est possible de scanner une séquence d'ADN d'un grand nombre d'individus sans avoir recours au séquençage. Cette technique, étant dans le passé moins dispendieuse que le séquençage, a été utilisée pour séparer et identifier des espèces de *Chironomus* en Australie (Carew *et al.*, 2003, Sharley *et al.*, 2004). Elle consiste à couper les amplicons[8] à l'aide d'enzymes de restriction et à séparer les fragments d'amplicons obtenus à l'aide d'un gel d'électrophorèse. L'idée derrière cette technique est que si la séquence de nucléotides diffère entre deux espèces, certaines enzymes de restrictions couperont les amplicons à des endroits différents créant ainsi des fragments d'amplicons qui diffèreront en nombre et en grandeur d'une espèce à l'autre.

Le premier objectif de la thèse était donc d'identifier les espèces de *Chironomus* récoltées lors de nos campagnes d'échantillonnage à l'aide d'évidences morphologiques, caryosystématique et moléculaires (*cox1* et *gb2β*).

2.2 Déterminer si les concentrations d'éléments traces diffèrent entre espèces de *Chironomus* vivant au même endroit

Comme mentionné ci-dessus (section 1.3), S. Martin *et al.* (2008) ont démontré que les concentrations de Cd et de Zn différaient entre deux espèces de *Chironomus* (*C. staegeri* et *C. « tigris »*) récoltées dans le lac Saint-Joseph (Québec, Canada). Dans les débuts de nos recherches, nous avons effectué une étude préliminaire afin d'évaluer si dans d'autres lacs, les concentrations de Cd différaient entre espèces sympatriques de *Chironomus*. En plus de récolter des larves de *Chironomus* au lac Saint-Joseph, nous avons récolté des larves de *Chironomus* dans 3 autres lacs situés dans la région minière de Rouyn-Noranda. Les larves de *Chironomus* ont été séparées hypothétiquement au niveau de l'espèce selon leur morphologie

[8] Séquences d'ADN amplifiées

et grâce à une technique génétique (*cox1* PCR-RFLP). Les résultats de cette étude sont présentés dans l'article 1. Dans tous les lacs, les concentrations de Cd différaient entre certaines « espèces » sympatriques[9] de *Chironomus*. Ces résultats indiquèrent que les différences de concentrations d'éléments traces entre espèces sympatriques devaient être davantage scrutées.

Le deuxième objectif de la thèse était donc de déterminer si les concentrations de Cd et d'autres éléments traces diffèrent entre espèces de *Chironomus* vivant au même endroit, et ce, dans plusieurs lacs. Notre hypothèse était que dans d'autres lacs, les concentrations de Cd et d'autres éléments traces allaient aussi différer entre espèces sympatriques de *Chironomus*.

Pour répondre à notre 2^{e} objectif, nous avons comparé les concentrations larvaires d'arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), cuivre (Cu), manganèse (Mn), nickel (Ni), sélénium (Se) et zinc (Zn) chez des larves de *Chironomus* récoltées dans des lacs des régions minières de Rouyn-Noranda et Sudbury. Tous ces éléments sont dits essentiels à l'exception du Ba et du Cd (Luoma *et al.*, 2008). Des études ont reporté des concentrations d'As, Ba, Cd, Co, Cu, Ni, Se et Zn plus élevées dans l'eau, les sédiments ou les animaux des lacs à Sudbury comparativement à celles mesurées dans des lacs non affectés par les activités minières (Pyle *et al.*, 2005, Shuhaimi-Othman *et al.*, 2006). À Rouyn-Noranda, le Cd et le Cu ont été identifiés comme étant les métaux les plus susceptibles de causer des effets toxiques chez les organismes (Borgmann *et al.*, 2004b) tandis qu'à Sudbury, l'élément trace considéré comme étant le plus problématique est le Ni (Borgmann *et al.*, 2001). Nous avons aussi tenté de mesurer les concentrations d'argent (Ag), bismuth (Bi), chrome (Cr), plomb (Pb), antimoine (Sb) et thallium (Tl) chez les larves. Cependant, puisque les concentrations larvaires de Bi, Sb et Tl étaient sous ou près de la limite de détection dans la plupart des lacs, que notre méthode de digestion ne permettait pas de libérer la totalité du Cr (seulement environ 40%) et du Pb et que les blancs étaient contaminés par l'Ag, nous avons laissé tomber l'analyse de ces contaminants.

[9] Espèces vivant au même endroit; partageant le même habitat.

2.3 **Déterminer le comportement alimentaire des larves des espèces de *Chironomus***

Afin d'expliquer pourquoi les larves de *C. staegeri* et *C. « tigris »* récoltées au même endroit différaient dans leurs concentrations de Cd et de Zn, S. Martin *et al.* (2008) ont comparé le cycle de vie des larves, leurs tailles et le temps de passage de la nourriture dans leur tractus digestif et ont conclu que ces facteurs n'expliquaient pas les différences de concentrations de Cd et de Zn mesurées entre les deux espèces. Cependant, ils ont observé que la couleur du contenu stomacal de *C. staegeri* était orangée comme celui des sédiments oxiques (dû à la présence d'oxyde de fer), tandis que celle du contenu stomacal de *C. « tigris »* était grise comme celui des sédiments anoxiques (dû à la présence de sulfures). Ces observations indiquaient que les larves de ces deux espèces ingéraient des particules de sédiments qui différaient. Afin de confirmer leurs observations, S. Martin *et al.* (2008) ont mesuré les isotopes stables de soufre (^{34}S et ^{32}S) chez les larves.

En écotoxicologie, les analyses d'isotopes stables peuvent s'avérer très utiles pour étudier les contaminants dans la chaîne alimentaire, mais aussi pour évaluer les sources de nourriture des animaux (Jardine *et al.*, 2006). Les valeurs des isotopes stables sont exprimées en ‰ où le δ représente la différence entre la mesure d'un échantillon par rapport à celle d'un matériel de référence utilisé lors de l'analyse (voir équation 2 à la p. 24).

Règle générale, les sédiments anoxiques ont une signature isotopique de soufre ($\delta^{34}S$) plus négative que les particules provenant des sédiments oxiques ou de la colonne d'eau (Croisetière *et al.*, 2009). Étant donné qu'il y a très peu de fractionnement isotopique de soufre entre les consommateurs et leur nourriture, les valeurs de $\delta^{34}S$ peuvent être utilisées pour déterminer si les animaux se nourrissent de particules oxiques ou anoxiques (Croisetière *et al.*, 2009, Peterson, 1999, Peterson *et al.*, 1985). Il faut mentionner qu'en eau douce, il n'est pas encore clair quels sont les processus responsables du fractionnement isotopique de soufre dans les sédiments. En milieu marin, on évalue que ce fractionnement isotopique est le résultat de la respiration anaérobique des bactéries sulfato-réductrices qui transforment les sulfates en sulfures (Brunner *et al.*, 2005). Cependant, comme les sulfates sont présents en quantité limitée en eau douce (comparativement à ce que l'on retrouve dans les océans; Habicht *et al.*, 2002), d'autres processus pourraient s'avérer être responsables du fractionnement isotopique du soufre (voir discussion dans Croisetière *et al.*, 2009). Donc, si certaines espèces de *Chironomus* ingèrent des particules provenant de la colonne d'eau ou fraîchement déposées à l'interface eau-sédiments tandis que d'autres ingèrent des particules dans les sédiments

anoxiques, leurs valeurs de $\delta^{34}S$ devraient différer. De compte fait, S. Martin *et al.* (2008) ont mesuré des signatures isotopiques de soufre plus élevées chez *C. staegeri* que chez *C. « tigris »* confirmant que *C. staegeri* se nourrit de particules plus oxiques que *C. « tigris »*.

Les signatures isotopiques de carbone ($\delta^{13}C$) peuvent aussi servir à différencier les sources de nourriture des animaux. Tout comme le S, le ratio d'isotopes de carbone ($^{13}C/^{12}C$) chez un animal tend à être le même que dans sa nourriture (McCutchan *et al.*, 2003). Les signatures isotopiques de carbone sont couramment utilisées pour déterminer si le carbone assimilé par les animaux aquatiques provient de sources allochtone ou autochtone (Jansson *et al.*, 2007, Jones, 1992, Reynolds, 2008) ou de chaines alimentaires basées sur les algues planctoniques ou benthiques (Hecky *et al.*, 1995, Vander Zanden *et al.*, 2006). Jones *et al.* (2011) ont démontré que les signatures isotopiques de carbone pouvaient aussi être utilisées pour différencier les espèces de *Chironomus* qui se nourrissent de bactéries méthanotrophes[10] de celles qui assimilent d'autres sources de carbone. Les larves qui se nourrissent de bactéries méthanotrophes sont appauvries en ^{13}C par rapport aux autres larves, car lors de la production du méthane par les méthanogènes[11] (Coplen *et al.*, 2002) et l'assimilation du méthane par les méthanotrophes (Deines *et al.*, 2007, Eller *et al.*, 2005, Summons *et al.*, 1994), l'isotope le plus léger (^{12}C) est favorisé.

Les isotopes stables d'azote (^{14}N et ^{15}N) sont aussi grandement utilisés lors d'études en écologie pour déterminer le niveau trophique des animaux étant donné que les consommateurs sont enrichis en ^{15}N par rapport à leur diète (McCutchan *et al.*, 2003). Toutefois, bien que les *Chironomus* soient des consommateurs primaires, Grey *et al.* (2004), Jones *et al.* (2004) et Kelly *et al.* (2004) ont observé que les signatures isotopiques d'azote ($\delta^{15}N$) variaient grandement entre espèces récoltées dans un même lac. Ils ont conclu que ces différences étaient liées à une différence de comportement alimentaire (Grey *et al.*, 2004, Jones *et al.*, 2004, Kelly *et al.*, 2004).

Donc, afin d'établir s'il y a un lien entre le comportement alimentaire des larves et leurs concentrations d'éléments traces, **le 3^{e} objectif de la thèse était de déterminer le comportement alimentaire des larves de *Chironomus* en mesurant leurs ratios isotopiques de soufre, de carbone et d'azote et en observant la couleur de leur contenu stomacal.**

[10] Procaryote qui métabolise le méthane en l'oxydant comme source de carbone et d'énergie.

[11] Procaryote qui produit du méthane à partir de la réduction du dioxyde de carbone.

Puisque dans la littérature des différences de comportement alimentaire ont été reportées entre certaines espèces (M. B. Berg, 1995), nous avions comme hypothèse que les signatures isotopiques de soufre, carbone et azote ainsi que la couleur des contenus stomacaux allaient différer entre certaines espèces sympatriques de *Chironomus*.

2.4 Évaluer le potentiel d'utiliser les larves de *Chironomus* comme biomoniteurs de la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments.

Pour être de bons biomoniteurs de la contamination des sédiments, les organismes doivent accumuler les contaminants provenant des sédiments dans leurs tissus et ne pas être en mesure de maintenir constantes leurs concentrations totales de ce contaminant. Une corrélation entre les concentrations d'un contaminant chez un organisme benthique et dans les sédiments permet généralement de démontrer l'efficacité de l'organisme en tant que biomoniteur des concentrations biodisponibles du contaminant dans les sédiments (Luoma *et al.*, 2008).

Le 4^{e} objectif de la thèse était donc d'évaluer le potentiel d'utiliser les larves de *Chironomus* comme biomoniteurs de la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments en comparant les concentrations de ces éléments traces chez les larves de *Chironomus* à celles dans les sédiments et dans l'eau.

Puisque les *Chironomus* sont des organismes benthiques, notre hypothèse était que leurs concentrations d'éléments traces allaient généralement refléter celles dans les sédiments que celles dans la colonne d'eau.

2.5 Utiliser les larves de *Chironomus* comme biomoniteurs pour établir si la contamination des sédiments a changé au cours des 20 dernières années dans les lacs de Rouyn-Noranda et Sudbury.

Notre dernier objectif était d'utiliser les larves de *Chironomus* comme biomoniteur pour établir si la contamination des sédiments a changé au cours des 20 dernières années dans les lacs des régions des villes minières de Rouyn-Noranda et Sudbury. Pour ce faire, nous avons comparé les concentrations d'éléments traces chez les larves que nous avons récoltées à celles de larves de *Chironomus* qui ont été récoltées de 1987 à 1993.

L'objectif de cette section n'était pas de faire une étude exhaustive de l'état de contamination des lacs à Sudbury et à Rouyn-Noranda, mais plutôt de tester la praticité d'utiliser les larves de *Chironomus* comme biomoniteurs de sédiments.

Les villes de Rouyn-Noranda et Sudbury sont situées sur le Bouclier canadien, une région géographique caractérisée par un sol très mince recouvrant un lit de roche datant du Précambrien. Les activités minières ont débuté à Rouyn-Noranda suite à la découverte d'importants gisements de minerai de cuivre et à Sudbury suite à la découverte de minerai de cuivre et de nickel. À Rouyn-Noranda, les minerais de Cu de la région ont été extraits et traités de 1927 jusqu'à la fermeture de la mine Horne en 1976. La fonderie est restée toutefois en activité et traite maintenant des matériaux riches en métaux provenant d'un peu partout dans le monde. À Sudbury, l'extraction et le raffinage de métaux ont lieu depuis 1888. Ces activités minières sont responsables de la contamination d'un grand nombre de lacs dans les deux régions. Cependant, depuis la réduction des émissions de sulfures et de métaux dans les années 1970 et 1990 à Sudbury et dans les années 1980 à Rouyn-Noranda, l'état des lacs s'est grandement amélioré (Keller *et al.*, 2004, Keller *et al.*, 1991, Nriagu *et al.*, 1982, Nriagu *et al.*, 1998, Perceval *et al.*, 2006, Shuhaimi-Othman *et al.*, 2006).

Puisque dans les années 1980 à 1990, des travaux dans les fonderies ont permis de réduire les émissions des contaminants à Rouyn-Noranda et à Sudbury, notre hypothèse est que l'état de la contamination des sédiments dans ces régions minières s'est amélioré depuis 20 ans.

3 MÉTHODOLOGIE

Cette section décrit brièvement les campagnes d'échantillonnages ainsi que les analyses effectuées pour répondre aux objectifs de l'étude. Pour chacun des objectifs du projet, les détails précis de la méthodologie utilisée se retrouvent dans les articles faisant partie de cette thèse.

3.1 Échantillonnage

Des larves de *Chironomus,* de 4^{e} stade larvaire, ont été récoltées durant les mois de mai et juin de 2006 à 2011, dans des lacs situés au Québec (dans les régions de Québec, Rouyn-Noranda et Trois-Rivières) et en Ontario (dans la région de Sudbury), Canada. Voir tableau 1 de l'article 2 pour tous les lieux d'échantillonnage lors de la thèse. L'échantillonnage a eu lieu qu'une seule fois (dans la plupart des lacs), et ce, à un endroit précis dans les lacs afin d'éliminer toute variabilité temporelle et spatiale. Les sites d'échantillonnages dans les lacs étaient choisis en fonction de la présence de *Chironomus*. Les larves ont été récoltées à l'aide d'une benne Ekman et séparées des sédiments à l'aide d'un filet de 0,5 mm de maillage et d'un tamis de 0,7 mm de maillage. Elles ont par la suite été placées à 4^{o}C dans un sac contenant de l'eau du lac. Les larves vivantes ont été triées sous un microscope à dissection selon leur type larvaire[12], leur taille et la couleur de leur *frontoclypeus*[13]. Elles ont été placées dans des pots contenant l'eau du lac d'où elles ont été récoltées, et ce, jusqu'à ce qu'elles aient vidé leur contenu intestinal (en moyenne 4 jours). Afin d'éviter que les larves ne consomment leurs excréments, ceux-ci étaient retirés journellement. Beaucoup de ces excréments ont été conservés pour des analyses d'éléments traces. Certaines larves non dépurées ont été disséquées pour permettre l'observation de leurs contenus stomacaux. Certains de ces contenus stomacaux ont aussi été préservés pour des analyses d'éléments traces. Pour chaque site d'échantillonnage, des larves de chaque groupe morphologique ont été préservées dans de l'éthanol 94 % afin de vérifier le tri et d'identifier les espèces. Quant au reste des larves, elles ont été congelées pour des analyses d'éléments traces ou d'isotopes stables.

[12] Classification basée sur la présence de tubules abdominaux (voir tableau 3 de l'article 2)

[13] C'est une structure de la partie dorsale de la capsule céphalique (voir figure 2 de l'article 2).

Dans certains lacs, afin de mieux élucider le comportement alimentaire des larves de *Chironomus*, des larves de *Chaoborus* et de chironomidés ainsi que des oligochètes récoltés avec les larves de *Chironomus* ont aussi été préservés. Au lac Ramsey du plancton a aussi été récolté à l'aide de tirs horizontaux de filet à plancton de 64 µm de maillage. Dans la plupart des lacs, afin de relier les concentrations d'éléments trace chez les larves à celles dans l'eau et les sédiments, trois échantillons d'eau ont été récoltés à l'aide de dialyseurs et trois 'échantillons de sédiments ont été récoltés à l'aide d'une benne. Les dialyseurs étaient placés à environ 1 m au-dessous de la surface de l'eau pour au moins 3 jours. Des mesures de température et de concentration d'oxygène ont démontré que dans tous les lacs, la colonne d'eau était bien mélangée. Une fois les dialyseurs remontés à la surface, des sous-échantillons d'eau étaient immédiatement prélevés pour des mesures de pH et d'éventuelles analyses de cations, d'anions, de carbone organique et de carbone inorganique. Les étapes de préparations des dialyseurs ainsi que la récolte des échantillons d'eau pour les diverses analyses sont décrites dans les travaux Croteau *et al.* (1998) et Ponton *et al.* (2009) et résumées dans l'article 4 de cette thèse. Pour l'analyse d'éléments trace dans les sédiments, des sédiments oxiques[14] (les premiers mm de sédiments de surface identifiable par leur couleur orangée ou verdâtre) et anoxiques (les sédiments plus profonds gris) ont été grossièrement prélevés des bennes à l'aide d'une spatule en plastique. En 2010, pour des analyses de SEM-AVS, les 5 premiers cm de sédiments ont été prélevés des bennes à l'aide d'une seringue. Tous sédiments récoltés ont été mis dans des sacs Whirl-Pak qui ont été refermés tout en prenant soin de bien enlever l'air. Ils ont été insérés à l'intérieur d'un gros sac contenant des sédiments anoxiques et gardés au froid (≈4 ^{o}C), jusqu'à ce que ce soit le temps de faire les analyses (délais de temps 1 semaine à un mois).

3.2 **Identification des espèces de *Chironomus***

Comme mentionnées plus haut, les larves ont premièrement été classées, alors qu'elles étaient encore vivantes, selon leur type larvaire, leur taille et la couleur de leur *frontoclypeus*. Les capsules céphaliques et la partie postérieure de la larve (incluant le 10^{e} segment) ont été retenues pour des analyses morphologiques tandis que le reste de la larve a servi à des analyses génétiques et caryosystématiques. Tous les individus ont été étudiés

[14] Bien que ce terme n'existe pas selon l'Office québécois de la langue française, nous l'utilisons dans la thèse pour désigner la présence d'oxygène dans les sédiments.

morphologiquement. Le gène mitochondrial *cox1* des larves récoltées en 2006 et 2007 a été initialement caractérisé à l'aide de la technique PCR-RFLP. Cette technique a permis de séparer les larves en groupe selon la longueur de leurs fragments de restriction. Le gène mitochondrial *cox1* et le gène nucléaire *gb2B* ont par la suite été séquencés chez des larves représentant chaque groupe généré par l'approche *cox1* PCR-RFLP. Les premières années de l'étude, nous avons utilisé cette technique pour réduire les coûts liés à l'analyse de brins d'ADN d'un grand nombre d'échantillons. Cependant, puisque les coûts reliés au séquençage étaient peu dispendieux, nous avons réalisé qu'il était plus rentable et moins fastidieux de séquencer directement les gènes amplifiés. C'est donc pour cette raison que les gènes *cox1* et *gb2β* des larves récoltées en 2009, 2010 et 2011 ont été directement séquencés. Les larves ont été séparées au niveau de l'espèce grâce aux analyses morphologiques et génétiques. L'identification des espèces s'est faite grâce à la comparaison des séquences d'ADN des larves récoltées avec celles de spécimens de référence et/ou l'analyse des chromosomes polytènes.

3.2.1 Analyses morphologiques

La classification des types larvaires est basée sur la présence/absence, la longueur et la forme de tubules ventraux et latéraux. Puisque dans la littérature, les définitions des types de larves différaient et étaient parfois même contradictoires (Andersen, 1949, Harnisch, 1942, Lindeberg *et al.*, 1979, Shobanov, 2002, Shobanov *et al.*, 1996), nous avons redéfini la classification des types de larves (tableau 3 de l'article 2). Les détails concernant la redéfinition des types de larves sont présentés dans la section méthodologie de l'article 2.

La longueur des larves de *Chironomus* et de leurs tubules ventraux et latéraux ainsi que la largeur de leur capsule céphalique (à la hauteur des tâches oculaires) ont été mesurées à l'aide d'un microscope à dissection, d'une caméra et d'un programme de traitement d'image. Les larves ont été étêtées et leur capsule céphalique a été disséquée et montée sur lame. Brièvement, les capsules céphaliques étaient coupées latéralement de façon à séparer la partie ventrale de la partie dorsale. Par la suite, les mandibules et le labre étaient respectivement détachés de la partie dorsale de la capsule céphalique avant que le tout soit monté sur lame dans du baume du Canada. Il est possible d'obtenir des montages de têtes de *Chironomus* plus clairs, en trempant préalablement les têtes dans de l'hydroxyde de potassium. Voir Bertrand (2012) pour de plus amples détails concernant la digestion des têtes de *Chironomus*. Cependant, pour des raisons pratico-pratiques, la plupart des têtes montées sur lame lors de la thèse n'ont pas été préalablement digérées. À l'aide d'un microscope à dissection, la couleur du

frontoclypeus et du *gula* ainsi que la texture de la limite antérieure des plaques paralabiales des larves ont été décrites. Le mentum, les mandibules et le *pecten epipharyngis* des larves ont été classés par type selon la forme, la coloration et la structure de leurs dents. Nous avons redéfini la classification des types de mentum et de mandibules (voir article 2) afin de mieux englober les variations morphologiques des dents que nous observions chez nos larves.

3.2.2 Analyses génétiques

3.2.2.1 Extraction de l'ADN

Nos larves étant de bonnes tailles, seulement 0,5 à 1,0 cm de longueur de corps était nécessaire pour l'extraction de l'ADN. L'extraction de l'ADN des larves s'est faite selon la méthodologie présentée dans Carew *et al.* (2003) et résumée dans l'article 2.

3.2.2.2 Amplification des gènes cox1 et gb2β

Brièvement, nous avons amplifié les séquences *cox1* et *gb2β* des larves grâce à la technique de réaction en chaine par polymérase (PCR, sigle du terme anglais *polymerase chain reaction*); une technique basée sur une répétition de cycles de transition de température. Cette technique permet de dupliquer en grand nombre un brin d'ADN spécifique à partir d'une faible quantité d'ADN et d'une solution tampon contenant des amorces, des acides nucléiques et de l'ADN polymérase[15]. La procédure d'amplification est décrite en détail dans l'article 2. Pour toutes les larves, l'amplification de la séquence *cox1* s'est faite aisément à l'aide des amorces 911 et 912 (tableau 2 de l'article 2). Tandis que l'amplification de la séquence *gb2β* a été beaucoup plus problématique que prévu. Bien que diverses amorces aient été testées (tableau 2 de l'article 2), la séquence *gb2β* n'a pu être amplifiée chez certaines espèces.

3.2.2.3 Analyse RFLP du gène cox1

Basée sur les publications de Carew *et al.* (2003), Sharley *et al.* (2004) et Carew *et al.* (2007), les enzymes de restrictions suivantes ont été utilisées pour fragmenter les amplicons de *cox1* : *Alu I*, *Hha I*, *Hinf I*, *Rsa I*, *Ssp I* et *Taq I*. La procédure pour fragmenter l'ADN à l'aide des enzymes de restriction est décrite dans l'article 2. Afin de vérifier les résultats de cette technique génétique, nous avons simulé la digestion de nos fragments d'ADN à l'aide du

[15] Enzyme permettant la duplication de l'ADN.

programme NEBCutter V2.0 de New England BioLabs (http://tools.neb.com/NEBcutter2/). Les simulations ont permis de démontrer la véracité de la technique (tableau 4 de l'article 2).

3.2.2.4 Analyse des séquences d'ADN

Les produits d'amplifications des gènes *cox1* et *gb2β* ont été purifiés et séquencés dans les deux directions à l'aide des amorces utilisées pour leur amplification. Le séquençage des amplicons dans les deux directions a été fait par la compagnie Macrogen à Séoul en Corée et par le centre de recherche du Centre hospitalier universitaire du Québec (Québec, Canada) à l'aide de l'analyseur d'ADN ABI3730 XL (Applied Biosystems). Les séquences du gène *cox1* ont été alignées à l'aide du programme ClustalW (Thompson *et al.*, 1994). En raison de la présence d'introns[16], les séquences du gène *gb2β* ont été alignées manuellement selon l'alignement du gène présenté dans Hankeln *et al.* (1997). Les séquences ont été analysées à l'aide du programme Mega 5.05 (Tamura *et al.*, 2011). Des arbres d'identification basés sur les gènes *cox1* et *gb2β* ont été construits à l'aide du l'algorithme Neighbor-Joining (Saitou *et al.*, 1987). La divergence des séquences a été calculée à l'aide du modèle Kimura 2-parameter (Kimura, 1980). Pour de plus amples détails, consulter l'article 2.

3.2.3 Analyses caryosystématiques

Les glandes salivaires des *Chironomus* ont été disséquées et les chromosomes polytènes ont été montés sur lame selon la méthode de coloration utilisant de l'orcéine et de l'acide acétique décrite dans J. Martin *et al.* (2006). Les analyses cytologiques ont été réalisées par Veronika Golygina de l'*Institute of Cytology and Genetics* (Novossibirsk, Russie) pour la séparation des larves de *C. plumosus* et *C. entis* et par Jon Martin de l'université de Melbourne (Melbourne, Australie) pour l'identification des autres espèces.

3.3 Analyses chimiques

Pour toutes les analyses chimiques, des précautions ont été prises afin de ne pas contaminer les échantillons. Les risques de contamination étant particulièrement élevés lors d'analyse d'éléments traces, la digestion et l'analyse d'échantillons se sont déroulées sous hotte à flux laminaire et en salle blanche. Tous les matériaux utilisés lors de ces analyses ont été

[16] Fragment non codant d'un gène, situé entre deux exons et qui ne se retrouve pas dans l'ARN cytoplasmique final.

préalablement trempés dans de l'acide nitrique 15 % (v/v) pendant 3 jours, rincés 7 fois à l'eau déminéralisée ultra-pure (18 MΩ cm) et séchés sous hotte à flux laminaire.

Lors des analyses chimiques énumérées ci-dessous, la qualité des résultats a été vérifiée par la réplication d'analyses d'échantillons et par l'analyse de blancs analytiques, de blanc de digestion, d'échantillons dosés, d'étalons analytiques, d'échantillons analytiques certifiés et de matériaux de digestion certifiés.

Les mesures obtenues par les analyses chimiques ont été analysées statistiquement à l'aide du programme SigmaPlot 11.0.

3.3.1 Mesures d'isotopes stables de soufre, carbone et azote

Les échantillons d'invertébrés, de plancton et de sédiments ont été lyophilisés, broyés, pesés et introduits dans une capsule d'étain. Pour les analyses d'isotopes stables de soufre, du pentoxyde de vanadium a aussi été ajouté aux échantillons afin de favoriser la combustion. Les échantillons ont été envoyés à Iso-Analytical ltée (Cheshire, Angleterre) où les isotopes ont été mesurés à l'aide d'un analyseur élémentaire couplé à un spectromètre de masse (EA-IRMS; Element Analysis-Isotope Ratio Mass Spectrometry). Pour de plus amples détails concernant la préparation d'échantillons pour les analyses d'isotopes stables, consulter l'article 3. Les résultats d'analyse ont été rapportés sous forme de rapports isotopiques selon l'équation suivante :

$$\delta x‰ = \left(\frac{R_{\text{échantillon}}}{R_{\text{matériel de référence}}} - 1\right) \times 1000 \qquad (2)$$

où $x = {}^{13}C, {}^{15}N$ ou ${}^{34}S$

$R = \frac{{}^{13}C}{{}^{12}C}, \frac{{}^{15}N}{{}^{14}N}$ ou $\frac{{}^{34}S}{{}^{32}S}$

Les matériaux de références utilisés pour l'expression des résultats sont le Vienna-Canyon Diablo Troilite (V-CDT; météorite qui a formé le cratère *Meteor Crater* en Arizona au États-Unis) pour le soufre, le Vienna-PeeDee Belemnite (V-PDB; coquille de *Belemnitella americana* du site fossilifère de Pee Dee en Caroline du Sud aux États-Unis) pour le carbone et l'air ambiant pour l'azote.

3.3.2 Mesures d'éléments traces chez les larves

Lors de cette thèse, les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn ont été mesurées chez les larves. Les larves de *Chironomus* ont été lyophilisées et pesées avant d'être digérées à température ambiante dans de l'acide nitrique (≈100 µL par mg de tissu sec) pendant 3 jours et du peroxyde d'hydrogène (≈40 µL par mg de tissu sec) pendant 5 jours. À la fin de la période de digestion, de l'eau déminéralisée ultra-pure a été ajoutée jusqu'à l'obtention d'un volume final de 1 ml par mg de tissu sec. Ces échantillons ont par la suite été analysés par spectrométrie de masse couplée à un plasma inductif (ICP-MS).

3.3.3 Mesures dans la colonne d'eau

Les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dissoutes ont été mesurées dans l'eau par ICP-MS. Cependant, n'avons pas été capable de mesurer les concentrations de Se dissouste par ICP-MS en raison d'interférence avec le Br. Les mesures de Se totale dissoutes ont été prises de Ponton *et al.* (2009) qui ont utilisé une autre méthode d'analyse afin de mesurer le Se dans nos échantillons d'eau. Les concentrations de Se organique, de sélénite et de séléniate ont aussi été prises de cette publication.

Les concentrations d'ions libres de Ba, Cd, Co, Cu, Mn, Ni et Zn ont été estimées à partir du modèle WHAM 7.1 et nos mesures des variables suivantes : le pH et les concentrations dissoutes de cations (Al, Ca, Ba, Cd, Co, Cu, Fe, K, Mg, Mn, Na, Ni et Zn), d'anions (SO_4^{2-}, NO_3^{2-} et Cl), de carbone inorganique et d'acides fulviques. Les cations majeurs (Al, Ca, Fe, K, Mg, Mn et Na) ont été mesurés par spectrométrie d'émission atomique couplée à un plasma inductif (ICP-AES) tandis que, comme mentionné plus haut, les autres cations (Ba, Cd, Co, Cu, Ni et Zn) ont été mesurés par ICP-MS. Les anions ont été mesurés par chromatographie ionique, le carbone inorganique a été mesuré par chromatographie en phase gazeuse et l'acide fulvique a été estimé à partir des concentrations de carbone organique dissous. Le carbone organique dissous a été mesuré par combustion sur un analyseur Shimatzu. Pour de plus amples détails, consulter l'article 4.

3.3.4 Mesures dans les sédiments, les excréments et les contenus stomacaux

3.3.4.1 Éléments traces

Les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn ont été mesurées dans les sédiments oxiques et anoxiques ainsi que dans les excréments et contenus stomacaux des larves. Les

échantillons ont été séchés et digérés partiellement selon la méthode de digestion décrite pour les larves de façon à ne pas libérer des éléments traces fortement liés et potentiellement non disponibles. Suite à la digestion, les échantillons ont été centrifugés (à 7 000 rpm pour 3-5 minutes) et les mesures d'éléments traces ont été faites dans le surnageant. Les mesures d'As, Cd, Co et Se ont été faites par ICP-MS tandis que les mesures de Ba, Cu, Mn, Ni et Zn ont été faites par ICP-AES.

3.3.4.2 Fraction de carbone organique total

Les sédiments séchés ont été digérés dans de l'acide sulfurique. Le mélange a été agité afin de permettre l'évaporation des carbonates et a, par la suite, été filtré. Le % de carbone organique total a été mesuré sur ce qui a été retenu par le filtre à l'aide de l'analyseur élémentaire CHN.

3.3.4.3 [SEM]-[AVS]

Les concentrations de SEM et d'AVS ont été mesurées dans des sédiments récoltés en 2010. Brièvement, pour les mesures d'AVS, les sulfures dans les échantillons de sédiments ont été convertis en sulfure d'hydrogène gazeux (H_2S) à la suite de la digestion de ces échantillons encore humides dans de l'acide chlorhydrique. Le gaz a été piégé dans une solution d'hydroxyde de sodium et mesuré par spectrophotométrie. Après la libération du H_2S, la solution de digestion a été filtrée et les métaux (par définition les SEM; Ag, Cd, Cu, Ni, Pb et Zn) dans cette solution finale ont été mesurés par ICP-AES. Pour de plus amples détails, consulter l'article 4.

4 RÉSULTATS/DISCUSSION

Cette section comporte un résumé des résultats et des discussions qui ont été présentés dans les articles 2, 3 et 4. Cette section comporte aussi des observations et des résultats d'analyses obtenus lors de la thèse, mais qui n'ont pas été publiés.

4.1 Identification des espèces de *Chironomus*

Le processus et les résultats d'identification de nos espèces ont été publiés dans l'article 2. Nous avons effectué des analyses morphologiques et génétiques sur un total de 404 larves de *Chironomus*. Ces analyses suggèrent que nous avons récolté 17 espèces de *Chironomus*. Parmi ces 17 espèces, nous avons pu en identifier 14 grâce au barcoding moléculaire et à des analyses chromosomiques. Les 14 espèces que nous avons identifiées sont *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. entis*, *C. frommeri*, *C. harpi*, *C. maturus*, *C.* nr. *atroviridis* sp. 2i, *C. ochreatus*, *C. plumosus*, *C. staegeri* et *C. « tigris »*. Trois autres espèces non identifiées ont été étiquetées *C*. sp. NAI, *C*. sp. NAII, *C*. sp. NAIII. Certaines espèces de *Chironomus* ont été récoltées dans plus d'un lac (*C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. entis*, *C. plumosus*, *C*. sp. NAIII, *C. staegeri* and *C. « tigris »*) tandis que d'autres ont été récoltées qu'à un seul endroit (*C. cucini*, *C. dilutus*, *C. frommeri*, *C. harpi*, *C. maturus*, *C*. nr. *atroviridis* sp. 2i, *C. ochreatus*, *C*. sp. NAI and *C*. sp. NAI). Toutes les espèces ont été retrouvées en compagnie d'autres espèces dans au moins un lac à l'exception de *C. maturus* et *C. frommeri* (tableau 7, article 2). Parmi les 14 espèces que nous avons récoltées et identifiées, 11 d'entre-elles (*C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. entis*, *C. maturus*, *C*. nr. *atroviridis* (sp. 2i), *C. plumosus*, *C. staegeri* et *C. « tigris »*) figurent parmi les 20 espèces de *Chironomus* répertoriées au Canada à l'est des Rocheuses (J. Martin, 2013). Les trois autres espèces de *Chironomus* (*C. frommeri*, *C. harpi* et *C. ochreatus*) que nous avons identifiées ont déjà été répertoriées aux États-Unis, mais pas au Canada.

La morphologie des larves, la génétique et la cytologie des espèces de *Chironomus* récoltées sont décrites en détail dans l'article 2. Le poids et la longueur des larves de 4^{e} stade larvaire de chaque espèce récoltée sont présentés à la figure 4-1. Les larves de plus grande taille étaient généralement celles de *C. dilutus*, *C. entis* et *C. plumosus*. Cependant, il est à noter que la longueur et le poids des larves de certaines espèces dont celles de *C. plumosus* varient

grandement entre lacs. Nos larves figurent parmi les plus grosses au monde (selon une discussion avec Jon Martin, expert en taxonomie des *Chironomus*).

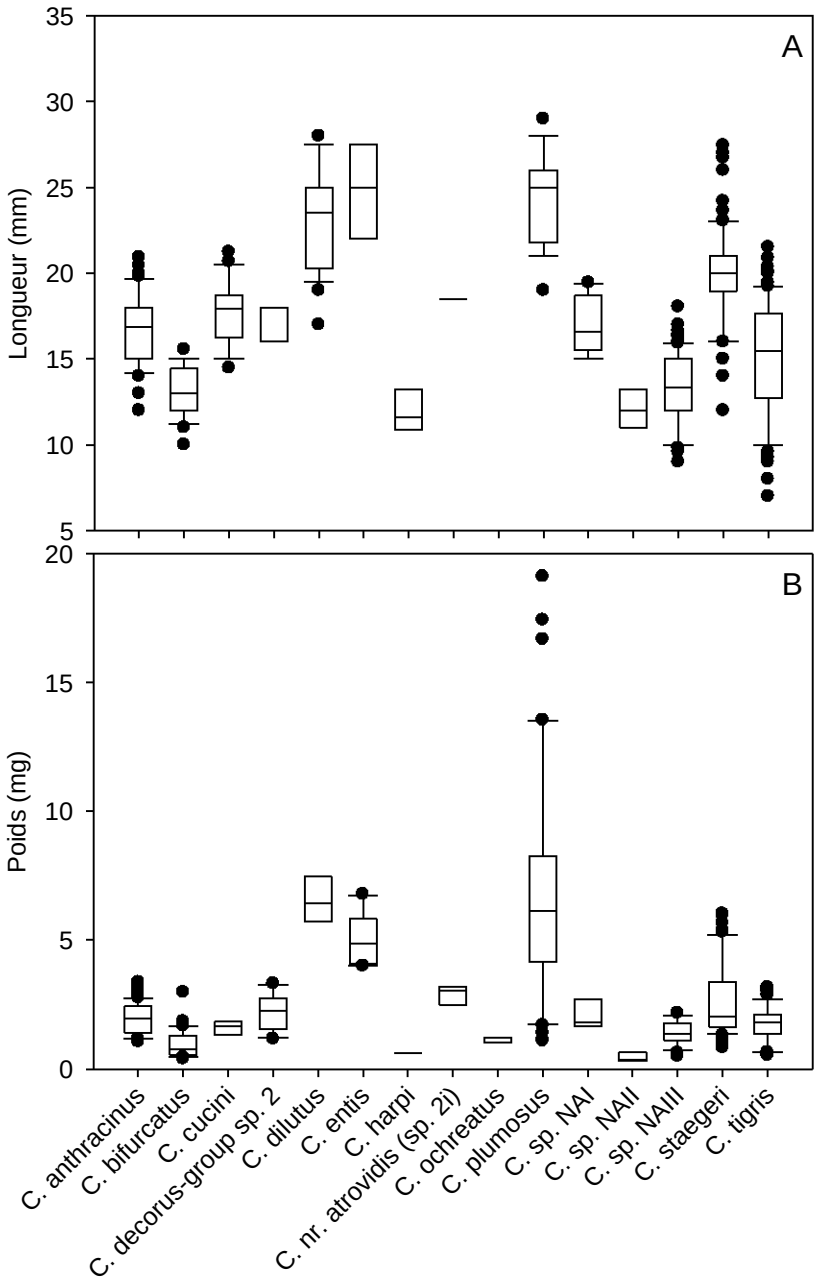


Figure 4-1 **Box plot de la longueur (A) (mm, n=2-97) et le poids sec (B) (mm, n=1-61) des larves de *Chironomus* récolées lors de la thèse.**
La médiane, le 25^{e} et 75^{e} percentile, le 10^{e} et 90^{e} percentile et les points aberrants sont représentés respectivement par une barre horizontale, les extrémités inférieures et supérieures des boîtes, les barres d'erreur et les points. Les 25^{e} et 75^{e} percentiles ne sont pas indiqués pour les espèces où n<3. De plus, les 10^{e} et 90^{e} percentiles ne sont pas indiqués pour les espèces où n<9.

Un détail que nous n'avons pas mentionné dans le 2e article est que certaines larves, morphologiquement identiques, différaient de couleur. Certaines étaient rouge orangé alors que d'autres étaient d'une couleur rouge vif. Notre hypothèse de départ était que ces larves représentaient des espèces différentes. Cependant, cette hypothèse s'est révélée fausse. Nous avons observé que certaines larves de la même espèce (p. ex. *C. staegeri*) pouvaient différer de teinte de rouge, et ce, même si elles étaient récoltées au même endroit. La coloration des larves n'est donc pas un bon critère pour séparer les larves de *Chironomus*.

En taxonomie il n'existe aucun consensus quant à la définition d'une espèce (Agapow *et al.*, 2004, Hausdorf, 2011). Le statut d'espèce est souvent établi selon un ensemble de preuves. Aucune des trois approches (morphologie, cytologie ou ADN) utilisées lors de l'étude n'a été capable de délimiter toutes les espèces de *Chironomus* récoltées. Le tableau 4-1 présente un résumé des cas où les approches morphologie, génétique ou cytologique ne peuvent être utilisées pour distinguer certains groupes d'espèces. Pour de plus amples détails, voir l'article 2.

Tableau 4-1 Résumé des cas où les approches morphologique, génétique (séquences *cox1* et *gb2β*) ou cytologique (chromosomes polytènes) ne peuvent être utilisées pour différencier certains groupes d'espèces.

	Morphologie des larves*	Séquences *cox1* et *gb2β*	Chromosomes polytènes
C. anthracinus	Identiques aux larves de *C*. sp. NAI	Séquences *gb2β* identiques à celles de *C*. sp. NAI	Similaires à ceux de *C*. sp. NAI
C. bifurcatus	Identiques aux larves de *C. decorus*-group sp. 2. Potentiellement aussi très similaires aux larves d'autres espèces faisant partie du groupe cytologique *decorus* (ex. *C. quinnitukqut*)		
C. cucini	Similaires aux larves de *C*. sp. NAII et *C*. sp. NAIII. Similaires aussi à celles de *C. atribia* et *C. major*, des espèces non récoltées lors de cette étude.		
C. decorus-group sp.2	Identiques aux larves de *C. bifurcatus.* Potentiellement aussi très similaires aux larves d'autres espèces faisant partie du groupe cytologique *decorus* (ex. *C. quinnitukqut*)		
C. dilutus		Séquences *cox1* identiques à celles de *C. pallidivittatus*	
C. entis	Identiques aux larves de *C. plumosus*	Séquences *cox1* identiques à celles de *C. plumosus*	
C. frommeri	Identiques aux larves de *C. staegeri* et *C. crassicaudatus* (espèce non récoltée lors de cette étude)	Séquences *cox1* similaires à celles de *C. staegeri* et *C. « tigris »*	
C. harpi			
C. maturus			
C. nr. *atroviridis (*sp. 2i)		Séquences identiques à celles de *C. usenicus* (espèce non récoltée lors de cette étude)	
C. ochreatus			

(suite du tableau 4-1)

	Morphologie des larves*	Séquences *cox1* et *gb2β*	Chromosomes polytènes
C. plumosus	Identiques aux larves de *C. entis*	Séquences *cox1* identiques à celles de *C. entis*	
C. sp. NAI	Identiques aux larves de *C. anthracinus*	Séquences *cox1* identiques à celles de *C. anthracinus*	Similaires à ceux de *C. anthracinus*
C. sp. NAII	Similaires aux larves de *C*. sp. NAIII et *C. cucini*. Similaires aussi à celles de *C. atribia* et *C. major*, des espèces non récoltées lors de cette étude.		
C. sp. NAIII	Similaires aux larves de *C*. sp. NAII et *C. cucini*. Similaires aussi à celles de *C. atribia* et *C. major*, des espèces qui n'ont pas été récoltées lors de cette étude.		
C. staegeri	Identiques aux larves de *C. frommeri* et *C. crassicaudatus* (espèce non récoltée lors de cette étude)	Séquences *cox1* similaires à celles de *C. frommeri* et *C. « tigris »*	
C. « tigris »		Séquences *cox1* similaires à celles de *C. frommeri* et *C. staegeri*	

* Selon les traits morphologiques que nous avons observés.

Brièvement, selon les traits morphologiques que nous avons observés, il est impossible de différentier les larves de *C. cucini* et *C*. sp. NAIII, *C. bifurcatus* et *C. decorus*-group sp. 2, *C. anthracinus* et *C*. sp. NAI, *C. staegeri* et *C. frommeri* ainsi que celles de *C. entis* et *C. plumosus*.

De plus, nos résultats démontrent que le gène *cox1* ne peut être utilisé pour séparer les larves de *C. entis* et *C. plumosus* ainsi que celles de *C. dilutus* et *C. pallidivittatus*, car ces paires d'espèces possèdent la même séquence *cox1*. Une information que nous avons omis de mentionner dans l'article est que dans la région paléarctique, Polukonova *et al.* (2009) a aussi démontré que les séquences *cox1* de *C. usenicus* sont identique celles de *C. plumosus*. De plus, après avoir publié notre article sur l'identification des espèces de *Chironomus*, le coauteur Jon Martin a découvert que *C.* nr. *atroviridis* (sp. 2i) partageaient aussi la même séquence *cox1* (à deux nucléotides près) que celle de *C. bitumineus* (séquence non publiée) et ce, bien que les deux espèces soient très distinctes au niveau de leurs chromosomes (*C. bitumineus* n'a que 3 chromosomes alors que *C*. nr. *atroviridis* (sp. 2i) en possède 4). Bref, ces résultats démontrent qu'il n'est pas toujours possible de séparer les espèces de *Chironomus* à l'aide de la séquence standard en barcoding moléculaire (*cox1*). Un autre brin d'ADN couramment utilisé en taxonomie moléculaire est celui du gène mitochondrial *cytochrome b* (séquence *cytb*) (p. ex. Carew *et al.*, 2013). Cependant, comme pour les séquences *cox1*, les séquences *cytb* de *C. entis* et *C. plumosus* ainsi que celles de *C. dilutus* et *C. pallidivittatus* sont identiques (données non publiées, V. P. Guryev *et al.*, 2002, J. Martin *et al.*, 2002). Ce partage des séquences mitochondriales entre espèces de *Chironomus* serait dû à un transfert horizontal de gènes mitochondriaux lors de croisement entre espèces sympatriques (introgression) (V. P. Guryev *et al.*, 2002, J. Martin *et al.*, 2002). Selon nos résultats, les larves de *C. entis* et *C. plumosus* ainsi que celles de *C. dilutus* et *C. pallidivittatus* peuvent toutefois être séparées à l'aide de la séquence nucléaire *gb2β*.

Puisque le génome nucléaire et le génome mitochondrial diffèrent conformément à leur mode de transmission[17] et leur taux de mutation (Freeland, 2005), l'utilisation de brins d'ADN provenant de génomes différant s'avère parfois un bon outil pour séparer les espèces. L'utilisation de plus d'un gène est de plus en plus monnaie courante lors d'étude en taxonomie moléculaire (ex. Carew *et al.*, 2009, Li *et al.*, 2010). Un avantage d'utiliser l'ADN du génome nucléaire est qu'il est généralement moins affecté par le phénomène d'introgression que celui

[17] À quelques exceptions près, l'ADN mitochondrial n'est transmis que par la mère tandis que l'ADN nucléaire est hérité des deux parents (Freeland, 2005)

du génome mitochondrial (J. Martin, 2011). Cependant, nous avons découvert que le désavantage d'utiliser la séquence *gb2β* est que l'alignement des séquences est compliqué en raison de la présence d'introns chez certaines espèces. De plus, l'amplification des séquences est ardue. Aucune des paires d'amorces que nous avons testées ne sont universelles. Aucune n'a été capable d'amplifier la séquence *gb2β* de toutes les espèces de *Chironomus*. En fait, pour certaines espèces (*C. decorus*-group sp. 2, *C. frommeri*, *C*. nr. *atroviridis* sp. 2i, C*. ochreatus*, *C*. sp. NAII et *C. staegeri*), nous n'avons pas été capables d'obtenir des produits d'amplification. Il est à noter, qu'après que l'article a été publié, Jon Martin a réussi à séquencer le gène *gb2β* de *C*. nr. *atroviridis* sp. 2i à l'aide des amorces wyk1B et 2*β*–A. Finalement, puisque les gènes *globine 7A* (*gb7A*) et *globine 9* (*gb9*) ont des séquences similaires à celle de *gb2β*, nous avons obtenu à quelques reprises des produits d'amplification des gènes *gb7A* et *gb9* alors que nous cherchions à obtenir un produit d'amplification du gène *gb2β*. Ceci dit, bien que la séquence *gb2β* ait été utile pour séparer et identifier certaines espèces de *Chironomus*, nous recommandons à l'avenir que d'autres brins d'ADN nucléaire soient explorés pour séparer les espèces de *Chironomus*.

Lors de notre étude, l'analyse des chromosomes polythènes a permis d'identifier ou de confirmer l'identification de *C. anthracinus*, *C. bifurcatus*, *C. decorus*-group sp. 2i, *C. cucini*, *C. entis*, *C. frommeri*, *C*. nr. *atroviridis* sp. 2i, *C. ochreatus*, *C. staegeri* et *C. « tigris »*. Cependant, comme c'est le cas pour les autres techniques, ce n'est pas toujours simple d'établir si certaines différences de profils de bandes chromosomiques ou d'autres structures sont attribuables à des différences intraspécifiques ou interspécifiques (p. ex. *C*. sp. NAI et *C. anthracinus*, voir discussion dans l'article 2). Ceci dit, la plupart de nos conclusions cytologiques ont été confirmées par barcoding moléculaire.

Jusqu'à récemment, l'identification des espèces de *Chironomus* n'était possible que par l'analyse de leurs chromosomes et seul un nombre limité de personne mondialement était capable de séparer et identifier les larves au niveau de l'espèce à l'aide de cette méthode. Cependant, avec l'arrivée des barcodes moléculaires, nous croyons qu'il va devenir de plus en plus facile et faisable pour des non-experts en cytologie de séparer et d'identifier des larves de *Chironomus* au niveau de l'espèce. Nos résultats démontrent qu'il est possible de correctement séparer les espèces de *Chironomus* selon leur morphologie en combinaison avec leurs séquences *cox1* et *gb2β*.

Afin d'aider ceux et celles qui voudraient identifier des larves de *Chironomus* au niveau de l'espèce en Amérique du Nord, nous avons publié une clé permettant de séparer

morphologiquement les larves de la plupart des espèces que nous avons récoltées. Dans cette clé, nous utilisons des traits morphologiques simples et faciles à observer pour séparer les larves. Nous avons redéfini les classifications des types larvaires, des types de mentum et des types de mandibules afin qu'elles soient plus claires et faciles à utiliser. Toutes nos séquences *cox1* et *gb2β* ont été publiées. Il est à noter que selon nos résultats, deux individus sont membre d'espèces différentes si leurs séquences *cox1* divergent de plus de 3 % ou que leurs séquences *gb2β* divergent de plus de 2 %. Si les divergences sont inférieures à ces seuils, d'autres preuves sont nécessaires pour établir si les individus sont membres ou non de la même espèce.

4.2 **Concentrations d'éléments traces chez des larves d'espèces sympatriques de *Chironomus***

Nous avons mesuré les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn chez les larves de 4^{e} stade larvaire de toutes les espèces de *Chironomus* récoltées à l'exception de *C. maturus* et *C. frommeri*. Dans les figures 3 et 4 de l'article 4, nous présentons les mesures d'éléments trace dans les larves des lacs où plus d'une espèce a été retrouvée. Les mesures de concentrations d'éléments traces chez les larves dans les lacs où il n'y avait qu'une espèce sont présentées dans les tableaux S4-S12 de l'article 4.

En accord avec notre hypothèse, nous avons mesuré des différences des concentrations de certaines éléments traces entre espèces sympatriques de *Chironomus*. Pour ce qui est du Cd, nous avons mesuré des différences de concentrations de Cd énormes entre espèces de *Chironomus* récoltées au même site (ex. 3-520 nmol/g au lac Marlon). Fait intéressant, d'un lac à l'autre, certaines espèces avaient toujours des concentrations de Cd plus élevées que d'autres. Ces espèces sont *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri*. Tandis que celles dont les concentrations étaient plus basses sont *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C. ochreatus*, *C*. nr. *atroviridis* (sp. 2i), *C*. sp. NAI, *C*. sp. NAII, *C*. sp. NAIII et *C*. « *tigris* ». Dans certains lacs, les concentrations de Zn chez *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri* étaient aussi plus élevées que chez les autres espèces. Cependant, dans d'autres lacs, il n'y avait pas de différences de concentrations de Zn entre ces deux groupes d'espèces. Nous avons aussi mesuré des différences de concentrations de Se entre espèces de *Chironomus* vivant au même endroit. Cependant, la tendance était contraire au Cd et au Zn. Dans tous les lacs, ce sont les larves de *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri* qui avaient des concentrations de Se plus basses comparativement aux autres.

Généralement, les concentrations des autres éléments traces (As, Ba, Co, Cu, Mn et Ni) ne différaient pas entre espèces de *Chironomus* vivant au même endroit. Il faut dire que dans certains lacs, il y avait des différences, mais aucune espèce n'avait constamment des concentrations plus élevées que les autres. Nos résultats corroborent ceux de S. Martin *et al.* (2008) dont les concentrations de Cd et Zn étaient plus élevées chez *C. staegeri* que chez *C. « tigris »* mais dont les concentrations de Cu ne différaient pas entre ces deux espèces.

Nos résultats démontrent qu'il n'est pas toujours justifiable de regrouper les espèces de *Chironomus* lors d'analyses de contaminants. En effet, à un site donné, les concentrations de Cd étaient de 3 à 108 fois plus élevées chez certaines espèces que chez d'autres. Donc, la comparaison des concentrations de Cd chez les larves de *Chironomus* entre sites pourrait être grandement erronée si les espèces étaient regroupées. Nos résultats démontrent aussi que les concentrations de Se et de Zn diffèrent entre espèces sympatriques de *Chironomus*. Toutefois, étant donné que les concentrations de Se et de Zn différaient toujours que d'un facteur d'environ 2, la séparation d'espèces pour les analyses de ces éléments traces n'est peut être pas aussi cruciale que pour le Cd. Puisque les concentrations d'As, Ba, Co, Cu, Mn et Ni ne différaient pas entre espèces sympatriques, nos résultats suggèrent que dans nos régions d'échantillonnage, les espèces peuvent être regroupées lors d'analyses de ces contaminants.

4.3 **Comportement alimentaire des larves des différentes espèces de *Chironomus***

Afin de déterminer si le comportement alimentaire des larves diffère, nous avons mesuré leurs ratios isotopiques de soufre, de carbone et d'azote. Les résultats de ces analyses sont présentés dans l'article 3.

Dans cet article, les valeurs de $\delta^{34}S$ chez les espèces de *Chironomus* sont présentées à la figure 2. En accord avec notre hypothèse, les valeurs de $\delta^{34}S$ diffèrent entre espèces sympatriques de *Chironomus*. D'un lac à l'autre, *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri* avaient des signatures isotopiques de soufre plus élevées que celles de *C. anthracinus*, *C. bifurcatus*, *C. cucini*, C*. decorus*-group sp. 2, *C.* sp. NAI, *C*. sp. NAII, *C*. sp. NAIII et *C. « tigris »*. Ces résultats indiquent que dans les lacs, les larves de *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri* se nourrissent de particules plus oxiques comparativement aux autres espèces que nous avons récoltées. Nos mesures corroborent ceux d'autres études qui ont trouvé que les valeurs de $\delta^{34}S$ étaient plus élevées chez les larves de *C. staegeri* que chez celles de *C. « tigris*

» (S. Martin *et al.*, 2008) et plus élevées chez les larves de *C. plumosus* que chez celles de *C. anthracinus* (Grey *et al.*, 2005).

Les particules qu'ingèrent les larves de *Chironomus* viennent soit de la colonne d'eau, des sédiments oxiques ou des sédiments anoxiques. Afin de déterminer avec plus de précision ce qu'ingèrent les larves, nous avons comparé leurs valeurs de $\delta^{34}S$ à celles d'autres organismes, dont des *Chaoborus* et des Tubuficidae et à celles des sédiments oxiques et anoxiques récoltés aux mêmes sites (figure 3 de l'article 3). Les *Chaoborus* sont des organismes planctonivores tandis que les Tubuficidae sont des organismes benthiques fouisseurs. Selon McCall *et al.* (1982), les Tubuficidae se nourrissent de sédiments anoxiques à des profondeurs de 2 à 8 cm. Les valeurs de $\delta^{34}S$ chez le groupe de larves de *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C.* sp. NAI, *C*. sp. NAII, *C*. sp. NAIII et *C. « tigris »* étaient similaires à celles de Tubuficidae et beaucoup plus basses que celles de *Chaoborus* indiquant que les larves de ces espèces ingèrent des particules plutôt anoxiques. D'un autre côté, dans certains lacs, les valeurs de $\delta^{34}S$ chez *C. dilutus*, *C. entis*, *C. plumosus* et C. *staegeri* n'étaient pas statistiquement différentes de celles de *Chaoborus* indiquant que ces larves assimilent des particules oxiques provenant soit de la colonne d'eau ou de la couche oxygénée des sédiments. Il est à noter que dans d'autres lacs, les valeurs de $\delta^{34}S$ chez *C. dilutus*, *C. entis*, *C. plumosus* et C. *staegeri* étaient un peu plus basses que celles de *Chaoborus*. Ceci pourrait être dû au fait qu'à l'occasion, en se nourrissant à la surface des sédiments, les larves ingèrent un peu de sédiments anoxiques. Tel qu'anticipé, les valeurs de $\delta^{34}S$ étaient plus élevées dans les sédiments oxiques qu'anoxiques. Cependant, contrairement à nos attentes, les signatures isotopiques de soufre chez les larves de *Chironomus* n'étaient pas nécessairement les mêmes que dans la couche de sédiment dans laquelle elles se nourrissent. À l'exception de seulement deux lacs (Saint-Joseph et Duprat), les espèces de *Chironomus* avaient des signatures isotopiques plus élevées que les sédiments oxiques et anoxiques d'où elles ont été récoltées. Ces mesures suggèrent que les larves de *Chironomus* ne se nourrissent pas de sédiments en vrac, mais sélectionnent les particules de sédiments qu'elles ingèrent.

Afin de confirmer nos conclusions, nous avons disséqué certaines larves afin de voir si la couleur de leur contenu stomacal différait entre espèces. Au lac Crooked, nous avons noté que la couleur du contenu stomacal des larves de *C. staegeri* était brun orangé comme celle des sédiments oxiques tandis que celle des larves de type bathophilus[18] était plus gris et ne

[18] Nous n'avons pu identifier ces larves.

contenait pas d'orangé comme celle des sédiments anoxiques. Ces observations confirment nos conclusions que *C. staegeri* ingère des particules oxiques (dans ce cas-ci des sédiments) tandis que les autres larves de type bathophilus (possiblement *C. bifurcatus* ou *C. decorus*-group sp. 2i) ingèrent des sédiments anoxiques. Cependant, règle générale, nous n'avons pas trouvé évident de déduire le comportement alimentaire des larves selon la couleur de leur contenu stomacal spécialement pour les larves récoltées dans les lacs de Rouyn-Noranda où la couleur de sédiments oxiques et anoxiques différait de très peu. C'est pour cette raison que nous n'avons pas établi le comportement alimentaire des larves à partir de ces observations. À noter que selon la couleur des contenus stomacaux, toutes les larves que nous avons récoltées semblent ingérer des sédiments plutôt que du plancton.

Nos résultats corroborent aussi les conclusions de S. Martin *et al.* (2008) selon lesquelles *C. staegeri* se nourrit dans les sédiments oxiques tandis que *C. « tigris »* se nourrit dans les sédiments anoxiques. Ils vont aussi de pair avec ce qui a été reporté dans la littérature pour *C. plumosus* et *C. anthracinus*. Les larves de *C. plumosus* sont décrites comme étant des larves qui filtrent les particules de la colonne d'eau ou qui se nourrissent de détritus selon la disponibilité de la nourriture (Hodkinson *et al.*, 1980, Jónasson, 1972). Tandis que les larves de *C. anthracinus* sont décrites comme étant des larves qui se nourrissent directement dans les sédiments (Jónasson, 1972).

Certaines mesures isotopiques de soufre n'ont pas été publiées dans l'article 2 pour des lacs où des larves n'ont pu être identifiées, où plus d'une espèce a été regroupée, où les valeurs de Cd pour toutes les espèces n'étaient pas disponibles et où nous avions des mesures pour plus d'une année. Ces mesures isotopiques de soufre présentées à la figure 4-2 suivent les mêmes tendances que celles présentées dans l'article 2 (figures 2A et 3) et corroborent nos conclusions. Il est à noter cependant que contrairement aux autres lacs, les signatures isotopiques de soufre dans les sédiments anoxiques des lacs Bousquet, Fortune, Marlon et Opasatica échantillonnés en 2006 sont plus élevées que dans les sédiments oxiques. En 2006, les sédiments oxiques ont été échantillonnés plus grossièrement que dans les années suivantes. Il y a donc de fortes chances qu'en 2006, des sédiments anoxiques aient été incorporés aux sédiments oxiques lors de l'échantillonnage.

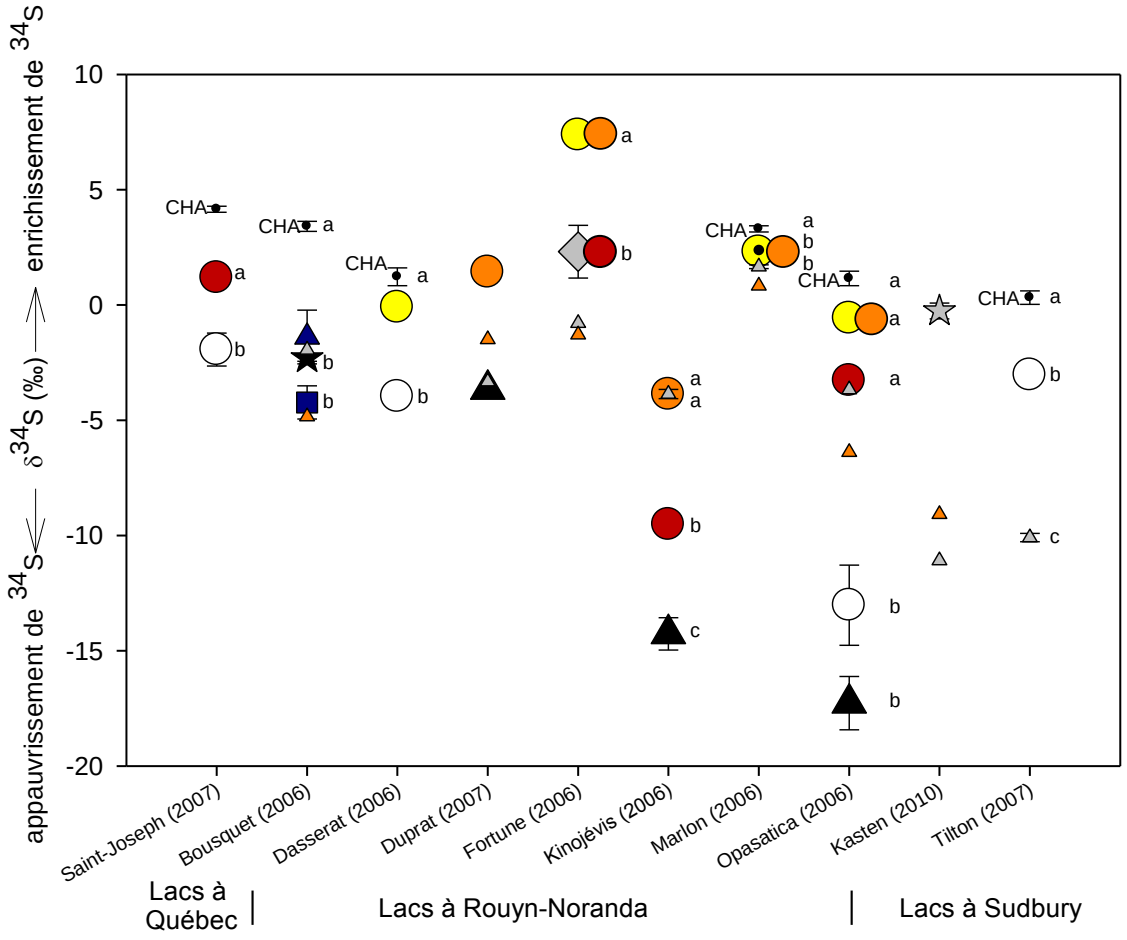


Figure 4-2 **Moyennes des signatures isotopiques de soufre ($\delta^{34}S$, ±écart-type, n=1-6) (‰) chez des larves de *Chironomus* (●●●○ ★ ☆ ▲ ◆; voir le tableau 4-2 pour l'identification des espèces, n=1-6), des larves de *Chironomus* de type larvaire thummi (■, n=2) et bathophilus (▲, n=3), des larves *C. entis* et *C. plumosus* (●●) regroupées (n=3-4), des larves de *C. decorus*-group sp. 2 et *C. staegeri* (◆●) regroupées (n=3), des larves de *Chaoborus* (CHA, n=2-4) ainsi que dans les sédiments oxiques (▲, n=1) et anoxiques (△, n=1-3) de lacs à Québec, Rouyn-Noranda et Sudbury. Pour chaque lac, les valeurs de $\delta^{34}S$ qui ne diffèrent pas significativement (p>0.05) sont suivit de la même lettre (les valeurs où n=1 ou 2 ne sont pas inclus dans les analyses).**

Nous avons aussi mesuré les ratios isotopiques de carbone et d'azote chez les larves. Les valeurs de $\delta^{13}C$ et de $\delta^{15}N$ sont présentées à la figure 5 de l'article 3. Dans certains lacs, les valeurs de $\delta^{13}C$ et $\delta^{15}N$ diffèrent significativement ($p<0.05$) entre espèces. Cependant, contrairement à quoi l'on s'attendait, les valeurs de $\delta^{13}C$ et de $\delta^{15}N$ ne sont pas constamment plus élevées chez certaines espèces que d'autres. Les signatures isotopiques de carbone et d'azote ne se sont donc pas avérées utiles pour expliquer les habitudes alimentaires des espèces de *Chironomus* dans nos lacs. Pour de plus amples détails concernant les valeurs de $\delta^{13}C$ et de $\delta^{15}N$ mesurées chez nos larves, voir l'article 3.

Dans l'ensemble, les valeurs de $\delta^{34}S$ indiquent que les larves de certaines espèces ingèrent des particules oxiques (*C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri*) alors que d'autres (*C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C*. spp. NAI-NAIII et *C.* « *tigris* ») ingèrent des sédiments anoxiques (tableau 4-2). Il a été intéressant de constater que les larves dont les concentrations de Cd et de Zn sont plus élevées et les concentrations de Se sont plus basses dans les lacs ingèrent des sédiments oxiques (tableau 4-2). Celles dont les concentrations de Cd et de Zn sont plus basses et les concentrations de Se sont plus élevées, ingèrent des sédiments anoxiques (tableau 4-2). Ces résultats indiquent fortement que les différences de concentrations de Cd, Zn et Se entre espèces sympatriques de *Chironomus* sont dues à leur comportement alimentaire. Selon cette tendance, les concentrations et/ou la biodisponibilité du Cd et parfois du Zn sont plus élevées dans les particules oxiques que dans les sédiments anoxiques tandis que pour le Se, c'est le contraire. Il est à noter que selon le modèle biodynamique (équation 1 présentés l'encadré 1-1), d'autres facteurs pourraient dicter des différences de concentrations entre espèces sympatriques de *Chironomus*. Cependant, nous avons émis comme hypothèse que ces facteurs sont négligeables comparativement au comportement alimentaire des larves (voir discussion dans l'article 4, pp.217-219).

Tableau 4-2 **Résumé des tendances des mesures d'isotopes stables de soufre ($\delta^{34}S$) et des concentrations de Cd, de Zn et de Se chez les larves de *Chironomus*.**

Espèces de *Chironomus*	Symboles utilisés dans les articles 3 et 4	Type de larve	Indication si les valeurs mesurées chez les larves étaient élevées ou basses par rapport à celles mesurées chez d'autres espèces récoltées au même endroit				Provenance des particules qu'ingèrent les larves
			$\delta^{34}S$	Cd	Zn	Se	
C. entis	●	semireductus	élevées	élevées	parfois élevées	basses	colonne d'eau et/ou sédiments oxiques
C. plumosus	●	semireductus et plumosus	élevées	élevées	parfois élevées	basses	colonne d'eau et/ou sédiments oxiques
C. dilutus	●	plumosus	élevées	élevées	parfois élevées	basses	colonne d'eau et/ou sédiments oxiques
C. staegeri	●	plumosus	élevées	élevées	parfois élevées	basses	colonne d'eau et/ou sédiments oxiques
C. harpi	●	plumosus	pas de mesures	basses	parfois basses	élevées	sédiments anoxiques
C. « tigris »	○	plumosus	basses	basses	parfois basses	élevées	sédiments anoxiques
C. decorus-group sp. 2	◇	bathophilus, fluviatilis ou melanotus	basses	basses	parfois basses	élevées	sédiments anoxiques
C. bifurcatus	▲	bathophilus	basses	basses	parfois basses	élevées	sédiments anoxiques
C. anthracinus	□	thummi	basses	basses	parfois basses	élevées	sédiments anoxiques
C. nr. *atroviridis* (sp. 2i)	■	thummi	pas de mesures	basses	parfois basses	élevées	sédiments anoxiques
C. ochreatus	■	thummi	pas de mesures	basses	parfois basses	élevées	sédiments anoxiques
C. sp. NAI	□	thummi	basses	basses	parfois basses	élevées	sédiments anoxiques
C. cucini	★	salinarius	basses	basses	parfois basses	élevées	sédiments anoxiques
C. sp. NAII	☆	salinarius	basses	basses	parfois basses	élevées	sédiments anoxiques
C. sp. NAIII	☆	salinarius	basses	basses	parfois basses	élevées	sédiments anoxiques

Nous n'avons pas été en mesure de mesurer les signatures isotopiques de soufre chez *C. harpi*, *C*. nr. *atroviridis* sp. 2i et *C. ochreatus*. Cependant, puisque les larves qui se nourrissent en zone oxique ont des concentrations de Cd plus élevées que les larves qui se nourrissent dans les sédiments anoxiques, nous avons inféré le comportement alimentaire des larves de *C. harpi*, *C*. nr. *atroviridis* sp. 2i et *C. ochreatus* en comparant leur concentration de Cd à celle de larves d'autres espèces récoltées au même endroit et dont le comportement alimentaire a été élucidé (pour de plus amples détails voir article 3). C'est ainsi que nous avons établi que *C. harpi*, *C*. nr. *atroviridis* sp. 2i et *C. ochreatus* ingèrent des sédiments anoxiques. Malheureusement, puisque *C. frommeri* et *C. maturus* n'ont pas été récoltés avec d'autres espèces de *Chironomus*, il nous a été impossible d'établir leur comportement alimentaire.

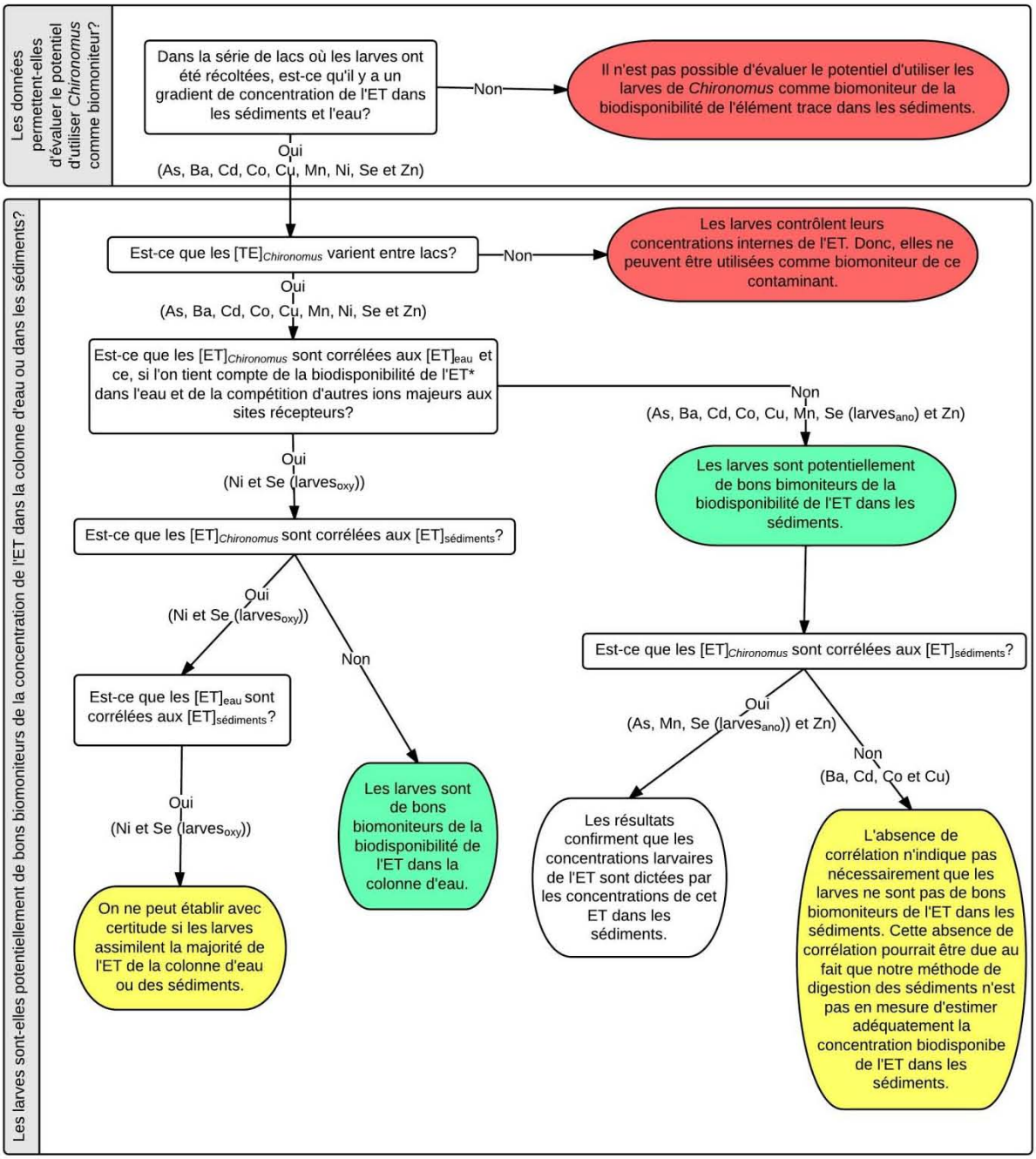
Encadré 4-1 Mesures de concentrations d'hémoglobine chez les larves

Comme le coût des analyses d'isotopes stables de soufre (22$/échantillon) est assez élevé, nous avons exploré la possibilité de séparer les larves de *Chironomus* qui se nourrissent de particules oxiques de celles qui se nourrissent de particules anoxiques en mesurant leurs taux d'hémoglobine (1$/échantillon). L'idée de mesurer les concentrations d'hémoglobine chez les larves provient du fait que l'étude de Panis *et al*. (2005) a démontré que les larves de *C. plumosus* présentes en eau profonde et peu oxygénée ont des concentrations d'hémoglobine plus élevées que celles vivant en eau moins profonde et oxygénée. Notre hypothèse était que les espèces de *Chironomus* qui se nourrissent de particules anoxiques sont exposées à des concentrations d'oxygène plus faibles que celles qui se nourrissent en zone oxique et, ont par conséquent, des concentrations d'hémoglobine plus élevées. Nous avons mesuré les concentrations d'hémoglobine chez les larves de 5 espèces de *Chironomus* récoltées dans trois lacs. Les résultats des mesures sont présentés à l'annexe A. Nos résultats confirment notre hypothèse et démontrent l'idée de mesurer les concentrations d'hémoglobine chez les larves mériterait d'être davantage explorée.

Les larves que nous avons récoltées et qui se nourrissent de particules oxiques sont morphologiquement différentes de celles qui se nourrissent de sédiments anoxiques. Il est donc possible de les distinguer aux binoculaires alors qu'elles sont toujours vivantes selon la présence/absence, la forme et la longueur de leurs tubules ventraux et latéraux et la couleur de leur *frontoclypeus*. Dans le but de faciliter la tâche à ceux et celles qui voudraient utiliser *Chironomus* comme biomoniteur des sédiments, nous avons donc développé une clé morphologique permettant de séparer (article 4, p. 248) les larves qui ingèrent des sédiments anoxiques de celles qui ingèrent des particules oxiques à Rouyn-Noranda et Sudbury.

4.4 **Potentiel d'utiliser les larves de *Chironomus* comme biomoniteurs de la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments.**

Nous avons évalué le potentiel d'utiliser les larves de *Chironomus* comme biomoniteur de la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments. Le raisonnement que nous avons utilisé pour faire cette évaluation est présenté sous forme d'organigramme dans l'encadré 4-2. Les larves de *Chironomus* ont été récoltées dans une série de lacs dont les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn variaient dans la colonne d'eau et les sédiments (voir figures 1 et 2 de l'article 4). D'un lac à l'autre, les concentrations larvaires de chacun de ces éléments traces variaient, et ce, même si certains éléments sont considérés comme étant essentiels (figures 3 et 4 de l'article 4). Ces différences de concentrations indiquent que les larves de *Chironomus* ne sont pas en mesure de garder constantes leurs concentrations internes de ces contaminants et sont donc de potentiels biomoniteurs de tous ces éléments traces.



* La biodisponibilité des cations (Ba, Cd, Co, Cu, Mn, Ni et Zn) dans l'eau a été établie selon les concentrations de l'ion libre. Pour ces éléments, nous avons aussi vérifié que les cations majeurs de H^+ et Ca^{2+} n'influaient pas sur les concentrations larvaires. Les concentrations larvaires de Se ont été comparées aux concentrations des diverses espèces de Se dans la colonne d'eau. Pour ce qui est de l'As, nous n'avons pas été en mesure d'estimer les concentrations biodisponibles dans l'eau.

Encadré 4-2 **Organigramme du raisonnement utilisé pour évaluer le potentiel d'utiliser les larves de *Chironomus* comme biomoniteurs de la disponibilité d'un élément trace (ET) dans les sédiments oxiques et anoxiques. Nous avons suivi cette suite logique d'idée pour chaque élément trace (As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn) investigué lors de cette étude.**

De façon générale, les éléments traces qu'assimilent les larves proviennent soit de la colonne d'eau ou des sédiments. Comme mentionnées plus haut, toutes les larves de *Chironomus* creusent des tunnels dans les sédiments dans lesquels elles vivent et se nourrissent. Elles se nourrissent soit de particules en suspension dans la colonne d'eau ou des particules sédimentaires. Bien qu'elles habitent dans les sédiments, les larves de *Chironomus* sont exposées à l'eau de la colonne d'eau puisqu'elles irriguent leur tunnel pour le garder oxygéné. Ceci dit, une larve de *Chironomus* peut assimiler un élément trace de la colonne d'eau par son exposition à cette eau ou par l'ingestion de particules en suspension dans la colonne d'eau (ex. phytoplancton) (Croteau *et al.*, 1998, Munger *et al.*, 1997, Ponton *et al.*, 2009, Ponton *et al.*, 2013). D'un autre côté, cette larve de *Chironomus* peut aussi assimiler cet élément trace des sédiments par l'ingestion de particules sédimentaires. Nous considérons que l'exposition des larves à l'eau interstitielle des sédiments est négligeable.

Pour déterminer si les larves assimilent principalement l'As, le Ba, le Cd, le Co, le Cu, le Mn, le Ni, le Se et le Zn de la colonne d'eau ou des sédiments, nous avons comparé les concentrations de chacun de ces éléments traces chez les larves à ceux dans la colonne d'eau et les sédiments. Pour ce faire, nous avons regroupé les larves qui se nourrissent de particules oxiques et celles qui se nourrissent de sédiments anoxiques.

Puisqu'il est possible d'adéquatement estimer la biodisponibilité de la plupart des éléments traces dans la colonne d'eau, nous considérions que les larves assimilent majoritairement un élément trace du compartiment sédimentaire lorsque les concentrations larvaires de cet élément ne sont pas corrélées à celles biodisponibles dans la colonne d'eau. Cette estimation est valide même si les larves assimilent majoritairement un élément trace de la colonne d'eau par l'ingestion de particules en suspension puisque de fortes corrélations ont déjà été reportées entre les concentrations d'éléments traces dans la colonne d'eau et celles chez *Chaoborus*, un organisme planctonivore qui assimilent majoritairement les éléments traces de sa nourriture (Croteau *et al.*, 1998, Munger *et al.*, 1997, Ponton *et al.*, 2009, Ponton *et al.*, 2013). La biodisponibilité des cations (Ba, Cd, Co, Cu, Mn, Ni et Zn) dans l'eau a été établie à partir de la concentration de leur ion libre. De plus, comme des études ont déjà démontré que les ions de H^+ et Ca^{2+} pouvaient avoir une influence sur la prise en charge de certains métaux par les larves (Bervoets *et al.*, 2000, Craig *et al.*, 1999, Gillis *et al.*, 2008) ou leur nourriture (Croteau *et al.*, 1998, Munger *et al.*, 1997, Ponton *et al.*, 2009, Ponton *et al.*, 2013), nous avons aussi vérifié que ces cations majeurs n'influaient pas sur les concentrations de métaux chez les larves. Quant aux anions dans l'eau, leur biodisponibilité est dictée par leur état d'oxydation (Luoma *et*

al., 2008). Afin de déterminer si les larves assimilent principalement le Se de la colonne d'eau, nous avons comparé les concentrations des diverses espèces de Se à celles chez les larves. Pour l'As, les concentrations larvaires n'ont été comparées qu'avec les concentrations totales dissoutes de l'As dans la colonne d'eau.

Les concentrations larvaires d'As, Ba, Cd, Co, Cu, Mn et Zn n'étaient pas corrélées ($p>0.05$) à celles dans la colonne d'eau et ce, autant pour les larves qui se nourrissent de sédiments anoxiques que celles qui se nourrissent de particules oxiques (tableau S13, article 4). Ces résultats indiquent que les larves n'assimilent pas majoritairement l'As, le Ba, le Cd, le Co, le Cu, le Mn et le Zn de la colonne d'eau et sont donc de potentiels biomoniteurs de ces contaminants dans les sédiments. De plus, les concentrations de Se chez les larves qui se nourrissent de sédiments anoxiques n'étaient pas corrélées à celles dans l'eau, indiquant que ces larves assimilent majoritairement le Se du compartiment sédimentaire (figure S3 de l'article 4). Seules les concentrations de Se chez les larves qui se nourrissent de particules oxiques et les concentrations de Ni chez les larves qui se nourrissent de particules oxiques et anoxiques étaient corrélées à celles dans l'eau (tableau S13, article 4). Toutefois, leurs concentrations étaient aussi reliées à celles dans les sédiments. À vrai dire, leurs concentrations étaient généralement plus fortement corrélées à celles dans les sédiments que celles dans l'eau (tableau S13 de l'article 4). Nous croyons que les corrélations entre les concentrations de Ni et de Se chez les larves et l'eau sont tout simplement dues au fait que les concentrations de ces éléments traces dans l'eau sont aussi reliées à celles dans les sédiments (voir article 4 pour plus de détails).

Nous avons mesuré les concentrations d'éléments traces dans la matière fécale (excrété et non excrété) des larves. À quelques exceptions près, les concentrations d'éléments traces dans les matières fécales des larves sont corrélées à celles dans les sédiments où les larves ont été récoltées. Ces résultats indiquent que les larves qui se nourrissent de particules oxiques et celles qui se nourrissent de particules anoxiques ingèrent des sédiments. Ces résultats corroborent nos observations des contenus intestinaux (voir p. 36-37). Le fait que les concentrations d'éléments traces dans les sédiments ne sont pas toujours les mêmes que dans les matières fécales des larves indique que les larves ne se nourrissent pas de sédiments en vrac, mais sélectionne les particules de sédiments qu'elles ingèrent (pour de plus amples détails, voir l'article 4).

Bref, selon nos attentes, les larves ne semblent pas assimiler la majorité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn de la colonne d'eau. Cela s'applique aussi pour les larves qui ingèrent des

particules oxiques. Le fait que les concentrations d'As, Mn, Ni, Se et Zn chez les larves sont corrélées à celles dans les sédiments confirment nos conclusions. Cependant, l'absence de corrélations entre les concentrations larvaires de Ba, Cd, Co et Cu, n'indique pas nécessairement que les larves ne sont pas de bons biomoniteurs de ces contaminants dans les sédiments. Ces résultats indiquent plutôt que notre méthode de digestion des sédiments ne permet pas d'adéquatement estimer la concentration biodisponible de ces éléments traces.

Dans l'article 4, nous avons vérifié si normaliser les concentrations d'éléments trace par le % de matière organique permettait de mieux prédire la biodisponibilité des éléments traces dans les sédiments oxiques et anoxiques. Nous avons aussi testé l'efficacité de la méthode SEM-AVS pour prédire la biodisponibilité des cations dans les sédiments anoxiques. Aucune de ces méthodes ne s'est avérée plus efficace pour prédire les concentrations d'éléments traces chez les larves comparativement aux mesures dans les sédiments.

Le manque d'efficacité de ces méthodes non biologiques démontre l'avantage d'utiliser des organismes comme biomoniteur pour évaluer la disponibilité des contaminants dans les sédiments. Conformément à leurs comportements alimentaires, nous concluons que les larves de *C. dilutus*, *C. entis*, *C. plumosus* et *C. staegeri* sont de bons biomoniteurs de la contamination des sédiments oxiques, tandis que les larves de *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C*. sp. NAI, *C*. sp. NAII, *C*. sp. NAIII et *C*. « *tigris* » sont de bons biomoniteurs de la contamination des sédiments anoxiques. À chaque site, les concentrations de Cd et de Zn n'étaient pas nécessairement plus élevées dans les sédiments oxiques qu'anoxiques, les concentrations de Se n'étaient pas nécessairement plus basse dans les sédiments oxiques qu'anoxiques et les concentrations d'As, Ba, Co, Cu, Mn et Ni n'étaient pas nécessairement les mêmes dans les sédiments oxiques qu'anoxiques. Ces résultats indiquent que les concentrations d'éléments traces chez les espèces sympatriques de *Chironomus* sont régies par la biodisponibilité de ces éléments traces dans les sédiments oxiques et anoxiques plutôt que par leur concentration totale. Il est à noter que les concentrations de Cd chez les larves qui se nourrissent dans les sédiments anoxiques variaient moins entre lacs (2-167 nmol/g) que chez les larves qui ingèrent des particules oxiques (1-470 nmol/g). Ces résultats indiquent que dans les sédiments anoxiques à Rouyn-Noranda et Sudbury, le Cd est très peu biodisponible.

4.5 Utilisation de larves de *Chironomus* comme biomoniteurs

Puisque nos résultats indiquent que certaines larves de *Chironomus* peuvent être utilisées comme biomoniteurs des sédiments oxiques tandis que d'autres peuvent être utilisées comme biomoniteurs des sédiments anoxiques, nous nous sommes servis de ces larves pour évaluer si la biodisponibilité des éléments traces dans les sédiments oxiques et anoxiques des lacs à Rouyn-Noranda et Sudbury à changer depuis les 20 dernières années.

De 1987 à 1993, des larves de *Chironomus* ont été récoltées dans les lacs D'Alembert, Duprat et Marlon à Rouyn-Noranda et McFarlane, Raft et Tilton à Sudbury.

Tableau 4-3 Années durant lesquelles les larves ont été récoltées.

Lacs	Codes	Années durant lesquelles les larves ont été récoltées dans le passé	Années durant lesquelles les larves ont été récoltées durant cette étude
ROUYN-NORANDA (QC)			
D'Alembert	DA	1988 et 1990	2006
Duprat	DP	1990, 1992	2006, 2007, 2010
Marlon	MN	1993	2006, 2007, 2010
SUDBURY (ON)			
Clearwater	CL	1987	2007
McFarlane	MC	1987, 1992, 1993	2007
Raft	RA	1993	2010
Tilton	TI	1992, 1993	2007

Les larves ont été séparées selon la longueur et la présence/absence de leurs tubules ventraux et latéraux et la couleur de leur *frontoclypeus*. Les larves ont été digérées selon la méthode présentée plus haut et les produits de digestions ont été entreposés à température ambiante. En 2012, nous avons ressorti les produits de digestion et avons mesuré les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn par ICP-MS. Dans le passé, des concentrations de Cd, Cu et Zn ont aussi été mesurées dans ces échantillons. Afin de s'assurer les concentrations d'éléments traces dans les produits de digestions n'aient pas changé au cours des vingt dernières années, nous avons comparé, pour chaque échantillon, les concentrations de Cd, Cu et Zn mesurées dans le passé à celles mesurées en 2012. Le coefficient de variation entre les mesures de Cd, Cu et Zn fait dans le passé et celles faites environ 20 ans plus tard était moins de <10%.

Selon leur morphologie, le comportement alimentaire des larves récoltées entre 1987 et 1993 a été déduit grâce à la clé morphologique présentée dans l'article 3. Dans chaque lac et pour chaque groupe de larves ayant le même comportement alimentaire, les concentrations d'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn chez les larves récoltées lors de cette étude (2006-2010) ont été comparées à celles récoltées dans le passé (1987 à 1993) (figures 4-3 à 4-11 et tableau 8-1 de l'annexe B).

D'un lac à l'autre, comme c'est le cas pour les larves récoltées entre 2006 et 2010, les larves récoltées entre 1987 et 1993, qui se nourrissent dans les sédiments oxiques, avaient des concentrations de Cd (figure 4-5) et de Zn (figure 4-11) plus élevées et des concentrations de Se (figure 4-10) plus bases que les larves qui se nourrissent dans les sédiments anoxiques. De plus, comme pour les larves récoltées de 2006 à 2010, les concentrations de Cd chez les larves qui se nourrissent dans les sédiments anoxiques variaient très peu entre les lacs, tandis que les concentrations chez les larves qui ingèrent des particules oxiques variaient considérablement d'un site à l'autre. Ces résultats indiquent qu'au cours des années, la biodisponibilité des éléments traces dans les sédiments oxiques par rapport à ceux dans les sédiments anoxiques n'a pas réellement changé pour ces éléments.

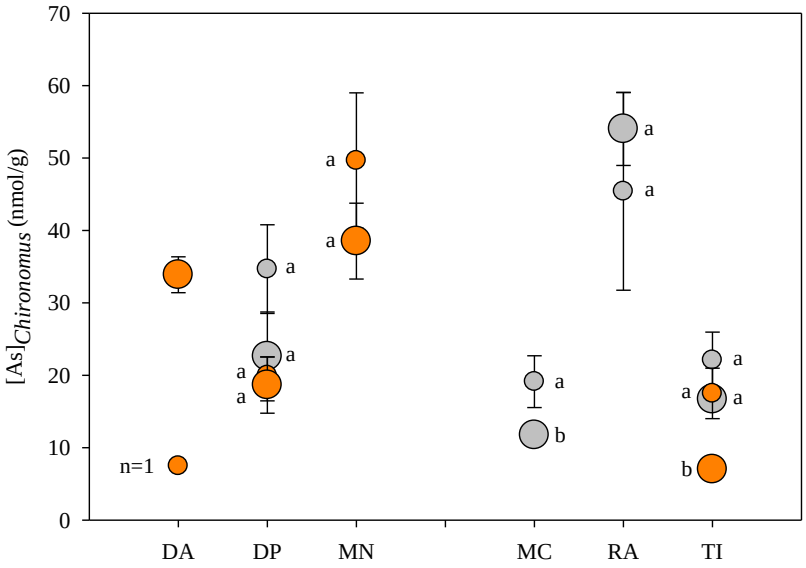


Figure 4-3 **Concentrations moyennes (± écart-type, n=1-23) d'As chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (MC, RA, TI) (voir tableau 4-3 pour les acronymes).**
Dans chaque lac, les concentrations d'éléments traces ne diffèrent pas significativement (p>0.05) entre les larves récoltées durant les périodes 1987-1993 et 2006-2010 et ayant le même comportement si les symboles sont suivis de la même lettre (les moyennes où n=1 ou 2 ne sont pas incluses dans les analyses). Les résultats des analyses statistiques pour les larves qui se nourrissent de sédiments oxiques sont présentés à gauche des symboles tandis que ceux des larves qui se nourrissent de sédiments anoxiques sont présentés à droite des symboles.

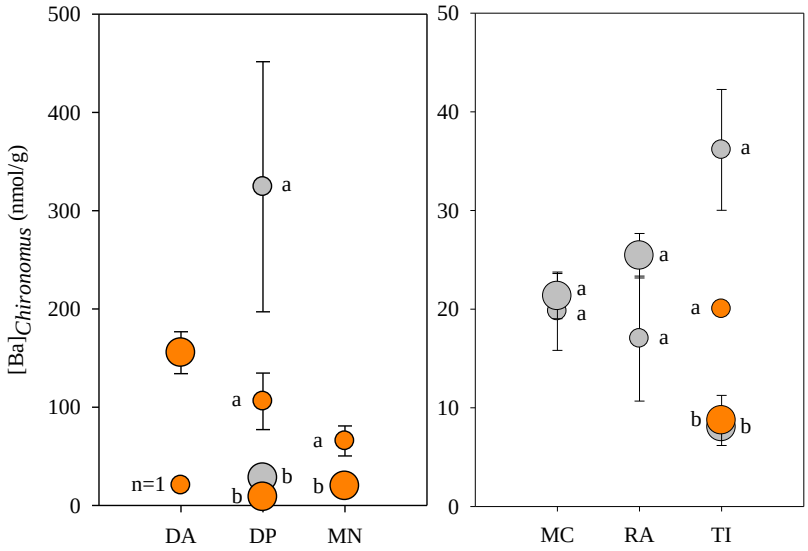


Figure 4-4 **Concentrations moyennes (± écart-type, n=1-23) de Ba chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

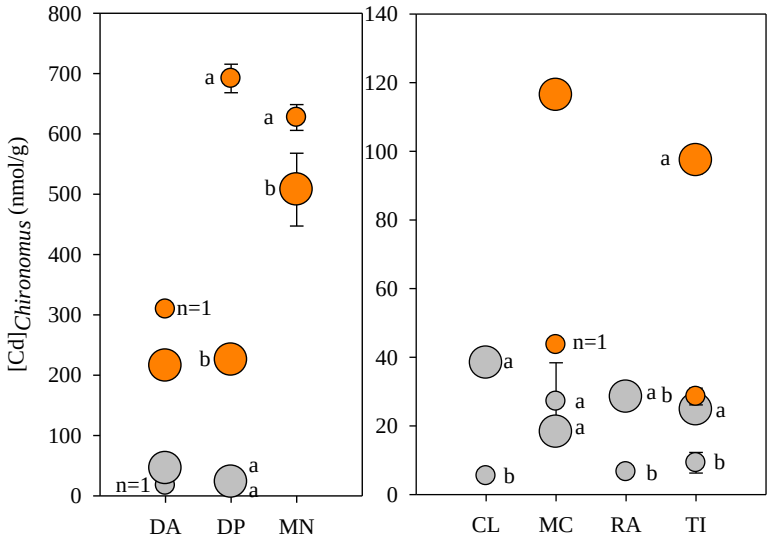


Figure 4-5 **Concentrations moyennes (± écart-type, n=1-23) de Cd chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (CL, MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

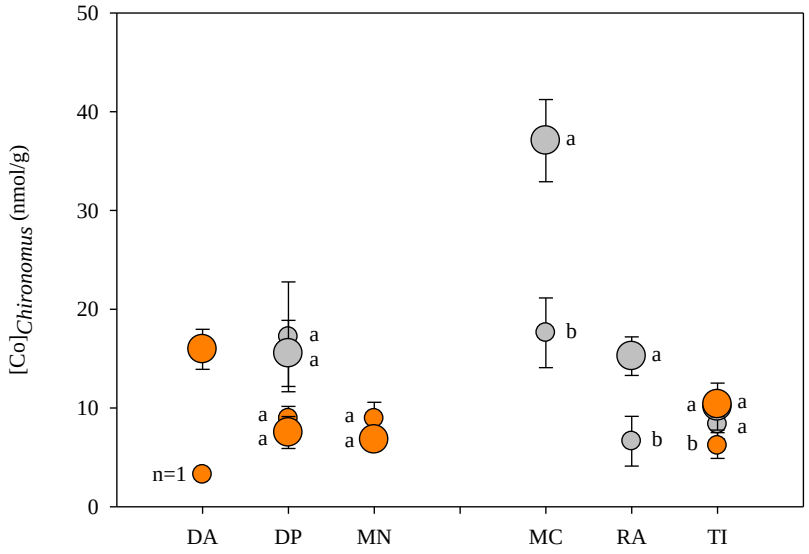


Figure 4-6 **Concentrations moyennes (± écart-type, n=1-23) de Co chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

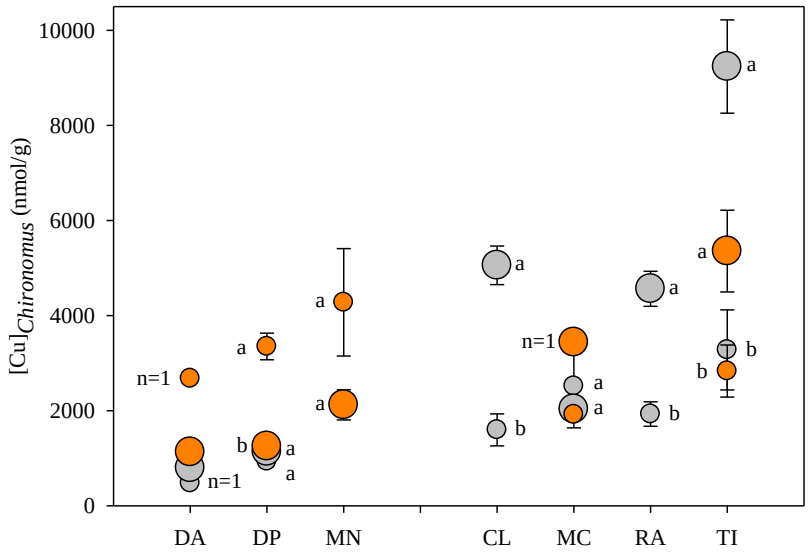


Figure 4-7 **Concentrations moyennes (± écart-type, n=1-23) de Cu chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (CL, MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

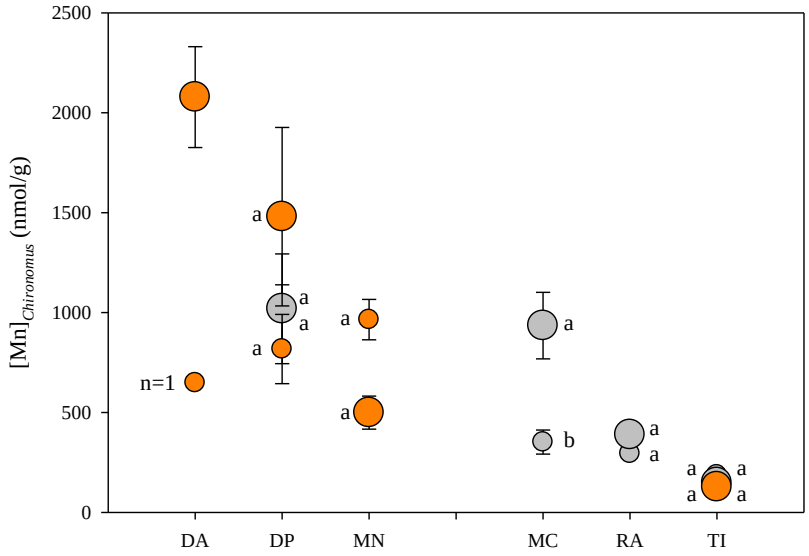


Figure 4-8 **Concentrations moyennes (± écart-type, n=1-23) de Mn chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

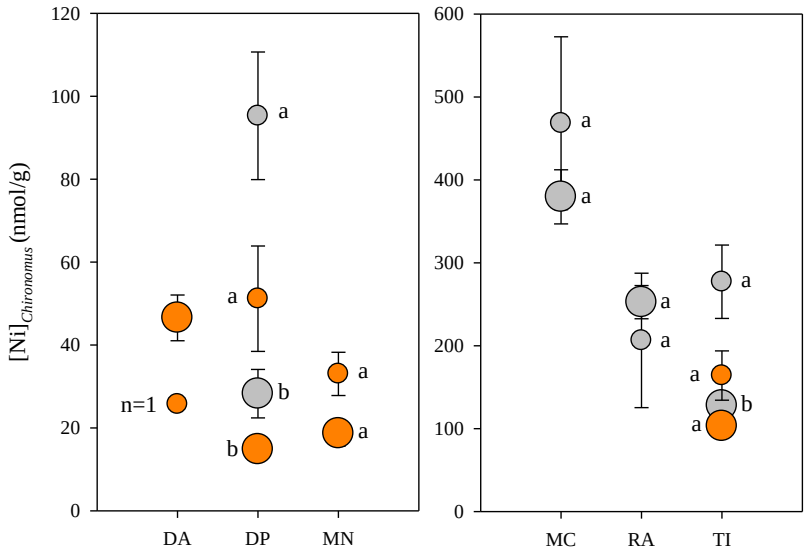


Figure 4-9 **Concentrations moyennes (± écart-type, n=1-23) de Ni chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

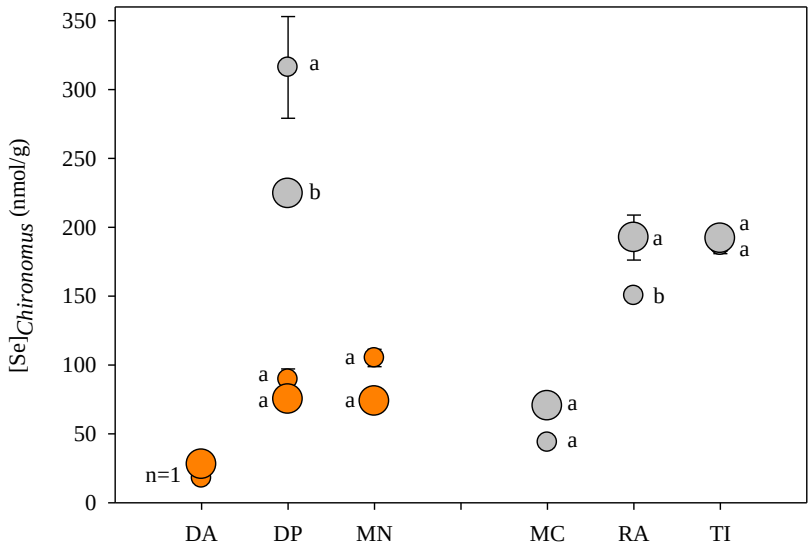


Figure 4-10 **Concentrations moyennes (± écart-type, n=1-23) de Se chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

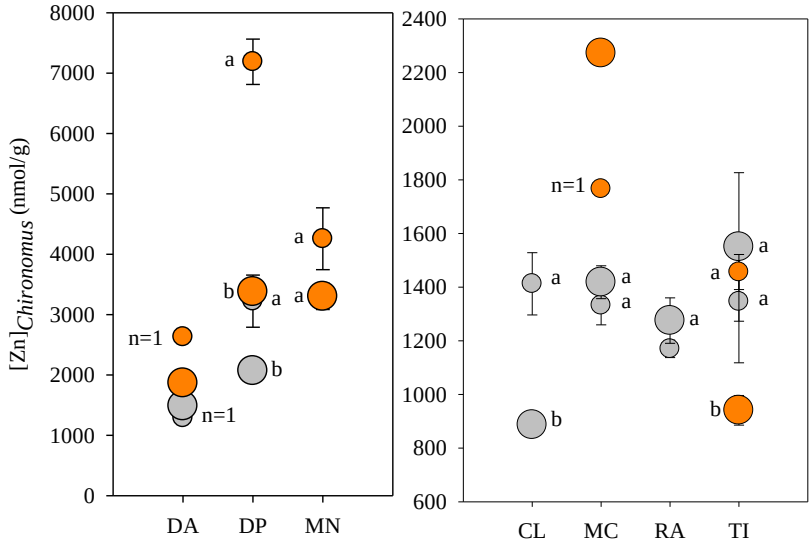


Figure 4-11 **Concentrations moyennes (± écart-type, n=1-23) de Zn chez des larves de *Chironomus* qui ingèrent des sédiments oxiques (symboles orange) et chez des larves qui ingèrent des sédiments anoxiques (symboles gris) récoltées entre 1987 et 1993 (petit cercle) et entre 2006 et 2010 (gros cercle) dans des lacs à Rouyn-Noranda (DA, DP et MN) et Sudbury (CL, MC, RA, TI) (voir tableau 4-3 pour les acronymes). Voir la figure 4-3 pour des précisions sur les comparaisons statistiques des moyennes.**

À Rouyn-Noranda, depuis environ 20 ans, les concentrations de Cd et de Cu ont diminué chez les larves se nourrissant dans les sédiments oxiques et sont restées semblables chez celles se nourrissant dans les sédiments anoxiques (Figs. 4-5 et 4-7). Donc selon ces résultats, la biodisponibilité du Cd et du Cu serait moindre depuis 20 ans dans les sédiments de surface et serait semblable dans les sédiments plus en profondeur. Nos résultats vont dans le même sens de ceux de Lacharité (2011) qui a comparé des mesures de Cd et Cu chez des larves d'*Hexagenia limbata* récoltées dans des lacs de Rouyn-Noranda en 2009 et durant la période 1987 à 1992. Les larves de *H. limbata* sont des insectes benthiques fouisseurs qui ingèrent des sédiments (Merritt *et al.*, 2008). Comme les *Chironomus* qui se nourrissent de particules oxiques, Lacharité (2011) a noté que dans la grande majorité des lacs, les concentrations de Cd et Cu étaient plus bases chez les larves *H. limbata* récoltées en 2009 que celles récoltées de 1987-1992. Cette tendance à la baisse de la biodisponibilité du Cd et du Cu dans les sédiments de surface à Rouyn-Noranda pourrait être reliée à la fermeture de la mine de Cu en 1976 et à la réduction des émissions de contaminant par la raffinerie Horne dans les années 1980. Dans les sédiments oxiques, la biodisponibilité du Se est restée la même, mais a diminué dans les sédiments anoxiques (Fig. 4-10). Pour ce qui est des autres éléments traces, les biodisponibilités ont soit diminué ou sont restées les mêmes dans les sédiments oxiques et anoxiques des lacs Duprat et Marlon (Figs. 4-3, 4-4, 4-6, 4-8, 4-9 et 4-11). Tandis que dans le lac D'Alembert, la biodisponibilité de l'As, Ba, Co, Mn, et Ni a augmenté dans les sédiments oxiques (Figs. 4-3, 4-4, 4-6, 4-8 et 4-9).

À Sudbury, à quelques exceptions près, les concentrations de Cd, Co et Cu chez les larves récoltées entre 2006 et 2010 sont plus élevées que chez celles récoltées entre 1987 et 1993 (Figs. 4-5 à 4-7). Donc, la biodisponibilité du Cd, Co et Cu aurait généralement augmenté dans les sédiments à Sudbury depuis environ 20 ans, et ce, autant dans les sédiments oxiques que dans les sédiments anoxiques. D'un autre côté, selon les concentrations larvaires d'éléments traces, la biodisponibilité de l'As, du Ba et du Ni est restée la même dans certains lacs tandis que dans d'autres, elle a diminué (Figs. 4-3, 4-4 et 4-9). La biodisponibilité du Se dans les sédiments anoxique n'a pas changé dans le lac McFarlane et Tilton mais a augmenté dans le lac Raft (Fig. 4-10). Pour ce qui est du Mn et du Zn, il n'y a pas de tendance générale (Figs. 4-8 et 4-11). Dans certains lacs, les concentrations chez les larves sont les mêmes depuis 20 ans tandis que dans d'autres les concentrations sont plus élevées ou plus basses. Règle générale, contrairement à nos attentes, à plusieurs endroits, la biodisponibilité de certains éléments traces dans les sédiments à Sudbury n'a pas diminué depuis 20 ans. Il serait intéressant

d’évaluer si cette tendance est due aux émissions de ces contaminants par les raffineries ou par le recyclage de ces contaminants provenant des sédiments.

En conclusion, cette étude démontre comment il est possible et faisable d’utiliser les larves de *Chironomus* en tant que biomoniteurs des sédiments oxiques et anoxiques, et ce, sans avoir à identifier les larves au niveau de l’espèce.

5 CONCLUSION

En conclusion, nos résultats indiquent que les larves de *Chironomus* peuvent être utilisées comme biomoniteurs pour évaluer la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments. Certaines espèces de *Chironomus* se nourrissent de particules oxiques tandis que d'autres se nourrissent de sédiments anoxiques. Bien que celles qui se nourrissent de particules oxiques, peuvent ingérer des particules de la colonne d'eau, nos mesures suggèrent que ces larves assimilent principalement les éléments traces des sédiments oxiques. Donc les espèces qui se nourrissent de particules oxiques peuvent être utilisée pour évaluer la biodisponibilité de l'As, Ba, Cd, Co, Cu, Mn, Ni, Se et Zn dans les sédiments oxiques tandis que les larves qui se nourrissent de particules anoxiques peuvent être utilisées pour évaluer la biodisponibilité de ces éléments dans les sédiments anoxiques. Compte tenu de la variété des processus géochimiques qui peuvent influer sur la biodisponibilité d'éléments traces dans les sédiments oxiques et anoxiques, il est quasi impossible de comparer la biodisponibilité de ces éléments traces dans ces deux types de sédiments sans l'aide d'un biomoniteur comme *Chironomus*. En fait, à preuve du contraire, aucune autre étude n'a démontré qu'un autre invertébré benthique pouvait être utilisé comme *Chironomus* pour comparer la biodisponibilité des éléments traces dans les sédiments oxiques et anoxiques à un site donné.

Nos résultats démontrent que par leur comportement alimentaire, il n'est pas toujours justifiable de regrouper les espèces de *Chironomus* lors d'analyses de contaminants. À un même site, les espèces qui se nourrissent de particules oxiques avaient des concentrations de Cd et parfois de Zn plus élevées et des concentrations de Se plus basses que celles qui ingèrent des sédiments anoxiques. Ces concentrations larvaires indiquent que la biodisponibilité du Cd et parfois du Zn est plus élevée dans les sédiments oxiques que dans les sédiments anoxiques et que la biodisponibilité du Se est plus élevée dans les sédiments anoxique que dans les sédiments oxiques. La faible biodisponibilité du Cd dans les sédiments anoxique pourrait dû au fait que dans cette couche de sédiment, le Cd est précipité avec les sulfures. Tandis que pour le Se, on émet comme hypothèse qu'à la surface des sédiments le Se est peu biodisponible en raison de son association à de la matière organique, mais qu'en profondeur dans les sédiments il est plus biodisponible en raison de son relargage suite à la décomposition de cette matière organique.

Ne pas être en mesure de regrouper les larves de *Chironomus* lors d'analyses de contaminant peut être vu comme un désavantage pour utiliser cet organisme comme biomoniteur de la

contamination des sédiments. Cependant, comme les concentrations d'éléments traces chez les espèces sympatriques de *Chironomus* diffèrent en fonction de leur comportement alimentaire et que dans nos lacs, ces deux groupes de larves étaient distincts morphologiquement, la séparation des larves au niveau de l'espèce n'est pas nécessaire. Donc, nos résultats démontrent que les larves de *Chironomus* peuvent être utilisées comme biomoniteurs de la contamination des sédiments, et ce, de façon pratico-pratique.

5.1 **Sommaire des contributions à la connaissance**

Notre étude figure parmi l'une des rares en écologie et en écotoxicologie à avoir séparé des espèces de *Chironomus*. Ceci est dû en grande partie au fait qu'il n'existe aucun guide complet permettant l'identification des larves de *Chironomus* au niveau de l'espèce. D'autres études en écologie où les larves de *Chironomus* ont été séparées au niveau de l'espèce avec preuve à l'appui sont celles de M. G. Butler (1982), MG Butler *et al.* (2000) et Dinsmore *et al.* (1997). Comme l'identification des espèces de *Chironomus* est problématique, il est difficile d'affirmer hors de tout doute, que des larves de *Chironomus* ont été bien identifiées, dans des études comme celles de Grey *et al.* (2004), Jones *et al.* (2008) et Kelly *et al.* (2004) où les auteurs omettent de mentionner sous quels critères les larves ont été identifiées au niveau de l'espèce. En fait, dans les études citées en exemple, il est peu probable que les espèces de *Chironomus* aient été bien identifiées étant donné que les auteurs n'ont trouvé que deux espèces de *Chironomus* dans des dizaines de lacs en peu partout en Europe[19] alors que nous en avons trouvé 14 dans des lacs semblables.

Afin d'aider tous ceux et celles qui voudraient séparer et identifier des larves de *Chironomus* au niveau de l'espèce en Amérique du Nord, nous présentons dans l'article 2 des outils morphologiques et moléculaires pouvant être utilisés pour séparer les espèces. Nous avons construit une clé morphologique permettant de séparer la plupart des espèces de *Chironomus* que nous avons récoltées. Dans cette clé, nous avons utilisé des critères morphologiques simples à observer et qui peuvent être utilisés pour séparer morphologiquement d'autres espèces de *Chironomus* ailleurs dans le monde. Dans la littérature, il y avait un manque de consensus quant à la définition des types de larves. Nous avons donc clarifié la classification des types larvaires. Nous avons aussi redéfini les classifications des types de mentum et de

[19] Il y a plus de 175 espèces de *Chironomus* répertoriées dans le monde (selon la liste des chironomidés de la collection à Munich, *Zoologische Staatssammlung München* (http://www.zsm.mwn.de/dip/Chiro_web.pdf).

mandibules de façon à ce qu'elles soient plus claires et faciles à utiliser. Avant notre étude, moins d'une dizaine de séquences *cox1* de *Chironomus* en Amérique du Nord avaient été publiées. Il y en avait encore moins pour le gène *gb2β*. Notre étude a grandement contribué à augmenter la banque de codes-barres pour les espèces de *Chironomus*. Nous avons séquencé l'ADN de 23 espèces. Toutes les séquences *cox1* ou *gb2β* obtenues (241 séquences) lors de l'étude ont été publiées dans GenBank. Nous avons été les premiers à publier des séquences *cox1* pour les espèces suivantes : *C. acidophilus, C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. frommeri*, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C. plumosus*, *C. quinnitukqut*, *C*. sp. g. *C*. sp. h et *C*. « *tigris* ». Nous avons aussi été les premiers à publier des séquences *gb2β* pour les espèces suivantes : *C. anthracinus*, *C. bifurcatus*, *C. calligraphus*, *C. cucini*, *C. harpi*, *C. maturus*, *C.* sp. u et *C.* « *tigris* ». Finalement, nos recherches sur la taxonomie des *Chironomus* ont aussi permis plus précisément de :

- répertorier des espèces (*C. frommeri*, *C. harpi* et *C. ochreatus*) qui n'avaient pas été listées auparavant à l'est du Canada;
- relancer le débat sur le statut d'espèce de *C. rempelli* (voir article 2);
- démontrer que plusieurs espèces partagent la même séquence *cox1* et donc, que ce barcode moléculaire doit être utilisé avec précaution lors de l'identification d'espèces de *Chironomus;*
- d'investiguer l'utilisation de la séquence *gb2β* comme barcode moléculaire.

Grâce à notre étude, nous avons pu établir le comportement alimentaire de 7 espèces de *Chironomus*. En fait, nous avons été les premiers à étudier le comportement alimentaire de *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. entis*, *C. harpi*, *C*. nr. *atroviridis* (sp.2i) et *C. ochreatus.*

Notre étude a démontré qu'il n'est pas toujours justifiable de regrouper des *Chironomus* lors d'analyses de contaminants et que des différences comportementales entre espèces influencent leur exposition aux éléments traces. Toutefois, nous avons démontré que les larves de *Chironomus* ayant un comportement alimentaire différant pouvaient être séparées grâce à leur morphologie.

Les mesures d'éléments traces chez les larves ont démontré que la biodisponibilité de certains éléments traces peut grandement différer dans les sédiments selon la présence ou l'absence d'oxygène. Notre étude suggère que certaines espèces de *Chironomus* peuvent être utilisées pour évaluer la biodisponibilité des éléments traces dans les sédiments oxiques tandis que d'autres larves peuvent être utilisées pour évaluer la biodisponibilité des éléments traces dans

les sédiments anoxiques. À notre connaissance, les *Chironomus* sont les meilleurs outils qu'on ait jusqu'à présent pour évaluer de façon comparable la différence de biodisponibilité des éléments traces dans les sédiments oxiques et anoxiques à un site donné.

5.2 **Nouvelles avenues de recherche**

Il va sans dire qu'il reste beaucoup à découvrir quant à la taxonomie et la biologie des *Chironomus* et le devenir des éléments traces dans l'environnement aquatique. Pour faire suite à notre étude, voici d'autres idées de recherche qui seraient intéressantes à faire ou à poursuivre :

Identification des espèces de *Chironomus*

- Identifier les larves qui ont été étiquetées *C*. sp. NAI, *C*. sp. NAII et *C*. sp. NAIII lors de cette étude.
- Continuer à explorer l'idée que les larves étiquetées comme étant *C. bifurcatus* puissent représenter deux espèces différentes.
- Séquencer toutes les espèces de *Chironomus* listées pour que leur identification soit plus accessible. Je tiens à préciser que la taxonomie des *Chironomus* est en grande partie basée sur l'analyse des chromosomes polytènes, une technique qui ne compte que quelques experts dans le monde qui approchent un certain âge. Il y a donc un besoin criant de former d'autres gens dans ce domaine.
- Puisque le gène mitochondrial *cox1* ne peut être utilisé pour séparer toutes les espèces de *Chironomus* et que l'utilisation et l'amplification de la séquence nucléaire *gb2β* sont problématiques, il serait intéressant d'explorer l'utilisation d'un autre code-barre nucléaire pour l'identification d'espèces de *Chironomus.*

Comportement alimentaire des espèces de *Chironomus*

- Confirmer le comportement alimentaire de *C. dilutus*, *C. harpi*, *C*. nr *atroviridis* (sp. 2i), *C. ochreatus, C*. sp. NAI et *C*. sp. NAII à l'aide de signatures isotopiques de soufre dans d'autres lacs, puisque dans notre étude, ces espèces n'ont été récoltées qu'à un seul endroit.
- Établir le comportement alimentaire d'autres espèces afin que notre clé morphologique sur le comportement alimentaire des larves de *Chironomus* puisse englober d'autres espèces et ainsi être utilisée dans d'autres régions.

Différences de concentrations d'éléments traces entre espèces sympatriques de *Chironomus*

- À l'aide d'expériences, confirmer que les différences de concentrations de Cd, Zn et Se entre espèces sympatriques de *Chironomus* ne sont pas aussi dictés par des différences physiologiques pouvant affecter le taux d'assimilation (k_u, EA, TI) ou de perte (k_e, k_g) de ces éléments traces par les larves.

Chironomus en tant que biomoniteurs des sédiments

- Par le biais d'expériences, confirmer que les larves de *Chironomus* assimilent majoritairement l'As, Ba, Cd, Co, Cu, Mn, Ni et Zn de leur nourriture et non de leur exposition à l'eau.
- Établir les seuils pour lesquels les concentrations d'éléments traces chez les larves deviennent létales. Ces données pourraient être grandement utiles lors d'analyse d'impact environnemental.

Biodisponibilité des éléments traces dans les sédiments

- À l'aide de mesures d'éléments traces chez les larves de *Chironomus*, déterminer si dans d'autres régions le Cd est toujours plus biodisponible dans les sédiments oxiques et le sélénium est toujours plus biodisponible dans les sédiments anoxiques.
- Puisqu'on a maintenant une idée du comportement alimentaire des larves, on pourrait essayer de relier les concentrations larvaires d'éléments traces à celles d'autres phases sédimentaires.

6 RÉFÉRENCES

Agapow, P. M., Bininda-Emonds O. R. P., Crandall K. A., Gittleman J. L., Mace G. M., Marshall J. C. & Purvis A. (2004) The impact of species concept on biodiversity studies. *Quarterly Review of Biology* 79(2):161-179.

Alexander, L. C., Delion M., Hawthorne D. J., Lamp W. O. & Funk D. H. (2009) Mitochondrial lineages and DNA barcoding of closely related species in the mayfly genus *Ephemerella* (Ephemeroptera: Ephemerellidae). *Journal of the North American Benthological Society* 28(3):584-595.

Andersen, F. S. (1949) On the subgenus *Chironomus*. Studies on the systematics and biology of Chironomidae III. *Videnskabelige Meddelser fra Dansk Naturhistorisk Forening* III:1-66.

Ankley, G. T. (1996) Evaluation of metal/acid-volatile sulfide relationships in the prediction of metal bioaccumulation by benthic macroinvertebrates. *Environmental Toxicology and Chemistry* 15(12):2138-2146.

Ankley, G. T., Di Toro D. M., Hansen D. J. & Berry W. J. (1996) Technical basis and proposal for deriving sediment quality criteria for metals. *Environmental Toxicology and Chemistry* 15(12):2056-2066.

Armitage, P., Cranston P. S. & Pinder L. C. V. (1995) *The Chironomidae - The biology and ecology of non-biting midges.* Chapman & Hall. 1-572 p.

Ball, S. L., Hebert P. D. N., Burian S. K. & Webb J. M. (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society* 24(3):508-524.

Béchard, K. M., Gillis P. L. & Wood C. M. (2008) Trophic transfer of Cd from larval chironomids (*Chironomus riparius*) exposed via sediment or waterborne routes, to zebrafish (*Danio rerio*): Tissue-specific and subcellular comparisons. *Aquatic Toxicology* 90(4):310-321.

Beeby, A. (2001) What do sentinels stand for? *Environmental Pollution* 112:285-298.

Berg, K (1938) Studies on the bottom animals of Esrom Lake. *Kungelige Danske Videnskabernes Selskabs Skrifter* 9:1-255.

Berg, M. B. (1995) Larval food and feeding behaviour. *The Chironomidae: The biology and ecology of non-biting midges*, Armitage P, Cranston PS & Pinder LCV (Édit.) Chapman & Hall, London. p 136-168.

Bertrand, K. (2012) *Suivi temporel des effets d'un déversement de mazout sur la communauté benthique de la zone portuaire de Parry Sound*. Mémoire de maîtrise en Sciences de l'eau (Université du Québec. Institut national de la recherche scientifique, Québec). 95 p

Bervoets, L. & Blust R. (2000) Effects of pH on cadmium and zinc uptake by the midge larvae *Chironomus riparius*. *Aquatic Toxicology* 49(1-2):145-157.

Bervoets, L., Blust R. & Verheyen R. (1996) Effect of temperature on cadmium and zinc uptake by the midge larvae *Chironomus riparius*. *Archives of Environmental Contamination and Toxicology* 31(4):502-511.

Bervoets, L., Solis D., Romero A. M., Damme P. A. V. & Ollevier F. (1998) Trace metal levels in chironomid larvae and sediments from a Bolivian river: Impact of mining activities. *Ecotoxicology and Environmental Safety* 41(3):275-283.

Bidwell, J. R. & Gorrie J. R. (2006) The influence of salinity on metal uptake and effects in the midge *Chironomus maddeni*. *Environmental Pollution* 139(2):206-213.

Bonham-Carter, G. F., Henderson P. J., Kliza D. A. & Kettles I. M. (2006) Comparison of metal distributions in snow, peat, lakes and humus around a Cu smelter in western Quebec, Canada. *Geochemistry: Exploration, Environment, Analysis* 6(2-3):215-228.

Borgmann, U., Grapentine L., Norwood W. P., Bird G., Dixon D. G., Lindeman D., Chapman P. M., Goulet R. R. & Wang F. (2005) Sediment toxicity testing with the freshwater amphipod *Hyalella azteca*: Relevance and application. *Chemosphere* 61(11):1740-1745.

Borgmann, U., Norwood W. P. & Dixon D. G. (2004a) Re-evaluation of metal bioaccumulation and chronic toxicity in *Hyalella azteca* using saturation curves and the biotic ligand model. *Environmental Pollution* 131(3):469-484.

Borgmann, U., Norwood W. P., Reynoldson T. B. & Rosa F. (2001) Identifying cause in sediment assessments: bioavailability and the Sediment Quality Triad. *Canadian Journal of Fisheries and Aquatic Sciences* 58(5):950-960.

Borgmann, U., Nowierski M., Grapentine L. C. & Dixon D. G. (2004b) Assessing the cause of impacts on benthic organisms near Rouyn-Noranda, Quebec. *Environmental Pollution* 129(1):39-48.

Brunner, B. & Bernasconi S. M. (2005) A revised isotope fractionation model for dissimilatory sulfate reduction in sulfate reducing bacteria. *Geochimica et Cosmochimica Acta* 69(20):4759-4771.

Bryan, G. W. & Gibbs P. E. (1987) Polychaetes as indicators of heavy-metal availability in marine deposits. *Oceanic Processes in Marine Pollution*, Capuzzo JM, Kester DR & Duedall IW (Édit.) Kreiger Publishing Compagny, Malabar, Florida Vol 1. p 37-49.

Butler, M. G. (1982) Production dynamics of some artic *Chironomus* larvae. *Limnology and Oceanography* 27(4):728-736.

Butler, M. G., Kiknadze I. I., Cooper J. K. & Siirin M. T. (1995) Cytologically identified *Chironomus* species from lakes in North Dakota and Minnesota, USA. *Chironomids, from Gene to Ecosystems - Proceedings of the 12th International Symposium on Chironomidae (Canberra, January 23-26, 1994)*, Cranston PS (Édit.) CSIRO, Canberra. p 31-37.

Butler, MG, Rezanka RL & Giovannelli KM (2000) Utility of species level identification of *Chironomus* larvae in biological assessment of prairie lakes. *Verhandlungen der Internationalen Vereinigung für Limnologie* 27:273-277.

Cain, D. J., Luoma S. N. & Wallace W. G. (2004) Linking metal bioaccumulation of aquatic insects to their distribution patterns in a mining-impacted river. *Environmental Toxicology and Chemistry* 23(6):1463-1473.

Campbell, P. G. C. (1995) Interaction between trace metals and aquatic organisms: a critique of the free-ion activity model. *Metal speciation and aquatic systems*, Tessier A & Turner DR (Édit.) John Wiley, New York. p 45-102.

Campbell, P. G. C., Chapman P. M. & Hale B. A. (2006) Risk assessment of metals in the environment. *Chemicals in the Environment: Assessing and Managing Risk*, Hester RE & Harrison RM (Édit.) Royal Society of Chemistry, Cambridge (UK). p 102-131.

Campbell, P. G. C. & Couillard Y. (2004) Prise en charge et détoxification des métaux chez les organismes aquatiques. *Écotoxicologie moléculaire - Principes fondamentaux et perspectives de développement*, Pelletier E, Campbell PGC & Denizeau F (Édit.) Presses de l'Université du Québec, Québec. p 10-61.

Carew, M. E., Marshall S. E. & Hoffmann A. A. (2011) A combination of molecular and morphological approaches resolves species in the taxonomically difficult genus *Procladius* Skuse (Diptera: Chironomidae) despite high intra-specific morphological variation. *Bulletin of Entomological Research* 101(5):505-519.

Carew, M. E., Pettigrove V., Cox R. L. & Hoffmann A. A. (2007) DNA identification of urban Tanytarsini chironomids (Diptera:Chironomidae). *Journal of the North American Benthological Society* 26(4):587-600.

Carew, M. E., Pettigrove V. & Hoffmann A. A. (2003) Identifying chironomids (Diptera: Chironomidae) for biological monitoring with PCR-RFLP. *Bulletin of Entomological Research* 93(6):483-490.

Carew, M. E., Pettigrove V. J., Metzeling L. & Hoffmann A. A. (2013) Environmental monitoring using next generation sequencing: Rapid identification of macroinvertebrate bioindicator species. *Frontiers in Zoology* 10(1).

Carew, M. E., Schiffer M., Umina P., Weeks A. & Hoffmann A. (2009) Molecular markers indicate that the wheat curl mite, *Aceria tosichella* Keifer, may represent a species complex in Australia. *Bulletin of Entomological Research* 99(5):479-486.

CCME (2014) *Canadian Environmental Quality Guidelines.* http://ceqg-rcqe.ccme.ca/ (consulté le May 27, 2014).

Charbonneau, P. & Hare L. (1998) Burrowing behavior and biogenic structures of mud-dwelling insects. *Journal of the North American Benthological Society* 17(2):239-249.

Chételat, J., Amyot M., Cloutier L. & Poulain A. (2008) Metamorphosis in chironomids, more than mercury supply, controls methylmercury transfer to fish in high Arctic lakes. *Environmental Science and Technology* 42(24):9110-9115.

City of Greater Sudbury (2013) *Individual Lake Information.* http://www.greatersudbury.ca/living/lakes-facts/local-lake-descriptions/ (consulté le 17 September 2013).

Coplen, T. B., Hopple J. A. & Böhlke J. K. (2002) *Compilation of Minimum and Maximum Isotope Ratios of Selected Elements in Naturally Occuring Terrestrial Materials and Reagents.* U.S. Geological Survey Water-Resources Investigations Report 01-4222, Reston, Virginia

Craig, A., Hare L., Charest P. M. & Tessier A. (1998) Effect of exposure regime on the internal distribution of cadmium in *Chironomus staegeri* larvae (insecta, diptera). *Aquatic Toxicology* 41(3):265-275.

Craig, A., Hare L. & Tessier A. (1999) Experimental evidence for cadmium uptake via calcium channels in the aquatic insect *Chironomus staegeri*. *Aquatic Toxicology* 44(4):255-262.

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(5):1006-1015.

Croteau, M. N., Hare L. & Tessier A. (1998) Refining and testing a trace metal biomonitor (*Chaoborus*) in highly acidic lakes. *Environmental Science and Technology* 32(9):1348-1353.

Croteau, M. N., Hare L. & Tessier A. (2001) Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*. *Canadian Journal of Fisheries and Aquatic Sciences* 58(9):1737-1746.

Davies-Colley, R. J., Nelson P. O. & Williamson K. J. (1984) Copper and cadmium uptake by estuarine sedimentary phases. *Environmental Science and Technology* 18(7):491-499.

Davis, G. A., Havill N. P., Adelman Z. N., Caccone A., Kok L. T. & Salom S. M. (2011) DNA barcodes and molecular diagnostics to distinguish an introduced and native *Laricobius* (Coleoptera: Derodontidae) species in eastern North America. *Biological Control* 58(1):53-59.

De Jonge, M., Dreesen F., De Paepe J., Blust R. & Bervoets L. (2009) Do acid volatile sulfides (AVS) influence the accumulation of sediment-bound metals to benthic invertebrates under natural field conditions? *Environmental Science and Technology* 43(12):4510-4516.

Deines, P., Bodelier P. L. E. & Eller G. (2007) Methane-derived carbon flows through methane-oxidizing bacteria to higher trophic levels in aquatic systems. *Environmental Microbiology* 9(5):1126-1134.

Desrosiers, M., Gagnon C., Masson S., Martel L. & Babut M. P. (2008) Relationships among total recoverable and reactive metals and metalloid in St. Lawrence River sediment: Bioaccumulation by chironomids and implications for ecological risk assessment. *Science of the Total Environment* 389(1):101-114.

Di Toro, D. M., McGrath J. A., Hansen D. J., Berry W. J., Paquin P. R., Mathew R., Wu K. B. & Santore R. C. (2005) Predicting sediment metal toxicity using a sediment biotic ligand model: Methodology and initial application. *Environmental Toxicology and Chemistry* 24(10):2410-2427.

Dinsmore, W. P. & Prepas E. E. (1997) Impact of hypolimnetic oxygenation on profundal macroinvertebrates in a eutrophic lake in central Alberta. II Changes in *Chironomus* spp. abundance and biomass. *Canadian Journal of Fisheries and Aquatic Sciences* 54(9):2170-2181.

Dubois, M. & Hare L. (2009a) Selenium assimilation and loss by an insect predator and its relationship to Se subcellular partitioning in two prey types. *Environmental Pollution* 157(3):772-777.

Dubois, M. & Hare L. (2009b) Subcellular distribution of cadmium in two aquatic invertebrates: Change over time and relationship to Cd assimilation and loss by a predatory insect. *Environmental Science and Technology* 43(2):356-361.

Dumas, J. & Hare L. (2008) The internal distribution of nickel and thallium in two freshwater invertebrates and its relevance to trophic transfer. *Environmental Science and Technology* 42(14):5144-5149.

Ekrem, T., Willassen E. & Stur E. (2007) A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution* 43(2):530-542.

Ekrem, T., Willassen E. & Stur E. (2010) Phylogenetic utility of five genes for dipteran phylogeny: A test case in the Chironomidae leads to generic synonymies. *Molecular Phylogenetics and Evolution* 57(2):561-571.

Elbaz-Poulichet, F., Garnier J. M., Guan D. M., Martin J. M. & Thomas A. J. (1996) The conservative behaviour of trace metals (Cd, Cu, Ni and Pb) and as in the surface plume of stratified estuaries: Example of the Rhone river (France). *Estuarine, Coastal and Shelf Science* 42(3):289-310.

Elderkin, C. L., Corkum L. D., Bustos C., Cunningham E. L. & Berg D. J. (2012) DNA barcoding to confirm morphological traits and determine relative abundance of burrowing mayfly species in western Lake Erie. *Journal of Great Lakes Research* 38(1):180-186.

Eller, G., Deines P., Grey J., Richnow H. H. & Krüger M. (2005) Methane cycling in lake sediments and its influence on chironomid larval $\delta^{13}C$. *FEMS Microbiology Ecology* 54(3):339-350.

Environnement Canada (1997) *Méthode d'essai biologique: essaie de survie et de croissance des larves dulcicoles de chironomes (Chironomus tentans ou Chironomus riparius) dans les sédiments.* Série de la Protection de l'environnement, Ottawa (Canada). 156 p.

Epler, J. H. (2001) *Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida.* Special Publication SJ2001-SP13 North Carolina Department of Environmental and Natural Resources, Raleigh, NC, and St. John's River Water Management District, Palatka, FL. 526 p.

Fialkowski, W., Klonowska-Olejnik M., Smith B. D. & Rainbow P. S. (2003) Mayfly larvae (*Baetis rhodani* and *B. vernus*) as biomonitors of trace metal pollution in streams of a catchment draining a zinc and lead mining area of Upper Silesia, Poland. *Environmental Pollution* 121(2):253-267.

Filion, A. & Morin A. (2000) Effect of local sources on metal concentrations in littoral sediments and aquatic macroinvertebrates of the St. Lawrence River, near Cornwall, Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 57(SUPPL. 1):113-125.

Folmer, O., Black M., Hoeh W., Lutz R. & Vrijenhoek R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3(5):294-299.

Fox, H.M. (1955) The effect of oxygen on the concentration of haem in invertebrates. *Proceedings of the Royal Society of London* 143:203-214.

Freeland, J. R. (2005) *Molecular Ecology.* Wiley, Chichester (England). 388 p.

Gillis, P. L., Diener L. C., Reynoldson T. B. & Dixon D. G. (2002) Cadmium-induced production of a metallothioneinlike protein in *Tubifex tubifex* (oligochaeta) and *Chironomus riparius* (diptera): Correlation with reproduction and growth. *Environmental Toxicology and Chemistry* 21(9):1836-1844.

Gillis, P. L., Reynoldson T. B. & Dixon D. G. (2006) Metallothionein-like protein and tissue metal concentrations in invertebrates (Oligochaetes and Chironomids) collected from reference and metal contaminated field sediments. *Journal of Great Lakes Research* 32(3):565-577.

Gillis, P. L. & Wood C. M. (2008) Investigating a potential mechanism of Cd resistance in *Chironomus riparius* larvae using kinetic analysis of calcium and cadmium uptake. *Aquatic Toxicology* 89(3):180-187.

Grey, J. & Deines P. (2005) Differential assimilation of methanotrophic and chemoautotrophic bacteria by lake chironomid larvae. *Aquatic Microbial Ecology* 40(1):61-66.

Grey, J., Kelly A. & Jones R. I. (2004) High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. *Limnology and Oceanography* 49(1):239-244.

Guryev, V., Makarevitch I., Blinov A. & Martin J. (2001) Phylogeny of the genus *Chironomus* (Diptera) inferred from DNA sequences of mitochondrial *Cytochrome b* and *Cytochrome oxidase I*. *Molecular Phylogenetics and Evolution* 19(1):9-21.

Guryev, V. P. & Blinov A. G. (2002) Phylogenetic relationships among Holarctic populations of *Chironomus entis* and *Chironomus plumosus* in view of possible horisontal transfer of mitochondrial genes. *Russian Journal of Genetics* 38(3):239-243.

Habicht, K.S., Gade M., Thamdrup B., Berg P. & Canfield D. E. (2002) Calibration of sulfate levels in the Archean ocean. *Science* 298(5602):2372-2374.

Hankeln, T., Friedl H., Ebersberger I., Martin J. & Schmidt E. R. (1997) A variable intron distribution in globin genes of *Chironomus*: Evidence for recent intron gain. *Gene* 205(1-2):151-160.

Hare, L., Tessier A. & Warren L. (2001) Cadmium accumulation by invertebrates living at the sediment-water interface. *Environmental Toxicology and Chemistry* 20(4):880-889.

Harnisch, O. (1942) Die sogenannten "Blutkiemen" der Larven der Gattung *Chironomus* Mg. *Biologia Generalis* 16:593-609.

Hausdorf, B. (2011) Progress toward a general species concept. *Evolution* 65(4):923-931.

Hebert, P. D. N., Cywinska A., Ball S. L. & DeWaard J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270(1512):313-321.

Hecky, R. E. & Hesslein R. H. (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society* 14(4):631-653.

Hilsenhoff, W. L. (1966) The biology of *Chironomus plumosus* in Lake Winnebago, Wisconsin. *Annals of the Entomological Society of America* 59:465-473.

Hodkinson, I. D. & Williams K. A. (1980) Tube formation and distribution of *Chironomus plumosus* L. (Diptera: Chironomidae) in a eutrophic woodland pond. *Chironomidae: Ecology, Systematics, Cytology and Physiology*, Murray DA (Édit.) Pergamon Press, Oxford. p 331-337.

Hogg, I. D. & Hebert P. D. N. (2004) Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology* 82(5):749-754.

Jansson, M., Persson L., De Roos A. M., Jones R. I. & Tranvik L. J. (2007) Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends in Ecology and Evolution* 22(6):316-322.

Jardine, T. D., Kidd K. A. & Fisk A. T. (2006) Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environmental Science and Technology* 40(24):7501-7511.

Jónasson, P. M. (1972) Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. *Oikos Supplement* 14:1-148.

Jónasson, P. M. (2003) Hypolimnetic eutrophication of the N-limited dimictic L. Esrom 1908-1998: Pelagic-benthic coupling effects between phytoplancton and profundal zoobenthos, its growth, respiration and survival. *Archiv für Hydrobiologie. Supplement volumes, Monographic studies* 139(4):449-512.

Jones, R. I. (1992) The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia* 229(1):73-91.

Jones, R. I., Carter C. E., Kelly A., Ward S., Kelly D. J. & Grey J. (2008) Widespread contribution of methane-cycle bacteria to the diets of lake profundal chironomid larvae. *Ecology* 89(3):857-864.

Jones, R. I. & Grey J. (2004) Stable isotope analysis of chironomid larvae from some Finnish forest lakes indicates dietary contribution from biogenic methane. *Boreal Environment Research* 9(1):17-23.

Jones, R. I. & Grey J. (2011) Biogenic methane in freshwater food webs. *Freshwater Biology* 56(2):213-229.

Kao, W. Y., Trewitt P. M. & Bergtrom G. (1994) Intron-containing globin genes in the insect *Chironomus thummi*. *Journal of Molecular Evolution* 38:241-249.

Keller, W., Heneberry J., Gunn J. M., Morgan G. & Leduc J. (2004) Recovery of acid and metal - damaged lakes near Sudbury Ontario: trends and status. (Cooperative Freswater Ecology Unit, Department of Biology, Laurentian University, Sudbury, Ontario), p 1-53.

Keller, W. & Yan N. D. (1991) Recovery of crustacean zooplankton species richness in Sudbury area lakes following water quality improvements. *Canadian Journal of Fisheries and Aquatic Sciences* 48(9):1635-1644.

Kelly, A., Jones R. I. & Grey J. (2004) Stable isotope analysis provides fresh insights into dietary separation between *Chironomus anthracinus* and *C. plumosus*. *Journal of the North American Benthological Society* 23(2):287-296.

Kiknadze, I. I., Aimanova K. G., Butler M. & Cooper K. (1993) The pattern of the reduction of the chromosome number in the chironomid evolution (In Russian). *Tsitologiya* 35:96-104.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16(2):111-120.

Lacharité, J (2011) *Éléments traces dans le biomoniteur Hexagenia limbata provenant de lacs de la région de Rouyn-Noranda, Québec*. Mémoire de maîtrise en Sciences de l'eau (Université du Québec. Institut national de la recherche scientifique, Québec). 94 p

Langston, W. J. (1980) Arsenic in U.K. estuarine sediments and its availability to benthic organisms. *Journal of the Marine Biological Association of the United Kingdom* 60(4):869-881.

Langston, W. J. (1982) The distribution of mercury in British estuarine sediments and its availability to deposit-feeding bivalves. *Journal - Marine Biological Association* 62(3):667-684.

Lemly, A. D. (2002) A procedure for setting environmentally safe total maximum daily loads (TMDLs) for selenium. *Ecotoxicology and Environmental Safety* 52(2):123-127.

Leonard, E. M., Pierce L. M., Gillis P. L., Wood C. M. & O'Donnell M. J. (2009) Cadmium transport by the gut and Malpighian tubules of *Chironomus riparius*. *Aquatic Toxicology* 92(3):179-186.

Li, Y., Zhou X., Feng G., Hu H., Niu L., Hebert P. D. N. & Huang D. (2010) COI and ITS2 sequences delimit species, reveal cryptic taxa and host specificity of fig-associated *Sycophila* (Hymenoptera, Eurytomidae). *Molecular Ecology Resources* 10(1):31-40.

Lindeberg, B. & Wiederholm T. (1979) Notes on the taxonomy of European species of *Chironomus* (Diptera Chironomidae). *Entomologica Scandinavica Supplements* 10:99-116.

Linnaeus, C. (1758) *Systema naturae per regna tria naturae (Vol. 1, 10th ed.).* Laurentii Salvii, Stockholm

Lobel, P. B., Belkhode S. P., Jackson S. E. & Longerich H. P. (1990) Recent taxonomic discoveries concerning the mussel *Mytilus*: Implications for biomonitoring. *Archives of Environmental Contamination and Toxicology* 19(4):508-512.

Lundbeck, W. (1898) Diptera groenlandica. *Videnskabelige Meddelelser fra den Naturhistorisk Forening i Kjøbenhavn* 1898:236-314.

Luoma, S. N. (1989) Can we determine the biological availability of sediment-bound trace elements? *Sediment/water interactions. Proc 4th symposium, Melbourne, 1987*:379-396.

Luoma, S. N. & Bryan G. W. (1978) Factors controlling the availability of sediment-bound lead to the estuarine bivalve *Scrobicularia plana*. *Journal of the Marine Biological Association of the United Kingdom* 58(4):793-802.

Luoma, S. N. & Rainbow P. S. (2005) Why is metal bioaccumulation so variable? Biodynamics as a unifying concept. *Environmental Science and Technology* 39(7):1921-1931.

Luoma, S. N. & Rainbow P. S. (2008) *Metal contamination in aquatic environments: science and lateral management.* Cambrige University Press, New York. 573 p.

Ma, J., Sengupta M. K., Yuan D. & Dasgupta P. K. (2014) Speciation and detection of arsenic in aqueous samples: A review of recent progress in non-atomic spectrometric methods. *Anal. Chim. Acta* 831:1-23.

Mahony, J. D., Di Toro D. M., Gonzalez A. M., Curto M., Dilg M., De Rosa L. D. & Sparrow L. A. (1996) Partitioning of metals to sediment organic carbon. *Environmental Toxicology and Chemistry* 15(12):2187-2197.

Malloch, J.R. (1915) The Chironomidae, or midges, of Illinois, with particular reference to the species occuring in the Illinois River. *Bulletin of the Illinois State Laboratory of Natural History* 10:275-543.

Malloch, J.R. (1934) III. Chironomidae, Sciaridae, Phoridae, Syrphidae, Oestridae, Piophilidae, Helomyzidae, Calliphoridae and Tachinidae. *The Exploration of Southampton Island, Hudson Bay, by G.M. Sutton Supposed by J.B. Semple 1929-1930*, Holland WJ (Édit.) Memoirs of the Carnegie Museum, Vol 12. p 13-32.

Martin, J. (1979) Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera). *Entomologica Scandinavica Supplements* 10:67-74.

Martin, J. (2011) From bands to base pairs: Problems in the identification of species using the example of *Chironomus oppositus* Walker (Honorary Thienemann Lecture). *Contemporary Chironomid Studies - Proceedings of the 17th International Symposium on Chironomidae (July 6-9, 2009 Nankai University, China)*, Wang X & Liu W (Édit.) Nankai University Press, Tianjin. p 126-143.

Martin, J. (2013) *North American species of the genus Chironomus.* http://genetics.unimelb.edu.au/Martin/NACytfiles/NAChiron.html (consulté le 17 septembre 2013).

Martin, J., Andreeva E. N., Kiknadze I. I. & Wülker W. F. (2006) Polytene chromosomes and phylogenetic relationships of *Chironomus atrella* (Diptera: Chironomidae) in North America. *Genome* 49(11):1384-1392.

Martin, J., Guryev V. & Blinov A. (2002) Population variability in *Chironomus* (*Camptochironomus*) species (Diptera, Nematocera) with a Holarctic distribution: Evidence of mitochondrial gene flow. *Insect Molecular Biology* 11(5):387-397.

Martin, S., Proulx I. & Hare L. (2008) Explaining metal concentrations in sympatric *Chironomus* species. *Limnology and Oceanography* 53(2):411-419.

McCall, P. L. & Tevesz M. J. S. (1982) The Effects of Benthos on Physical Properties of Freshwater Sediments. *Animal-Sediment Relations. The Biogenic Alteration of Sediments*, Mccall PL & Tevesz MJS (Édit.) Plenum Press, New York. p 105-176.

McCutchan, J. H, Lewis W. M., Kendall C. & McGrath C. C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102(2):378-390.

Merritt, R.W., Cummins K.W. & Berg M.B. (2008) *An Introduction to the Aquatic Insects of North America.* Kendall/Hunt Publishing, Iowa, 4th

Ministère du Développement durable de l'Environnement et des Parcs (2012) *Le réseau de surveillance volontaire des lacs.* http://www.mddep.gouv.qc.ca/eau/rsvl (consulté le 20 August 2012).

Moore, P. G. & Rainbow P. S. (1987) Copper and zinc in an ecological series of talitroidean Amphipoda (Crustacea). *Oecologia* 73(1):120-126.
Munger, C. & Hare L. (1997) Relative importance of water and food as cadmium sources to an aquatic insect (Chaoborus punctipennis): Implications for predicting Cd bioaccumulation in nature. *Environmental Science and Technology* 31(3):891-895.
Muscatello, J. R. & Janz D. M. (2009) Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. *Science of the Total Environment* 407(4):1318-1325.
Norwood, W. P., Borgmann U. & Dixon D. G. (2007) Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyalella azteca* in relation to exposure and bioaccumulation. *Environmental Pollution* 147(1):262-272.
Nriagu, J. O. (1989) A global assessment of natural sources of atmospheric trace metals. *Nature* 338(6210):47-49.
Nriagu, J. O., Wong H. K. T. & Coker R. D. (1982) Deposition and chemistry of pollutant metals in lakes around the smelters at sudbury, Ontario. *ES and T Contents* 16(9):551-560.
Nriagu, J. O., Wong H. K. T., Lawson G. & Daniel P. (1998) Saturation of ecosystems with toxic metals in Sudbury basin, Ontario, Canada. *Science of the Total Environment* 223(2-3):99-117.
Osmulski, P. A. & Leyko W. (1986) Structure, function and physiological role of *Chironomus* haemoglobin. *Comparative Biochemistry and Physiology* 85B(4):701-722.
Panis, L.I., Goddeeris B. & Verheyen R. (1995) The hemoglobin concentration of *Chironomus* cf. *plumosus* L. (Diptera: Chironomidae) larvae from two lentic habitats. *Netherlands Journal of Aquatic Ecology* 29(1):1-4.
Perceval, O., Couillard Y., Pinel-Alloul B., Bonneris E. & Campbell P. G. C. (2006) Long-term trends in accumulated metals (Cd, Cu and Zn) and metallothionein in bivalves from lakes within a smelter-impacted region. *Science of the Total Environment* 369(1-3):403-418.
Péry, A. R. R., Geffard A., Conrad A., Mons R. & Garric J. (2008) Assessing the risk of metal mixtures in contaminated sediments on Chironomus riparius based on cytosolic accumulation. *Ecotoxicology and Environmental Safety* 71(3):869-873.
Péry, A. R. R., Mons R. & Garric J. (2005) Chironomus riparius solid-phase assay. *Small-Scale Freshwater Toxicity Investigations: Volume 1 - Toxicity Test Methods,* Springer Netherlands. p 437-451.
Peterson, B. J. (1999) Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. *Acta Oecologica* 20(4):479-487.
Peterson, B. J., Howarth R. W. & Garritt R. H. (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science* 227(4692):1361-1363.
Petrin, Z., Laudon H. & Malmqvist B. (2007) Does freshwater macroinvertebrate diversity along a pH-gradient reflect adaptation to low pH? *Freshwater Biology* 52(11):2172-2183.
Pfenninger, M., Nowak C., Kley C., Steinke D. & Streit B. (2007) Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. *Molecular Ecology* 16(9):1957-1968.
Polukonova, N. V., Djomin A. G., Mugue N. S. & Shaikevich E. V. (2009) Comparison of *Chironomus usenicus* and *Chironomus curabilis* with species of the group *plumosus* (Diptera) inferred from the mitochondrial DNA gene *COI* and by the polytene chromosomes banding pattern. *Russian Journal of Genetics* 45(8):899-905.
Ponton, D. E. & Hare L. (2009) Assessment of nickel contamination in lakes using the phantom midge *Chaoborus* as a biomonitor. *Environmental Science and Technology* 43(17):6529-6534.
Ponton, D. E. & Hare L. (2013) Relating selenium concentrations in a planktivore to selenium speciation in lakewater. *Environmental Pollution* 176:254-260.
Proulx, I. & Hare L. (2012) Mesures des concentraitons en éléments traces dans des sédiments et des invertébrés benthiques afin d'estimer leurs biodisponibilité près des sites miniers dans la région d'Oujé-Bougoumou. *Rapport de recherche (R1195)* (INRS Eau Terre Environnement, Québec (Canada)), p 53.
Proulx, I. & Hare L. (2014) Differences in feeding behaviours among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae. *Freshwater Biology* 59:73-86.
Proulx, I., Martin J., Carew M. & Hare L. (2013) Using various lines of evidence to identify *Chironomus* species in eastern Canadian lakes. *Zootaxa* 3741(4):401-458.

Pyle, G. G., Rajotte J. W. & Couture P. (2005) Effects of industrial metals on wild fish populations along a metal contamination gradient. *Ecotoxicology and Environmental Safety* 61(3):287-312.
Rainbow, P. S., Huang Z. G., Yan S.-K. & Smith B. (1993) Barnacles as biomonitors of trace metals in the coastal waters near Xiamen, P. R. China. *Asian Marine Biology* 10:109-121.
Rainbow, P. S., Kriefman S., Smith B. D. & Luoma S. N. (2011) Have the bioavailabilities of trace metals to a suite of biomonitors changed over three decades in SW England estuaries historically affected by mining? *Science of the Total Environment* 409(8):1589-1602.
Rasmussen, J. B., Gunn J. M., Sherwood G. D., Iles A., Gagnon A., Campbell P. G. C. & Hontela A. (2008) Direct and indirect (foodweb mediated) effects of metal exposure on the growth of yellow perch (*Perca flavescens*): Implications for ecological risk assessment. *Human and Ecological Risk Assessment* 14(2):317-350.
Redecker, B. & Zebe E. (1988) Anaerobic metabolism in aquatic insect larvae: studies on Chironomus thummi and Culex pipiens. *J Comp Physiol B* 158(3):307-315.
Reynolds, C. S. (2008) A changing paradigm of pelagic food webs. *International Review of Hydrobiology* 93(4-5):517-531.
Sæther, O. A. (2012) The *Chironomus* group (Diptera: Chironomidae) in Lake Winnipeg, Canada. *Zootaxa* 3275:1-19.
Saitou, N. & Nei M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4):406-425.
Scholz, F. & Zerbst-Boroffka I. (1998) Environmental hypoxia affects osmotic and ionic regulation in freshwater midge-larvae. *J. Insect Physiol.* 44(5-6):427-436.
Sharley, D. J., Pettigrove V. & Parsons Y. M. (2004) Molecular identification of *Chironomus* spp. (Diptera) for biomonitoring of aquatic ecosystems. *Australian Journal of Entomology* 43(4):359-365.
Shobanov, N. A. (1989) The morphological differentiation of *Chironomus* species of *plumosus* group (Diptera, Chironomidae). Larvae. *Acta Biologica Debrecina, Supplementum Oecologica Hungarica* 2:335-344.
Shobanov, N. A. (2002) Evolution of the genus *Chironomus* (Diptera, Chironomidae). 1. Ancestral form and major lines of phylogenesis. *Entomological Review* 82:487-493.
Shobanov, N. A., Kiknadze I. I. & Butler M. G. (1999) Palearctic and Nearctic *Chironomus* (*Camptochironomus*) *tentans* (Fabricius) are different species (Diptera: Chironomidae). *Insect Systematics and Evolution* 30(3):311-322.
Shobanov, N. A., Shilova A. I. & Belyanina S. I. (1996) Extent and content of the genus *Chironomus* Meig. (Diptera, Chironomidae): review of world fauna (In Russian). *Ecology, Evolution and Systematics of Chironomids*, Shobanov NA & Zinchenko TD (Édit.) Inst. Biol. Inland Waters & Inst. Ecol. Volga Basin, Russian Academy of Sciences, Tolyatti (Boruk, Russia). p 44-96.
Shuhaimi-Othman, M., Pascoe D., Borgmann U. & Norwood W. P. (2006) Reduced metals concentrations of water, sediment and *Hyalella azteca* from lakes in the vicinity of the sudbury metal smelters, Ontario, Canada. *Environmental Monitoring and Assessment* 117(1-3):27-44.
Sinclair, C. S. & Gresens S. E. (2008) Discrimination of *Cricotopus* species (Diptera: Chironomidae) by DNA barcoding. *Bulletin of Entomological Research* 98(6):555-563.
Stur, E. & Ekrem T. (2011) Exploring unknown life stages of Arctic Tanytarsini (Diptera: Chironomidae) with DNA barcoding. *Zootaxa* 2743:27-39.
Summons, R. E., Jahnke L. L. & Roksandic Z. (1994) Carbon isotopic fractionation in lipids from methanotrophic bacteria: Relevance for interpretation of the geochemical record of biomarkers. *Geochimica et Cosmochimica Acta* 58(13):2853-2863.
Swartz, R. C., Ditsworth G. R., Schults D. W. & Lamberson J. O. (1986) Sediment toxicity to a marine infaunal amphipod: Cadmium and its interaction with sewage sludge. *Marine Environmental Research* 18(2):133-153.
Tachet, H., Richoux P., Bournaud M. & Usseglio-Polatera P. (2002) *Invertébrés d'eau douce - systématique, biologie, écologie.* CNRS Éditions, Paris. 587 p.
Tamura, K., Peterson D., Peterson N., Stecher G., Nei M. & Kumar S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28(10):2731-2739.
Tessier, A., Campbell P. G. C. & Blsson M. (1979) Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry* 51(7):844-851.

Tessier, A., Couillard Y., Campbell P. G. C. & Auclair J. C. (1993) Modeling Cd partitioning in oxic lake sediments and Cd concentrations in the freshwater bivalve *Anodonta grandis*. *Limnology & Oceanography* 38(1):1-17.

Tessier, A., Fortin D., Belzile N., DeVitre R. R. & Leppard G. G. (1996) Metal sorption to diagenetic iron and manganese oxyhydroxides and associated organic matter: Narrowing the gap between field and laboratory measurements. *Geochimica et Cosmochimica Acta* 60(3):387-404.

Thompson, J. D., Higgins D. G. & Gibson T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22(22):4673-4680.

Townes, H. K., Jr. (1945) The Nearctic species of Tendipedini [Diptera, Tendipedidae (= Chironomidae)]. *American Midland Naturalist* 34:1-206.

Vander Zanden, M. J., Chandra S., Park S. K., Vadeboncoeur Y. & Goldman C. R. (2006) Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Canadian Journal of Fisheries and Aquatic Sciences* 63(12):2608-2620.

Walshe, B. M. (1950) The function of haemoglobin in *Chironomus plumosus* under natural conditions. *The Journal of Experimental Biology* 27:73-95.

Walshe, B. M. (1951) The feeding habits of certain chironomid larvae (subfamily Tendipendinae). *Proceedings of the Zoological Society of London* 121:63-79.

Wang, F., Chen J. & Forsling W. (1997) Modeling sorption of trace metals on natural sediments by surface complexation model. *Environmental Science and Technology* 31(2):448-453.

Wang, F., Goulet R. R. & Chapman P. M. (2004) Testing sediment biological effects with the freshwater amphipod *Hyalella azteca*: The gap between laboratory and nature. *Chemosphere* 57(11):1713-1724.

Wang, F. & Tessier A. (2009) Zero-valent sulfur and metal speciation in sediment porewaters of freshwater lakes. *Environmental Science and Technology* 43(19):7252-7257.

Webb, D. W. (1969) New species of chironomids from Costello Lake, Ontario (Diptera: Chironomidae). *Journal of the Kansas Entomological Society* 42:91-108.

Weber, R. E. & Vinogradov S. N. (2001) Nonvertebrate Hemoglobins: Functions and Molecular Adaptations. *Physiological Reviews* 81(2):569-628.

Wuelker, W. (2010) The role of chromosomes in chironomid systematics, ecology and phylogeny. *Proceedings of the XV International Symposium on Chironomidae.* (University of Minnesota, Saint Paul, Minnesota, Ferrington LC (Édit.) Chironomidae Research Group, p 1-13.

Wuelker, W., Martin J., Kiknadze I. I., Sublette J. E. & Michiels S. (2009) *Chironomus blaylocki* sp. n. and *C. bifurcatus* sp. n., North American species near the base of the *decorus*-group (Diptera: Chironomidae). *Zootaxa* 2023:28-46.

Wülker, W. F. & Butler M. G. (1983) Karyosystematics and morphology of Northern *Chironomus* (Diptera: Chironomidae): Freshwater species with larvae of the *salinarius*-type. *Entomologica Scandinavica* 14:121-136.

Wülker, W., Sublette J.E. & Martin J. (1991) *Chironomus utahensis* Malloch and *Chironomus harpi* new species and their karyosystematic relationships to other species in the *decorus*-group of *Chironomus*. *Spixiana* 14(1):71-94.

Zetterstedt, J. W. (1860) *Diptera Scandinaviae disposita et descripta. Tomus decimus quartus seu ultimus, continens addenda, corrigena & emendada tomis prioribus, una cum indice alphabetico navarum specierum hujus & praecedentis tomi, atque generico omnium tomorum*. *Lundae* 14:6497-6512.

7 ANNEXE A

MESURES DE CONCENTRATIONS D'HÉMOGLOBINE CHEZ LES LARVES

Introduction

Des études ont démontré que les concentrations d'hémoglobine chez les larves augmentaient lorsque la concentration d'oxygène diminuait dans leur environnement (Fox, 1955, Panis *et al.*, 1995). Nous avons cherché à établir si des mesures de concentrations d'hémoglobine chez les larves pouvaient être utilisées pour déterminer si celles-ci se nourrissent en milieu oxique ou anoxique. Notre hypothèse était que les espèces de *Chironomus* qui se nourrissent dans les sédiments anoxiques sont exposées à des concentrations d'oxygène plus faibles que celles qui se nourrissent de particules oxiques et que par conséquent, celles qui se nourrissent dans les sédiments anoxiques ont des concentrations d'hémoglobine plus élevées que celles qui se nourrissent de particules oxiques.

Méthodologie

Les larves congelées ont été décongelées, épongées et pesées. Elles furent étêtées et écrasées avec des pinces afin d'extraire une bonne partie de l'hémolymphe. Afin d'extraire le reste de l'hémolymphe, les larves furent centrifugées à 12 000 rpm pendant 12 minutes. Le fluide extrait fut dilué 100 fois avec une solution de NaCl (5%). Les concentrations totales d'hémoglobine dans l'hémolymphe des larves ont été mesurées à l'aide de la trousse *QuantiChrom™ Hemoglobin Assay* (BioAssay Systems, Hayward, CA, USA) et d'un spectrophotomètre (Varian Cary 50 MP5; Varian Inc., Palo Alto, CA). La qualité des résultats a été vérifiée par l'analyse de blancs, d'un contrôle (sang humain) et triplicata.

Résultats /Discussion

Nous avons mesuré des concentrations d'hémoglobine qui variaient de 12 à 83 mg/dl chez les larves (figure 7-1). À chaque site, les concentrations d'hémoglobine variaient entre espèces. En accord avec notre hypothèse, les espèces qui se nourrissaient dans les sédiments anoxiques (*C. bifurcatus* (▲), *C*. sp. NAIII (☆) et *C. « tigris »* (○)) avaient des concentrations d'hémoglobine plus élevées que les espèces qui se nourrissaient dans les sédiments oxiques (*C. entis* (●), *C. plumosus* (●) et *C. staegeri* (●).

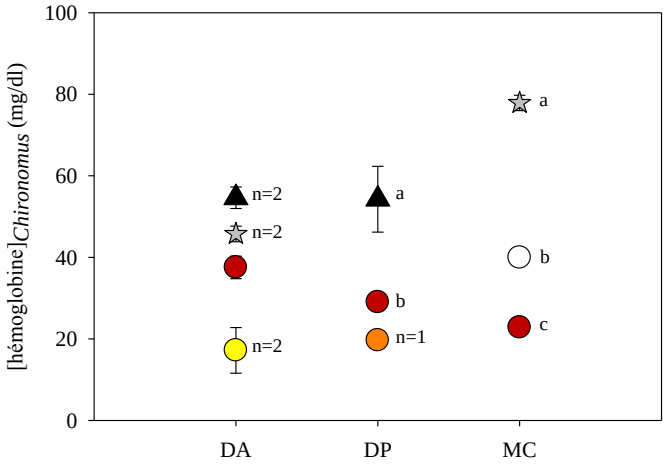


Figure 7-1 **Concentrations moyennes d'hémoglobine (mg/dl, ± écart-type, n=1-15) chez les larves d'espèces de *Chironomus* récoltées dans les lacs D'Alembert (DA), Duprat (DP) et McFarlane (MC). Chaque symbole représente une espèce différente (voir tableau 4-2). Dans un lac, il n'y a pas de différence significative (p>0.05) entre les concentrations d'hémoglobine chez les espèces dont les symboles sont suivis de la même lettre.**

Il est à noter que la taille et la grosseur des larves pourraient dicter les différences de concentrations d'hémoglobine entre espèces sympatriques de *Chironomus*. Si c'est le cas, on s'attendrait à ce que les larves plus grosses (c.-à-d., dont le ratio surface : volume est plus faible) aient des concentrations d'hémoglobine plus élevées que les autres larves. Cependant, dans notre étude, la tendance était contraire. Les larves généralement de plus grandes tailles et de plus grands poids (*C. entis*, *C. plumosus* et *C. staegeri*) avaient des concentrations d'hémoglobine moins élevées que les autres larves (*C. bifurcatus*, *C.* sp. NAIII et *C. « tigris »*).

Donc, nos résultats suggèrent que les larves qui mangent des sédiments anoxiques vivent dans un milieu moins bien oxygéné que celles qui ingèrent des particules oxiques. Voici quelques hypothèses pouvant expliquer cette tendance :

1- Les tunnels des espèces de *Chironomus* qui se nourrissent de sédiments anoxiques sont peut-être plus longs que ceux qui se nourrissent de particules oxiques et donc, plus difficile à oxygéner.
2- Les larves de *Chironomus* qui se nourrissent de sédiments anoxiques irriguent peut-être leurs tunnels moins souvent, moins longtemps et/ou moins efficacement que les larves qui se nourrissent de particules oxiques.
3- Pour se nourrir, les larves qui ingèrent des sédiments anoxiques doivent quitter leur tunnel oxygéné pour s'insérer dans les sédiments anoxiques.

Avant d'utiliser les concentrations d'hémoglobines chez les larves comme outil pour déterminer leur comportement alimentaire, ces diverses hypothèses mériteraient d'être testée afin de comprendre pourquoi les larves qui se nourrissent de particules anoxiques ont des concentrations d'hémoglobine plus basses que celles qui se nourrissent de particules oxiques. Il est à noter que différentes protéines d'hémoglobine ont été retrouvés dans l'hémolymphe d'individus de *Chironomus* (Weber *et al.*, 2001). La présence de ces différentes protéines peut différer selon l'espèce, le stade larvaire et les conditions environnementale (Weber *et al.*, 2001). Dans d'éventuels études, il serait intéressant de tester si la synthèse de différentes protéines d'hémoglobine change en fonction de la présence ou de l'absence d'oxygène. Finalement, la trousse utilisée pour mesurer les concentrations d'hémoglobine chez *Chironomus* a été développée pour mesurer les concentrations d'hémoglobine chez l'humain. Comme l'hémoglobine de *Chironomus* diffère de celle de l'humain[20], il resterait à vérifier que la méthode d'analyse permet de réellement mesurer les concentrations d'hémoglobine chez *Chironomus*.

Encadré 7-1 Mesures de concentrations d'hémoglobine pour expliquer des différences de couleur entre larves de la même espèce

Nous avons observé que la coloration des larves pouvait varier grandement entre individus d'une même espèce dont *C. staegeri*. Dans certains lacs, la coloration des larves de *C. staegeri* de 4^{e} stade larvaire vivant au même endroit variait d'un rouge orangé-pâle à un rouge pourpre-foncé. Puisque c'est la présence d'hémoglobine qui confère la couleur rouge aux larves, nous avons cherché à établir si cette différence de coloration pouvait être liée à une différence de taux d'hémoglobine chez les larves. Nous avons mesuré les concentrations d'hémoglobine chez des larves de *C. staegeri* de couleur différente récoltées au lac Duprat. Ces larves ne différaient pas au niveau de leur taux d'hémoglobine. Donc, selon ces résultats, il semblerait que les différences d'intensité de rouge chez les larves ne sont pas causées par des différences de concentrations d'hémoglobine.

[20] L'hémoglobine de *Chironomus* ne contient que deux unites d'hème alors que celle de l'humain en contient 4 (Merritt *etal*., 2008)

8 ANNEXE B

Tableau 8-1 **Comparaison des concentrations d'éléments traces chez des larves de *Chironomus* se nourrissant dans le même type de sédiment (oxique ou anoxique) récolté entre 2006 et 2010 par rapport à celles récoltées entre 1987 et 1993 dans des lacs à Rouyn-Noranda et à Sudbury. Augmentation (↑), diminution (↓) et pas de différence (=). n=1 signifie que nous avons des données que pour un lac.**

	Rouyn-Noranda		Sudbury	
	Larves qui ingèrent des sédiments oxiques	**Larves qui ingèrent des sédiments anoxiques**	**Larves qui ingèrent des sédiments oxiques**	**Larves qui ingèrent des sédiments anoxiques**
As	↑ dans DA = dans DP et MN	= (n=1)	↓ (n=1)	= dans RA et TI ↓ dans MC
Ba	↑ dans DA ↓ dans DP et MN	↓ (n=1)	↓ (n=1)	= dans MC et RA ↓ dans TI
Cd	↓	=	↑	↑ dans CL, RA et TI = dans MC
Co	↑ dans DA = dans DP et MN	= (n=1)	↑ (n=1)	↑ dans MC et RA = dans TI
Cu	↓	=	↑	↑ dans CL, RA et TI = dans MC
Mn	↑ dans DA = dans DP et MN	= (n=1)	= (n=1)	= dans RA et TI ↑ dans MC
Ni	↑ dans DA ↓ dans DP = MN	↓ (n=1)	= (n=1)	= dans MC et RA ↓ dans TI
Se	=	↓ (n=1)	pas de données	= dans MC et TI ↑ dans RA
Zn	= dans MN ↓ dans DA et DP	↓ dans DP = dans DA	↑ dans MC ↓ dans TI	= dans MC, RA et TI ↓ dans CL

SECTION 2 – Articles

ARTICLE 1

Why bother to identify animals used for contaminant monitoring?

Isabelle Proux et Landis Hare

Institut national de la recherche scientifique – Centre Eau Terre Environnement, 490 rue de la Couronne, Université du Québec, 490 rue de la Couronne, Québec (Québec), G1K 9A9, Canada

Publiée en 2008 dans Integrated *Environmental Assessment and Management* 4(1) : 125-126.

Measurements of contaminants in animals are an important component of ecological risk assessments because they provide the link between contaminant exposure and toxicity. Moreover, such measurements can provide a means of estimating the contamination level of whole ecosystems (Rainbow 2002, Hare *et al*. 2008). Before measuring bioaccumulation, animals need to be identified. However, detailed identifications take time and, where communities are diverse and animals are of small size, large numbers of individuals can be required to obtain a mass that is sufficient for contaminant analyses. One way around these constraints is to group related animal species. However, in doing so, one presumes that contaminant concentrations do not differ markedly among the pooled species. If they did, then changes in species composition from one site to another, or over time, could hide real trends in contaminant concentrations or create erroneous ones.

With these facts in mind, we measured cadmium (Cd) in the aquatic fly *Chironomus* (Diptera, Chironomidae) to determine if species in this widespread and ecologically-important genus vary markedly in their accumulation of this metal at a given site and among lakes. We chose Cd because it is readily accumulated by animals, is very toxic and is present at worrisome concentrations in some mining areas, agricultural soils and forest vegetation. *Chironomus* species were the object of our study because these larvae are widely used to assess sediment toxicity in the laboratory and because they are tolerant to and can accumulate metals in lakes and rivers, which makes them potential biomonitors for contaminants in these ecosystems. Because they are difficult to identify, *Chironomus* larvae are not generally separated into species but are pooled for contaminant analyses. This practice is reasonable only if *Chironomus* species sharing the same environment accumulate Cd to the same levels.

We compared Cd concentrations in *Chironomus* larvae collected at a single site in lakes located near either Quebec City (Lake St. Joseph) or a metals smelter at Rouyn-Noranda, Quebec (Lakes D'Alembert, Kinojévis and Opasatica). *Chironomus* species were separated on the basis of the morphology of their larval head capsule, tubuli on the posterior abdominal segments, and giant polytene chromosomes located in the salivary glands, as well as by using genetic techniques. Although these techniques allowed us to separate the species, we are not yet certain of their names (studies are ongoing).

More than one *Chironomus* species was present in all lakes (Figure 1), which means that in a given lake the species would have to be either separated or pooled for contaminant analyses. In a given lake, Cd concentrations differed widely among *Chironomus* species living at the same site; for example, the mean ratio of the most to the least contaminated species for the 4 lakes

was 12! These large inter-specific differences suggest that pooling species for Cd analyses would confound differences in contamination levels among the lakes (the degree to which this is likely to occur would depend on the species present and their population densities).

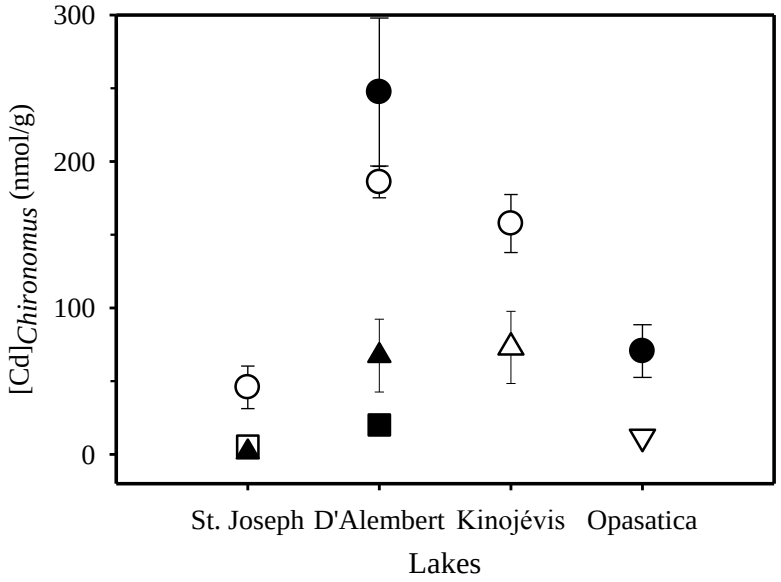


Figure 1. **Cadmium concentrations (mean ± SD) in *Chironomus* larvae collected from four Quebec lakes located either near Quebec City (St. Joseph) or upwind (Opasatica) or downwind (D'Alembert, Kinojévis) from a metals smelter at Rouyn-Noranda. Each type of symbol represents one as yet unidentified *Chironomus* species.**

Our results suggest that previously-published data on Cd concentrations in pooled samples of *Chironomus* (or for the family Chironomidae) should be evaluated critically. Likewise, caution should be used when comparing the results of laboratory studies using different *Chironomus* species (e.g., *C. riparius* and *C. tentans*) or when the results of laboratory studies are extrapolated to the field. We acknowledge that our results are for a single metal and insect genus; for some contaminants and animals, pooling related species might provide coherent results. However, the necessary studies must be done beforehand to show that this is indeed the case.

The up-side of our study is the revelation that some *Chironomus* species tend to consistently accumulate more Cd (circles in Figure 1) than do others (squares and triangles in Figure 1). Thus *Chironomus* species appear to respond to Cd contamination in a predictable way, which is a prerequisite for using this insect as a Cd biomonitor and in risk assessments. Given these differences among the species, our data allow us to suggest that the lakes we studied differ in their Cd-contamination levels (Figure 1), and that lakes located downwind of and in proximity to

a metals smelter (D'Alembert and Kinojévis) are more contaminated than those that are either upwind of the smelter (Opasatica) or distant from Cd point sources (St. Joseph).

If we are to use *Chironomus* larvae effectively, both as biomonitors and in laboratory experiments, we should know something about why *Chironomus* species differ so widely in their Cd concentrations. To answer this question, Martin *et al*. (2008) compared the morphology, life history and behavior of two of the several *Chironomus* species living in Lake St. Joseph (*C. staegeri* and *C. tigris*). They concluded that the former species accumulated an order of magnitude more Cd that did the latter species, largely because of differences in their feeding behaviors. By examining larval gut contents and by measuring stable sulfur isotopes in larvae they showed that although both species consume sediment, *C. staegeri* eats mostly recently-deposited oxic sediment whereas *C. tigris* consumes deeper anoxic sediment (Martin *et al*. 2008). The availability of Cd in these two sediment types is likely to differ because of vertical gradients of Cd binding phases. Martin *et al*. (2008) also showed that, in the laboratory, trends in metal concentrations in the two *Chironomus* species were reversed from those measured in the field, which underlines the difficulty of re-creating field conditions in the laboratory and thus in extrapolating from the laboratory to the field (Luoma 1995).

In conclusion, our results suggest that a "one-size fits all" approach for *Chironomus* species is not valid and that major behavioral differences among these morphologically-similar larvae can influence their exposure to contaminants. We hypothesize that *Chironomus* is not an isolated case and that species in other genera are also likely to differ in their accumulation of and susceptibility to contaminants (e.g., Buchwalter and Luoma 2005). Clearly more research is needed to test this hypothesis. Since differences in exposure influence contamination uptake by animals, such information is important for using animals as contaminant biomonitors and in toxicity tests. We suggest that understanding why related species differ in their contaminant concentrations will aid in producing more rigorous ecological risk assessments.

REFERENCES

Buchwalter DB, Luoma SN. 2005. Differences in dissolved cadmium and zinc uptake among stream insects: Mechanistic explanations. *Environmental Science & Technology* 39: 498-504.

Hare L, Tessier A, Croteau MN. 2008. A biomonitor for tracking changes in the availability of lakewater cadmium over space and time. *Human Ecological Risk Assessment* (forthcoming).

Luoma SN. 1995. Prediction of metal toxicity in nature from bioassays: limitations and research needs. In: Tessier A &Turner DR (Eds.), *Metal speciation and bioavailability in aquatic systems*. Wiley, Chichester, UK. p 609-659.

Martin S, Proulx I, Hare L. 2008. Explaining metal concentrations in sympatric *Chironomus* species. *Limnology and Oceanography* (forthcoming).

Rainbow PS. 2002. Trace metal concentrations in aquatic invertebrates: Why and so what? *Environmental Pollution* 120: 497-507.

ARTICLE 2

Using various lines of evidence to identify *Chironomus* species (Diptera : Chironomidae) in eastern Canadian lakes

Titre courant : Identifying *Chironomus* species

Isabelle Proulx[1], Jon Martin[2], Melissa Carew[3] et Landis Hare[1]

[1]Institut national de la recherche scientifique – Centre Eau Terre Environnement, 490 rue de la Couronne, Université du Québec, 490 rue de la Couronne, Québec (Québec), G1K 9A9, Canada

[2]Department of Genetics, University of Melbourne, Melbourne, Victoria, 3010, Australia

[3]Centre for Aquatic Pollution Identification and Management, Bio21 Institute, University of Melbourne, Melbourne, Victoria, 3010, Australia

Publié en 2013 dans Zootaxa 3741 (4): 401-458.

Spécification sur la contribution des auteurs :

Isabelle Proulx : Conceptualisé et réalisé (l'échantillonnage, l'analyse en laboratoire, l'interprétation des résultats) l'étude et rédigé l'article

Jon Martin : Réalisé les analyses cytologiques, séquencé les gènes *cox1* et *gb2β* de spécimens de référence, fourni des conseils sur l'interprétation des résultats et participé à la rédaction de l'article

Melissa Carew : Réalisé les analyses *cox1* PCR-RFLP pour les larves récollectées en 2006 et révisé la version finale de l'article

Landis Hare : Contribué à la rédaction de l'article

ABSTRACT

Chironomus Meigen (Diptera, Chironomidae) larvae are usually the largest sediment-burrowing chironomids, and as such often constitute a major part of the freshwater infaunal biomass. However, use of this genus in ecological, environmental and paleoecological studies is hampered by the fact that *Chironomus* larvae are difficult to identify to species because the larvae of many species are morphologically similar. We used a combination of morphological, cytological and genetic techniques to distinguish *Chironomus* larvae collected from 31 water bodies located in eastern Canada, producing 17 distinguishable groupings. These groups of larvae were ultimately identified as belonging to 14 known species (*C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. entis*, *C. frommeri*, *C. harpi*, *C. maturus*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C. plumosus*, *C. staegeri* and *C. 'tigris'*) and three other species that remain unidentified (*C*. sp. NAI-III). No single approach served to delimit and identify larvae of all 17 *Chironomus* species that we collected. Although we expected that morphological criteria alone would be insufficient, our results suggest that DNA barcoding, using either the mitochondrial *cox1* or the nuclear *gb2β* gene, was also inadequate for separating some *Chironomus* species. Thus we suggest that multiple approaches will often be needed to correctly identify *Chironomus* larvae to species.

INTRODUCTION

The insect genus *Chironomus* Meigen (Diptera, Chironomidae) is found in fresh waters on all continents except Antarctica. It includes several hundred species, now classified into three subgenera (*Chaetolabis*, *Chironomus*, *Lobochironomus*) (the subgenus *Camptochironomus* is no longer recognized—see Sæther (2012)), as well as other species that are yet to be described (Ryser *et al*. 1985; Ashe & Cranston 1990; Martin 2013). In lakes from the tropics (Hare & Carter 1986), to the temperate (Jónasson 1972), to the Arctic (Butler 1982), *Chironomus* larvae are usually the largest sediment-burrowing chironomid and often represent a major part of the infaunal biomass. Thus *Chironomus* larvae can be an important source of food for fish and are widely used in ecological (Jónasson 1972), environmental (Martin *et al*. 2008) and paleoecological (Brooks *et al*. 2007) studies of fresh waters. If we are to understand their roles in aquatic ecosystems, it is important to be able to correctly identify *Chironomus* species.

The identification of *Chironomus* larvae to species can be problematic because there are few conspicuous morphological differences among many *Chironomus* species (Lindeberg & Wiederholm 1979). As a result, larvae are often referred to simply as *Chironomus* spp. (Nyman *et al*. 2005) or at best are grouped into types according to the presence and form of their abdominal tubules (Shobanov *et al*. 1996) or the shape of their mouth parts (Brooks *et al*. 2007). Such groupings can limit the use of *Chironomus* larvae in ecological, environmental and paleoecological studies because behavioural and ecological differences among species are often important. For example, cadmium concentrations in sympatric *Chironomus* species can vary by an order of magnitude because of differences in their feeding habits and consequent contaminant exposure (Martin *et al*. 2008; Proulx & Hare 2008, 2013). Pooling such species would clearly limit their use as contaminant biomonitors. If we cannot correctly identify *Chironomus* larvae to species, then it is difficult to use them to infer environmental impacts.

In early studies, features of the head capsule and abdominal tubules were used to identify *Chironomus* larvae to species (Johannsen 1937). Subsequently, *Chironomus* species were also separated on the basis of the structure of polytene chromosomes located in their salivary glands (Keyl 1962; Martin 1979; Wülker *et al*. 1989). In the last decade or so, genetic techniques have been used to supplement these earlier taxonomic methods. For example, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) approach has been used to produce species-specific DNA profiles that can differentiate among *Chironomus* species (Carew *et al*. 2003; Sharley *et al*. 2004). This technique involves first amplifying specific genes or

regions with PCR, and then digesting the resulting PCR amplicons with restriction endonucleases. Restriction endonucleases cut PCR amplicons differentially based on nucleotide differences in their DNA sequence, thereby generating a species-specific RFLP or DNA profile. The DNA profiles are visualised by gel electrophoresis as DNA fragments of different lengths. Although this method is inexpensive and useful for screening large numbers of individuals, it only examines a subset of the variation present in PCR amplicons (Pfrender *et al*. 2010).

Another genetic technique used to separate and identify species is DNA sequencing (also known as Sanger sequencing) of PCR amplicons. This technique, referred to as DNA barcoding when used for identifying species, is more exact than PCR-RFLP as it detects all nucleotide differences. The standard gene used for DNA barcoding is the 3' end of the mitochondrial *cytochrome oxidase subunit I* (*cox1*; Hebert *et al*. 2003). Advantages of using the *cox1* gene are that universal primers are able to amplify this gene from many animal groups (Folmer *et al*. 1994) and sequence variations in *cox1* can be used to discriminate among many closely-related species (Hebert *et al*. 2004a). In insects, DNA barcoding using the *cox1* gene has been used to identify species from a range of groups including the Collembola (Hogg & Hebert 2004), the Ephemeroptera (Ball *et al*. 2005; Elderkin *et al*. 2012), the Coleoptera (Davis *et al*. 2011) and the Chironomidae (Carew *et al*. 2007; Ekrem *et al*. 2007; Pfenninger *et al*. 2007; Sinclair & Gresens 2008; Ekrem *et al*. 2010; Carew *et al*. 2011; Stur & Ekrem 2011). Although *cox1* sequences can be used to separate the majority of species, its mitochondrial origin is problematic for *Chironomus* because some species are known to hybridize (Martin 2011). Therefore, including sequence data from additional nuclear markers whose mode of inheritance differs from mitochondrial genes is required (Guryev *et al*. 2001; Martin *et al*. 2002; Martin 2011). To this end, the nuclear gene *globin 2β* (*gb2β*) has been used in several studies on *Chironomus* species (Kao *et al*. 1994; Hankeln *et al*. 1997; Guryev *et al*. 2001; Guryev & Blinov 2002; Martin *et al*. 2002).

We applied morphological, cytological and genetic techniques to identify *Chironomus* larvae collected in 31 water bodies in eastern Canada to determine what combination of techniques would allow us to accurately identify the *Chironomus* species. To date, very few studies have used multiple techniques to discriminate among *Chironomus* species. We anticipate that the results of our study will be useful to those wishing to identify North American *Chironomus* species and will provide useful tools to those wishing to identify *Chironomus* species on other

continents. The ability to accurately identify *Chironomus* species should facilitate future ecological and environmental studies in this and other geographical zones.

METHODS

Collection and dissection of larval *Chironomus*

We collected fourth-instar *Chironomus* larvae from 31 water bodies (Table 1) located in the provinces of Quebec (near Quebec City, Rouyn-Noranda and Trois-Rivières) and Ontario (near Sudbury), Canada. The collection period extended from ice-off in late spring (May) to early summer (June) in various years from 2006 to 2011 (Table 1). Exceptionally, in Lake Bédard, *Chironomus* were collected at the end of the summer (September). Sediments were collected using an Ekman grab and sieved through a net to eliminate fine sediment and retain *Chironomus* larvae. Larvae were preserved in 94% ethanol.

Depth and water chemistry were measured at each collecting site (Table 1). Water samples were filtered in situ using diffusion samplers (Ponton & Hare 2009). Dissolved organic carbon (DOC) was measured by combustion and transformation into CO_2 (TOC-VCPH, Shimadzu, Columbia, MD, USA) and magnesium (Mg) and calcium (Ca) concentrations were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Vista AX CCD, Varian, Mississauga, Ontario, Canada). Quality assurance of water chemistry measurements was assured through the use of blanks and appropriate standard reference materials. At the time of sampling, the water columns of all lakes were well mixed and oxygenated.

Head capsule and terminal abdominal segments with attached tubules were separated and kept for morphological studies, whereas the rest of the body was retained for genetic analyses. In addition, three individuals of each species (as determined by genetic analyses and morphology) were chosen at random for examination of their polytene chromosomes. For this purpose, the thoracic segments containing the salivary glands were preserved in a 3:1 mixture of 94% ethanol to glacial acetic acid (not all specimens showed chromosomal patterns of sufficient quality for species identification). Exceptionally, 41 larvae of *C. entis* and *C. plumosus* were examined cytologically to validate genetic results for these species.

Table 1. Location, year and depth of collection, as well as water chemistry and trophic status of the water bodies studied.

Water body	Code	Year	Depth (m)	Maximum depth (m)	Location	Water chemistry				Trophic status[b]
						pH	Ca (µM)	Mg (µM)	DOC (mg/L)	
QUEBEC CITY (QC)										
Lake Bédard	BE	2009	4-6	10	47°16'N, 71°07'W	5.7-8.3	35-69	11-17	3.6-5.3	mesotrophic[c]
Lake Saint Augustin	AU	2010	3,5		46°45'N, 71°24'W					eutrophic (2003)[d]
St. Charles River	SC	2010	1-3	3	46°49'N, 71°13'W					
Lake St. Joseph	SJ	2006	6-24	37	46°53'N, 71°38'W	7.1				oligo-mesotrophic[d]
ROUYN-NORANDA (QC)										
Lake Adéline	AD	2007			48°12'N, 79°10'W					
Lake Arnoux	AR	2010	1.5-4.5	4.5	48°15'N, 79°20'W	3.8-4.4	286-296	146-156	0.1-1.1	
Lake Bousquet	BO	2006	14	18	48°13'N, 78°39'W	6.9[a]				
Lake D'Alembert	DA	2006	5		48°23'N, 79°01'W					eutrophic (2009-2011)[d]
Lake Dasserat	DS	2006, 2010	3-5	17	48°17'N, 79°25'W	7.5-7.6	205-223	97	5.9-6.3	
Lake Dufault	DF	2006	4	19	48°17'N, 79°00'W	7.7	392	110	3.8	oligo-mesotrophic (2010)[d]
Lake Duprat	DP	2006, 2007, 2010	5-7	7.5	48°20'N, 79°07'W	6.8-7.6	140-178	45-57	2.9-6.6	
Lake Fortune	FO	2006	5-6		48°11'N, 79°19'W	7.6				oligo-mesotrophic (2008)[d]
Lake Kinojévis	KI	2006	7-8		48°08'N, 78°54'W	7	363	109	5.6	
Lake Marlon	MN	2006, 2007, 2009, 2010	1-2	2	48°16'N, 79°04'W	7.1-7.7	160-168	58-61	8.4-7.8	meso-eutrophic[d]
Lake Opasatica	OP	2006, 2007, 2009	2-9	60	48°10'N, 79°20'W	7.4-8.0	213-216	107-115	6.7-7.4	mesotrophic (2008)[d]
Lake Osisko	OS	2006, 2009, 2010	5.5, 6.5	6.5	48°15'N, 79°00'W	7.8-8.5	690	183	2.3	
Lake Pelletier	PE	2010	5		48°13'N, 79°03'W	8.3	826	258	3.7	meso-eutrophic[d]
Lake Rouyn	RO	2010	3.5-4		48°15'N, 78°57'W	8	2060	289	3.5	meso-eutrophic[d]
Lake Vaudray	VA	2010	35	35	48°04'N, 78°41'W	7.1	79	36	8.7	oligotrophic (2011)[d]
TROIS-RIVIERES (QC)										
unnamed pond	PO	2007	1	1	46°13'N, 72°39'W					
SUDBURY (ON)										
Kasten (Bibby) Lake	KA	2007	7.5	8	46°22'N, 80°58'W	6.8	69	45	4.4	oligotrophic (2008)[e]
Clearwater Lake	CL	2007	19	19	46°22'N, 80°03'W	6.2	109	43	2.3	oligotrophic[e]
Crooked Lake	CR	2007	5-6	8	46°25'N, 81°02'W	6.7	71	48	3.6	oligotrophic[e]

Table 1. Cont.

Water body	Code	Year	Depth (m)	Maximum depth (m)	Location	Water chemistry				Trophic status[b]
						pH	Ca (µM)	Mg (µM)	DOC (mg/L)	
Hannah Lake	HA	2007, 2010	7-7.5	7.5	46°27'N, 81°02'W	7.4-7.9	258-265	147-151	3.5-3.7	oligotrophic[e]
Kelly Lake	KE	2010, 2011	1.5-5	17	46°27'N, 81°04'W	7.5, 8.4, resp.	4596, 3683, resp.	1267, 614, resp.	7.0, 5.1, resp.	eutrophic (2008)[e]
McFarlane Lake	MC	2007	10	18	46°25'N, 80°57'W	7.8	430	227	4.2	oligo-mesotrophic[e]
Pine Lake	PI	2010	4-6	6	46°23'N, 81°01'W	5.7	25	15	1.7	
Raft Lake	RA	2010	10	14	46°25'N, 80°57'W	7.3	78	45	2.2	oligotrophic[e]
Ramsey Lake	RM	2007	12	18	46°28'N, 80°57'W	7.1	381	193	3.1	mesotrophic[e]
Silver Lake	SI	2007, 2011	4	10	46°26'N, 81°01'W	5.9, 7.0, resp.	194, 283, resp.	117, 170, resp.	2.7, 3.4, resp.	oligotrophic[e]
Tilton Lake	TI	2007-2011	4	12	46°21'N, 81°04'W	6.6, 7.1, resp.	89, 78, resp.	40, 37, resp.	2.3, 3.2, resp.	oligotrophic[e]

[a] Fortin *et al.* (2010)

[b] Trophic status determined for the collecting years, unless mentioned otherwise in parentheses

[c] Trophic status inferred from total phosphorus and chlorophyll a (data not published, personal communication from Jean-Christian Auclair, INRS – Centre Eau Terre Environnement)

[d] Trophic status inferred from total phosphorus, chlorophyll *a* and water transparency (Ministère du Développement durable de l'Environnement et des Parcs 2012)

[e] Trophic status inferred from total phosphorus measurements (City of Greater Sudbury 2013)

Genetic analyses

Polymerase chain reaction—restriction fragment length polymorphism (PCR-RFLP) analysis was performed on *Chironomus* larvae collected in 2006 and 2007. Specimens of each PCR-RFLP profile were subsequently sequenced for further DNA analysis. Specimens collected in subsequent years were sequenced directly.

DNA extraction

DNA was extracted from larvae using the modified Chelex method (Carew *et al*. 2003). Briefly, the larval body (minus the head and terminal segments) was dried using a paper towel and placed in a 0.5 mL plastic microcentrifuge tube. Individuals with large amounts of sediment in their gut were avoided as this can inhibit the PCR (Carew *et al*. 2003). Tubes were immersed in liquid nitrogen and the contents crushed into a powder using a pestle, 400 µL of suspended 5% Chelex-100 resin (BioRad) was added and samples were incubated at 90^{o}C for 30 min. Extracts were stored at -20^{o}C until required for the PCR procedure.

Polymerase chain reactions

PCR amplification of portions of the *cox1* and the *gb2β* genes was carried out in a 40 µL reaction mixture containing: 1x PCR pH 8.8 buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100; New England Biolabs (NEB)), 200 µM each of deoxynucleotide triphosphate (dNTPs), 0.4 mg/mL of bovine serum albumin (BSA), 0.5 µM of forward and reverse primers (see Table 2), 1 unit of Taq DNA polymerase (NEB), and 5 µL of Chelex DNA extraction supernatant taken from just above the resin after centrifugation at 15,000 relative centrifugal force for 2 min (Carew *et al*. 2003). All *gb2β* gene primers tested in our study are listed in Table 2. The primers used to amplify the *gb2β* gene for each species are given in Table 4. For the *cox1* gene, the PCR thermal regime consisted of an initial denaturation cycle of 94^{o}C for 3 min; followed by 40 cycles of denaturation at 94^{o}C for 15 s, annealing at 45^{o}C for 45 s, elongation at 72^{o}C for 1 min; and one cycle at 72^{o}C for 1 min. The PCR thermal regime for the *gb2β* gene was the same, but in some instances an annealing temperature of 50^{o}C was used. All PCRs had a negative control with no DNA template added. PCR products were verified by electrophoresis on a 1.5% Tris-Acetate-EDTA agarose gel with ethidium bromide. PCR product sizes were estimated using Hyper Ladder II (Bioline).

Table 2. Primers used in this study

Genes	Primers (forward (for) or reverse (rev))	Primers sequences (5'-3')	References
cox1	911 (for)	TTTCTACAAATCATAAAGATATTGG	Folmer *et al.* (1994)
	912 (rev)	TAAACTTCAGGGTGACCAAAAAATCA	
gb2β	wyk1b (for)	GAYATCCTTTACTACTYTT	Modified version of Kao *et al.* (1994) wyk1 primer
	wyk4 (rev)	GACCTTGTGTCCAGGC	Kao *et al.* (1994)
	wyk3 (rev)	GTGTTTCCATAGCTGGC	
	2β-B (for)	GATATCCTTTACTACATC	Hankeln *et al.* (1997)
	2β-A (rev)	CGATGTCAATAAATACATG	
	*2β*con for (for)	CCAGACATCATGGCTAA	
	*2β*con rev (rev)	CTTGACAACATCTTCGAC	

Polymerase chain reaction-restriction fragment length polymorphism analysis

Digest enzymes were chosen based on previous publications on chironomid identification using PCR-RFLP (Carew *et al*. 2003; Sharley *et al*. 2004; Carew *et al*. 2005; Carew *et al*. 2007). The PCR products from *cox1* were cleaved using restriction endonucleases with 4 base pairs (bp) (*Alu* I, *Rsa* I, *Taq* I) and 6 bp (*Hha* I, *Hinf* I, *Ssp* I) recognition sites. All digests were carried out as described by Carew *et al*. (2003) in a 20 µL reaction mixture containing: 10 µL of PCR product, 1x recommended buffer, 0.1 mg/mL BSA and variable units of restriction endonucleases (3 units for *Alu* I, *Rsa* I and *Ssp* I, 4 units for *Taq* I and 6 units for *Hha* I and *Hinf* I; NEB). Restriction digests were incubated at 37^{o}C overnight, with the exception of *Taq* I, which was incubated at 65^{o}C for 3h. Digest products were separated via electrophoresis for 2h at 100V on a 3% agarose gel stained with ethidium bromide and observed under UV light. The size of digest fragments was estimated with a 50 bp ladder (Promega). Fragment sizes below 100 bp were ignored, as they were not always clearly discernible on the agarose gels. To verify results obtained from these digests, we simulated digests of the corresponding *cox1* sequences using the New England BioLabs NEBCutter V2.0 program (http://tools.neb.com/NEBcutter2/). Simulation digests were also performed on the *cox1* sequences of larvae for which RFLP digests were not made.

DNA sequencing analysis

The *cox1* (709 bp) and *gb2β* (332–394 bp) gene products were purified and sequenced in both directions using the forward and reverse primers used for PCR amplification by Macrogen (Seoul, Korea) or by the research center at the Centre hospitalier universitaire de Québec (Quebec, Canada) on an ABI3730 XL automatic DNA sequencer (Applied Biosystems) and

were aligned using BioEdit 7.1.3.0 (Hall 1999). All sequences used for DNA analyses were submitted to GenBank (KF278208-KF278447; KF278449-KF278450). Sequences from *cox1* were aligned using CLUSTAL W (Thompson *et al*. 1994). Due to the presence of introns in some species, *gb2β*-sequences were aligned manually according to Hankeln *et al*. (1997). Sequences were analyzed in MEGA 5.05 (Tamura *et al*. 2011). Primer sequences for each gene were excluded from the analysis. Since our goal was to separate *Chironomus* species based on sequence similarities, rather than to infer interspecific phylogenetic relationships, identification trees (ID-trees) based on *cox1*-sequences and *gb2β*-sequences were built using the Neighbor-Joining (NJ) (Saitou & Nei 1987) algorithm. The pairwise distances were calculated from the Kimura 2-parameter (K2P) model (Kimura 1980), which is best suited when distances are low (Nei & Kumar 2000), as in our study. Bootstrap analysis was performed with 1000 replicates. For the *cox1* identification (ID) tree, *Polypedilum aviceps*, *Drosophila affinis* and *Glyptotendipes lobiferus* sequences from GenBank were added as outgroups. In the case of the *gb2β* ID-tree, because this gene is quite variable and because the only really conserved regions are also conserved in the *globin* genes *7A* (*gb7A*) and *9* (*gb9*) (Hankeln *et al*. 1997), *Chironomus* species sequences of the *gb7A* and *gb9* genes were also added to make sure that all sequences obtained for our specimens were of the *gb2β* gene. The *gb2β* primers did in fact amplify the *gb7A* gene from *C*. (*Chaetolabis*) nr. *atroviridis* (sp. 2i). Pairwise intraspecific and interspecific nucleotide-sequence divergences were also calculated for all sequences using the K2P model in MEGA 5.05. Since some authors have expressed reservations about using divergence thresholds to separate species (DeSalle *et al*. 2005), including those of *Chironomus* (Martin 2011), we also used specific base differences to quantify differences between some closely-related species.

Morphological analysis

Larval length was measured under a dissecting microscope and head capsule width (at the level of the eyes) and abdominal tubule lengths were measured using a microscope linked to an image-analysis system.

Chironomus were sorted according to larval type on the basis of the presence or absence, types and length of abdominal tubules using a dissecting microscope. Although there have been several attempts to classify *Chironomus* larvae into types based on the morphology of their tubules (Harnisch 1942; Andersen 1949; Lindeberg & Wiederholm 1979; Shobanov *et al*. 1996; Shobanov 2002), the definitions in these schemes have been inconsistent and in some cases

contradictory. In general we have returned to the original scheme of Harnisch (1942), for which many of the types were well-illustrated by Andersen (1949). To this scheme we have introduced two additional types, bathophilus-type and melanotus-type, from the more recent scheme of Shobanov (2002). While the early schemes clearly recognised the coiled nature of the ventral tubules of *C. thummi* (now *C. riparius*) and *C. plumosus*, this aspect was lost in later classifications, such as those of Lindeberg and Wiederholm (1979) and Shobanov (2002), which were based only on the length of the tubules. We have found the distinction between coiled and relatively straight tubules to be consistent within species, and also a useful distinction among species. The bathophilus- and melanotus-types can fill this gap but only if the Shobanov (2002) definition is broadened to cover larvae with long ventral tubules, but without the typical coiling of those seen in thummi-type and plumosus-type. It should be noted that Shobanov (2002) introduced the melanotus-type to replace the anthracinus-type because *C. anthracinus* does not have an anthracinus-type larva (i.e. with lateral tubules), but is a typical thummi-type (i.e. without lateral tubules) (see larval description in the "Results and discussion" section). Further, the anthracinus-type, along with the semi-thummi- or semi-bathophilus-types, were intended to define in part larvae with very small lateral tubules, but we have found it difficult to draw a clear line between short and long lateral tubules and so have ignored this criterion. Our amended version of the larval classification is presented in Table 3.

Table 3. Classification of *Chironomus* larval types. See Fig. 1 for illustrations of ventral tubules.

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 slightly curved; short
melanotus	present	straight or slightly curved; long	straight or be slightly curved; long
plumosus	present	with elbow; long	coiled; long

[a] long: ventral tubules $\geq$ the width of 11th segment
short: ventral tubules < the width of 11th segment
b Often hard to distinguish from bathophilus-type

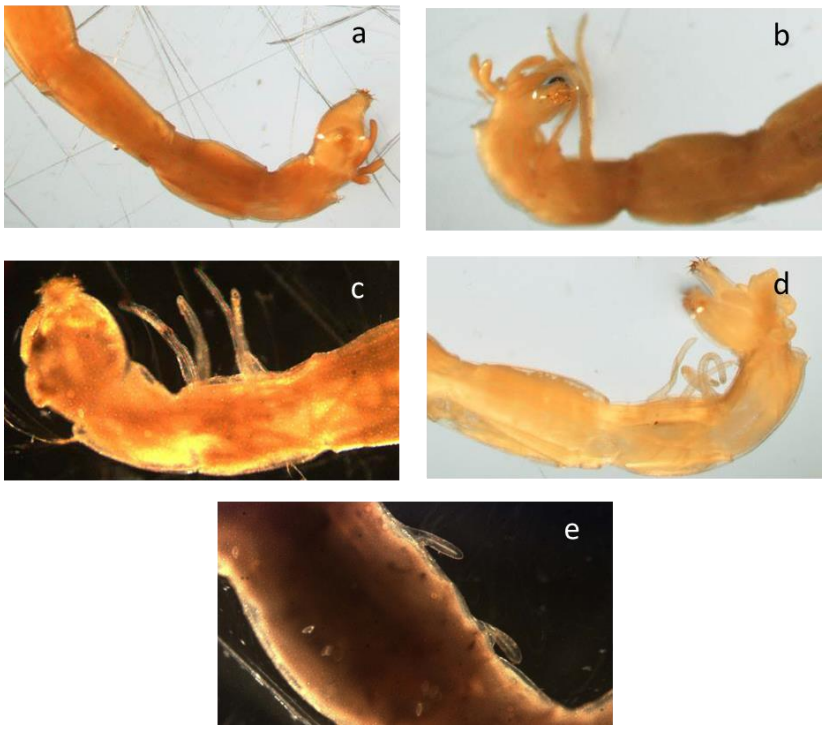


Figure 1. **Ventral tubules of the various larval types: salinarius and reductus (a), bathophilus and melanotus (b), fluviatilis (c), thummi and plumosus (d), as well as semireductus (e).**

Head capsules were separated into parts and mounted in Canada Balsam so as to determine the coloration of the frontoclypeus (Fig. 2) and the gula (Fig. 3) as well as the structure of: the central trifid tooth (Fig. 4) and 4th lateral teeth of the mentum (Webb & Scholl 1985; Vallenduuk & Moller Pillot 1997) (Fig. 6), the mandibles (Fig. 5), the pecten epipharyngis (Fig. 7) and the ventromental plates (Webb *et al*. 1985).

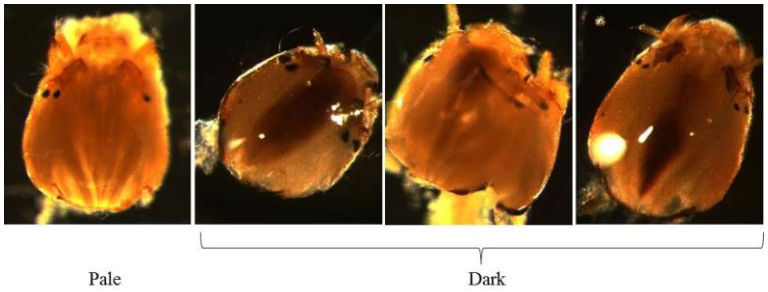


Figure 2. **Dorsal view of larval head-capsules showing variation among species in the color of the frontoclypeus from pale (*C.* sp. NAIII) to dark (from left to right: *C. 'tigris'*, *C. cucini*, *C. dilutus*).**

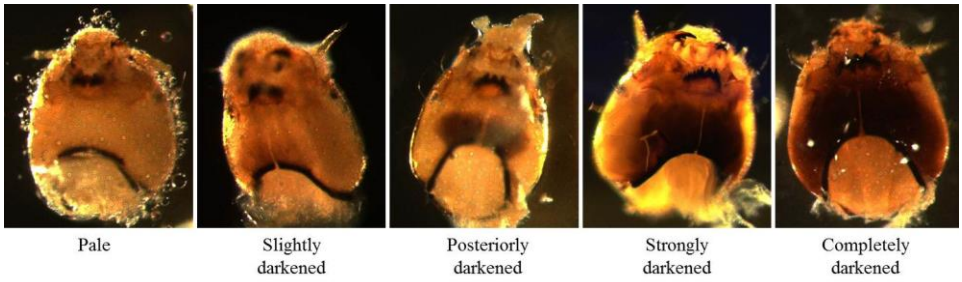


Figure 3. **Ventral view of larval head-capsules showing variation among species in the color of the gula from pale (*C. maturus*) to slightly darkened (*C. harpi*), to posteriorly darkened (*C.* nr. *atroviridis* (sp. 2i)), to strongly darkened (*C. staegeri*), to completely darkened (*C. 'tigris'*).**

We developed a classification scheme based on differences in the central trifid tooth of the mentum (Fig. 4) and teeth of the mandibles (Fig. 5) that is based in part on the previous classifications of Webb and Scholl (1985) and Vallenduuk and Moller Pillot (1997), but that better encompasses the range in variation we observed in these structures. For example, Webb and Scholl (1985) classified the central trifid tooth of the larval mentum according to the degree of fusion of its three component teeth, the width of the middle tooth, and the height of the outer teeth relative to the middle tooth. We found that the latter two criteria varied substantially within species and so considered only the first of these three criteria for that character. We note that although the degree of sharpness of the teeth of the mentum has been used for separating some *Chironomus* species (Martin 2013), this feature varied widely within the species under study and thus we did not use it for separating our study species.

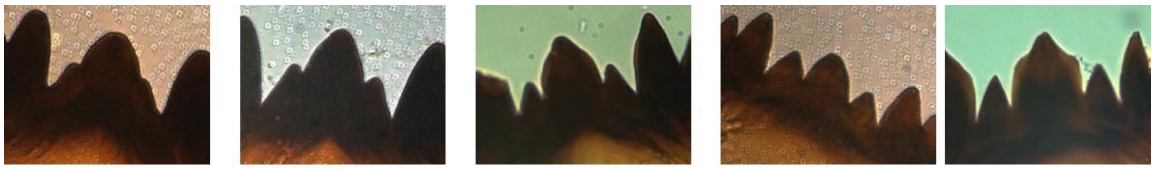
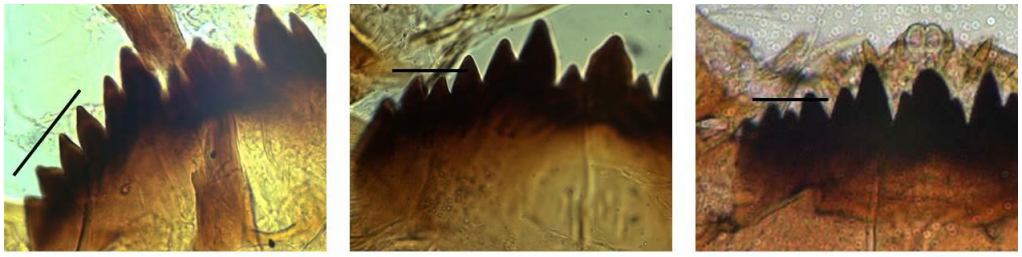


Figure 4. **Types of mentum middle trifid tooth: (type A) *C.* nr. *atroviridis* (sp. 2i), (type B) *C. cucini*, (type C) *C. staegeri*, (type D) *C. maturus* (left) and *C. plumosus* (right).**



Type I
4th lateral tooth the same size as the 3rd and 5th lateral teeth

Type II
4th lateral tooth reduced, it is the same height as the 5th lateral tooth

Type III
4th lateral tooth greatly reduced, it is shorter than the 5th lateral tooth

Figure 5. **Types of mentum 4th lateral teeth (Webb & Scholl 1985; Vallenduuk & Moller Pillot 1997): (type I) *C. plumosus*, (type II) *C. staegeri*, (type III) *C. cucini.***

Lastly, we used the coloration of the 3rd inner mandibular tooth and its degree of fusion with the lower mandibular margin to classify larvae.

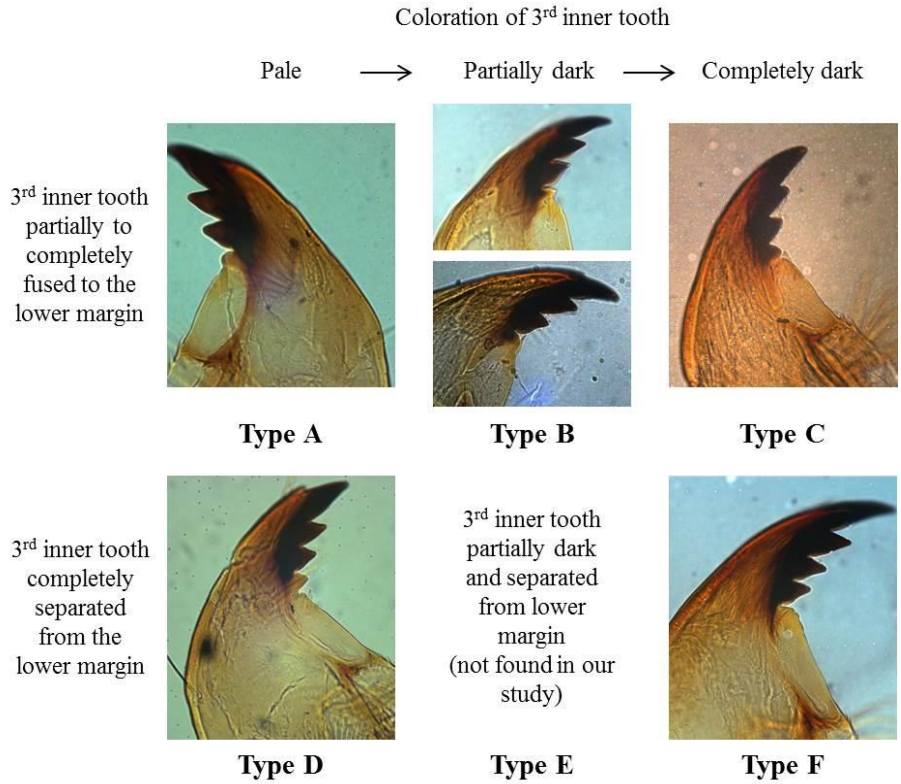


Figure 6. **Mandible types as defined by the degree of darkening and separation of the 3rd inner tooth: (type A) *C. bifurcatus*, (type B) *C.* sp. NAI (above) and *C. ochreatus* (below), (type C) *C.* nr. *atroviridis* (sp. 2i), (type D) *C. staegeri*, (type F) *C. plumosus.***

Type A
Pointed and rather uniform teeth

Type B
Pointed teeth with occasionally smaller teeth interspersed between larger teeth

Type C
Rounded and rather uniform teeth

Type D
Rounded teeth with smaller teeth interspersed between larger teeth

Figure 7. **Types of teeth on the pecten epipharyngis: (type A) *C. staegeri*, (type B) *C. anthracinus*, (type C) *C. dilutus*, (type D) *C. ochreatus.***

Cytological analysis

Isolated salivary glands were prepared for polytene chromosome analysis using the aceto-orcein method (Martin *et al*. 2006). Veronika Golygina (Institute of Cytology and Genetics, Novosibirsk, Russia) assisted in distinguishing cytogenetically between *C. entis* and *C. plumosus*. Preparations of these two species have been deposited at her Institute. Polytene chromosome mounts of the remaining species have been deposited, together with their respective head capsule mounts, at the Canadian National Collection of Insects, Arachnids and Nematodes in Ottawa, Ontario, Canada.

Species delimitation and identification

Larvae were sorted according to their morphology, their *cox1* PCR-RFLP profiles (larvae collected in 2006 and 2007) and their *cox1* and *gb2β* gene partial nucleotide sequences and then linked, via DNA sequences of cytologically-known species (either already in GenBank, or from karyotyping larvae also sequenced in this study) to recognized species.

RESULTS AND DISCUSSION

Species delimitation and identification

Species identifications are performed on 4th (final) instar larvae. We confirmed that *Chironomus* larvae were in the fourth instar by comparing the width of their head capsule (Table 6) to those of prepupal larvae and larval exuviae attached to pupae (data not shown). We did not measure the head capsule widths of *Chironomus* sp. NAII larvae, but these were undoubtedly 4th instars because we collected them just prior to adult emergence. Fourth instar larvae can also be recognized by the presence of developing imaginal discs in the thorax and/or posterior abdominal segments (Wülker & Götz 1968; Ineichen *et al*. 1983).

Analysis using PCR-RFLP of the *cox1* gene was performed on 296 larvae. The enzymes *Ssp* I, *Hinf* I, *Rsa* I and *Taq* I were used to cleave the partial *cox1* gene into different RFLP profiles (Table 4). These profiles were congruent with our groupings based on larval morphology (larval types and head-capsule features), with the exception of a single profile (*Ssp* I: 500,240; *Hinf* I: 710; *Rsa* I: 500,240; *Taq* I: 260,200,190) obtained for two larval types that differed in the coloration of their frontoclypeus. For these larvae, the partial *cox1* gene was cleaved with two additional restriction endonucleases, *Hha* I and *Alu* I, thereby creating three extra RFLP profiles. Results of these analyses are summarized in Table 4. To verify the accuracy of these results, larvae that included all of these RFLP profiles were sequenced.

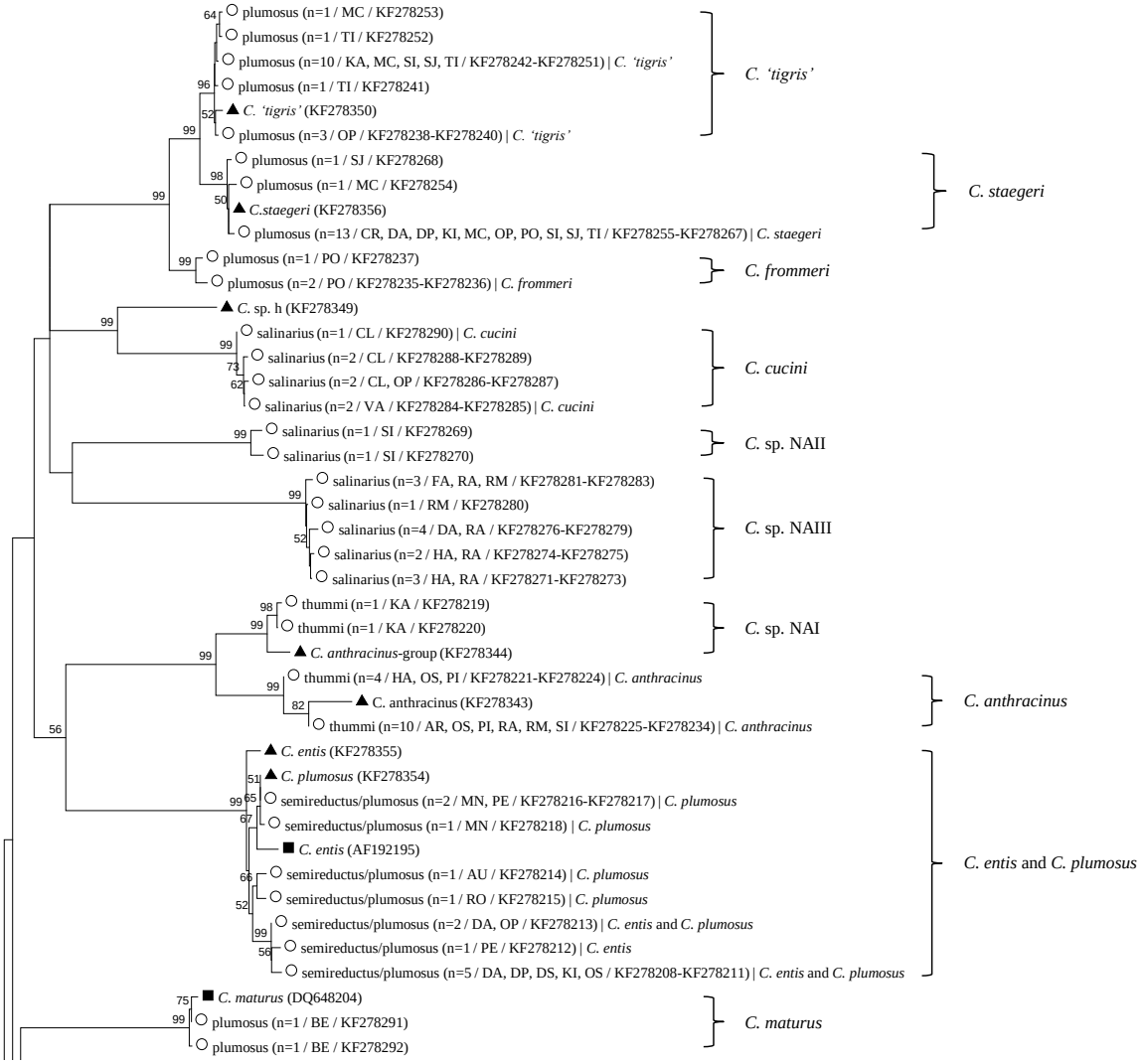
Table 4. **Summary of molecular results for the *cox1* and *gb2β* genes for each *Chironomus* species. *Cox1* gene: *cox1*-RFLP fragment sizes in base pairs (bp); 710-bp fragments are uncut; number of individuals analysed from real digests and number of individuals who subsequently had their *cox1* gene sequenced. *Gb2β* gene: primers used to amplify and sequence the *gb2β* gene; absence or presence of an intron (type I or type II) in the amplified *gb2β* sequence. ND; Not determined.**

Species	*cox1*								*gb2β*	
	RFLP analysis									
	cox1 RFLP fragment sizes in base pairs obtained from real digests (additional profiles obtained from simulation digests)						Number of individuals analysed from real digests	Number of individuals analysed through RFLP and subsequently sequenced	Primers used (refer to Table 2)	Intron: type or absence
	***Ssp* I**	***Hinf* I**	***Rsa I***	***Taq* I**	***Hha* I**	***Alu* I**				
C. anthracinus	710 (709)	710 (709)	710 (709)	380, 180, 100 (351, 174, 89, 72, 23)	ND	ND	16	3	wyk1b and wyk4	type II
C. bifurcatus (gr. 1)	710 (709)	510, 220 (489, 220)	610, 120 (596, 113)	550, 100 (525, 89, 72, 23)	(709)	(381, 214, 60, 33, 21)	12	4	wyk1b and wyk4	no intron
C. bifurcatus (gr. 2)	710 (709)	710 (709)	610, 120 (596, 113)	430, 100 or 550, 100 (426, 99, 89, 72, 23) or (525, 89, 72, 23)	(709)	(381, 150, 64, 60, 21, 18, 15 or 381, 214, 60, 21, 18, 15)	24	5	wyk1b and wyk4	no intron
C. cucini	710 (709)	710 (709)	460, 240 (443, 226, 40)	450, 100 (411, 99, 95, 89, 15)	ND	ND	26	5	wyk1b and wyk4	no intron
C. decorus-group sp. 2	710 (709)	710 (709)	500, 130 (483, 113, 113)	550, 100 (525, 95, 89)	ND	ND	10	3	no primer combination worked	ND
C. dilutus	(709)	(709)	(596, 87, 26) (683, 26)	(548, 89, 72) (533, 89, 72, 15)	(709)	(192, 150, 124, 93, 87, 63) (279, 150, 124, 93, 63)	0	0	wyk1b and wyk4	no intron
C. entis	710 (709)	450, 280 (439, 270)	500, 240 (483, 226)	250, 220, 100 (229, 208, 95, 89, 88)	ND	ND	1	0	wyk1b and wyk4	type II
C. frommeri	500, 240 (474, 235)	710 (709)	500, 240 (483, 226)	260, 200, 190 (252, 174, 99, 95, 89)	710 (709)	430, 220, 80 (414, 214, 81)	7	3	no primer combination worked	ND
C. harpi	(709)	(502, 207)	(330, 153, 113, 113)	(426, 194, 89)	ND	ND	0	0	wyk1b and wyk4	no intron

Table 4. Cont.

Species	*cox1*								*gb2β*	
	RFLP analysis									
	***cox1* RFLP fragment sizes in base pairs obtained from real digests (additional profiles obtained from simulation digests)**						**Number of individuals analysed from real digests**	**Number of individuals analysed through RFLP and subsequently sequenced**	**Primers used (refer to Table 3)**	**Intron: type or absence**
	Ssp I	***Hinf I***	***Rsa I***	***Taq I***	***Hha I***	***Alu I***				
C. maturus	500, 240 (474, 235)	710 (709)	500, 240 (483, 226)	450, 100 (426, 99, 95, 89)	ND	ND	10	2	wyk1b and wyk4	no intron
C. nr. *atroviridis* (sp. 2i)	(709)	(709)	(683, 26)	(525, 95, 89)	(709)	(414, 177, 118)	0	0	primer combinations either failed or gave *gb7A*	ND
C. ochreatus	(709)	(439, 270)	(346, 337, 26)	(620, 89)	ND	ND	0	0	no primer combination worked	ND
C. plumosus	710 (709)	450, 280 (439, 270)	500, 240 (483, 226)	250, 220, 100 or 332, 220, 100 (229, 208, 95, 89, 88 / 317, 208, 95, 89 or 525, 95, 89)	ND	ND	8	2	wyk1b and wyk4	type II
C. sp. NAI	710 (709)	710 (709)	710 (709)	260, 180, 100 (252, 174, 99, 89, 72, 23)	ND	ND	9	2	wyk1b and wyk4	type II
C. sp. NAII	710 (709)	510, 220 (502, 207)	500, 240 (483, 226)	470, 180 (446, 174, 89)	ND	ND	4	2	no primer combination worked	ND
C. sp. NAIII	500, 240 (474, 235)	710 (709)	710 (709)	450, 210, 100 (426, 99, 95, 89)	ND	ND	45	6	wyk1b and *2β*-A	type II
C. staegeri	500, 240 (474, 235)	710 (709)	500, 240 (483, 226)	260, 200, 190 (252, 194, 174, 89)	330, 440 (389, 320)	400, 130, 80 (378, 124, 90, 81, 36)	37	8	no primer combination worked	ND
C. 'tigris'	500, 240 (474, 235)	710 (709)	500, 240 (483, 226)	260, 200, 190 (252, 194, 174, 89)	710 (709)	430, 170, 120 (414, 171, 124 / 381, 171, 124, 33 or 255, 171, 159, 124)	61	12	wyk1b and wyk4	type II

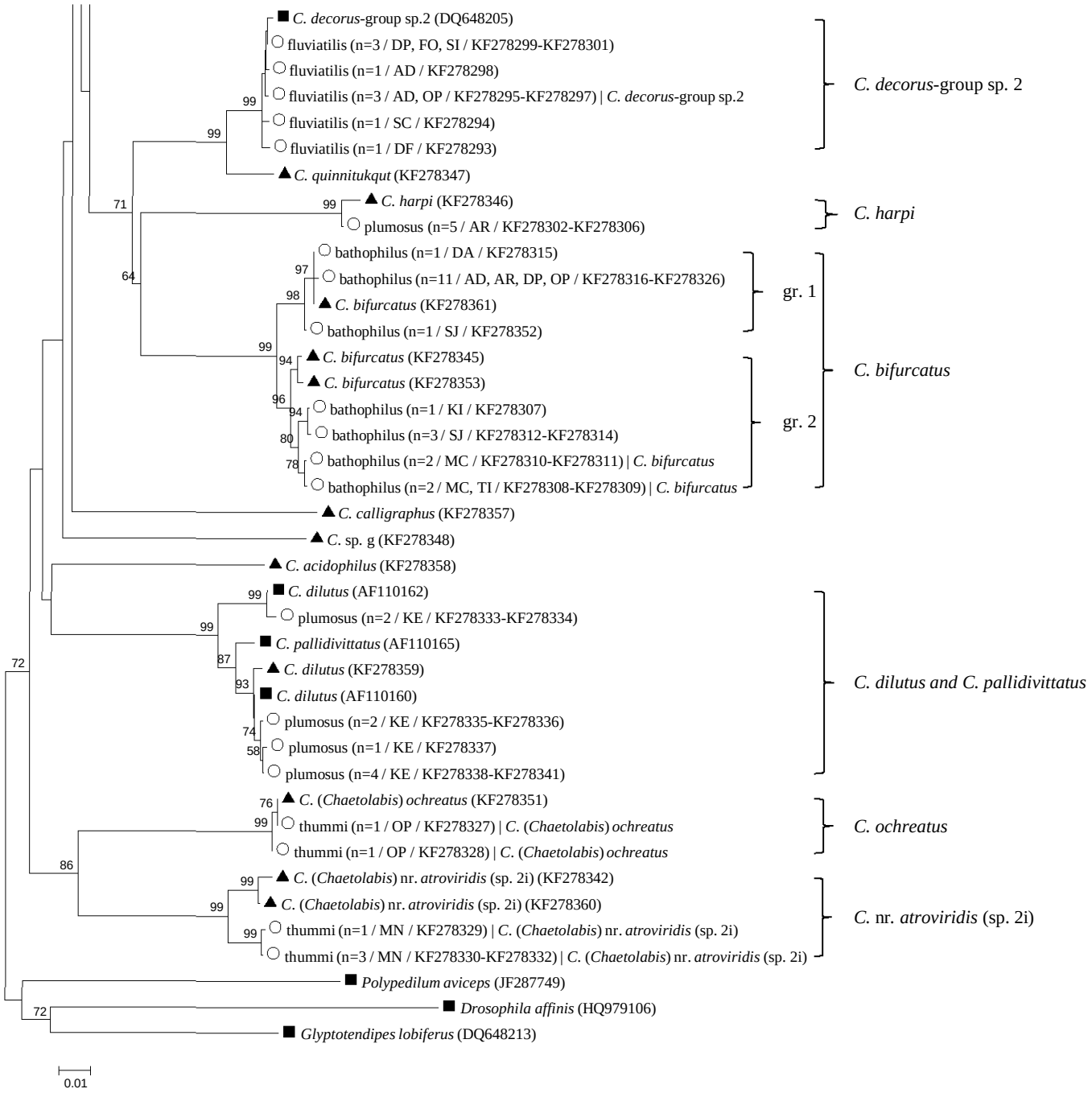
We sequenced the partial *cox1* gene of 59 larvae that included all 15 RFLP profiles, as well as that of 79 other larvae (Fig. 8). We also amplified and sequenced the partial *gb2β*-gene of 83 larvae (Fig. 9). However, we were unsuccessful in obtaining the *gb2β* sequence for all *Chironomus* species (Table 4) despite modifying PCR conditions and testing several primer combinations (Table 2).



(figure 8 continues on next page)

Figure 8. **Neighbor-joining identification tree (NJ ID-tree) based on partial *cox1* sequences and the K2P substitution model. Numbers on branches are bootstrap values >50%. ○ Sequences of *Chironomus* species collected from lakes in our study. Larval morpho-types are specified followed in parenthesis by: sample size (n = the number of individuals sequenced for each consensus sequence), lake abbreviations, and GenBank accession numbers. Some larvae were identified by examining their polytene chromosomes, and these results are indicated alongside the corresponding sequence next to the vertical line. ■ Sequences obtained from GenBank (species name and GenBank accession number in parenthesis). ▲ Sequences obtained from cytologically identified reference *Chironomus* specimens (species name and GenBank accession number in parenthesis).**

Figure 8. (continued)



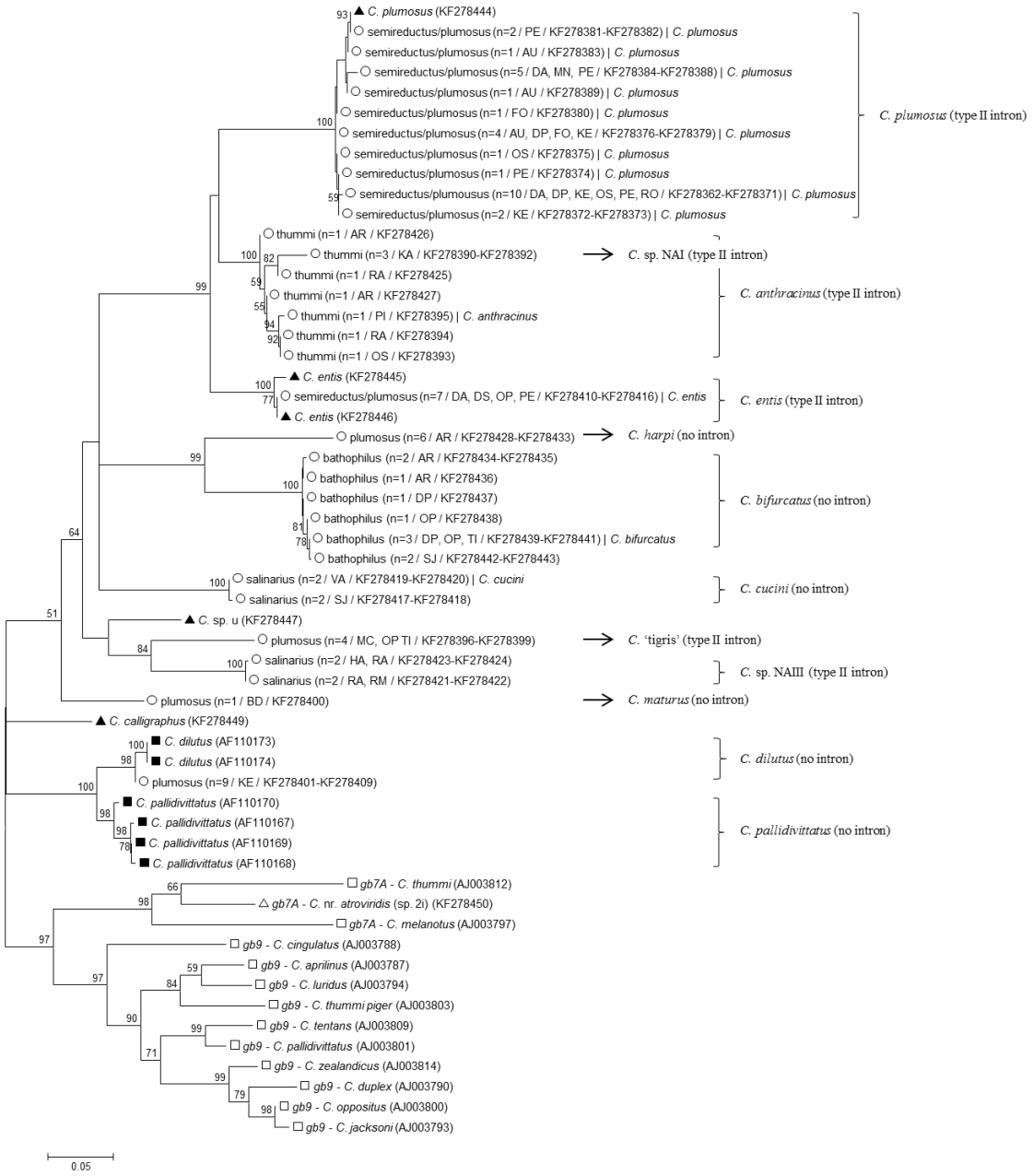


Figure 9. **Neighbor-joining identification tree (NJ ID-tree) based on partial *gb2β* sequences and the K2P substitution model. ○ Sequences of *Chironomus* species collected from lakes in our study. Larval morpho-types are specified followed in parentheses by: sample size (n = the number of individuals sequenced for each consensus sequence), lake abbreviations and GenBank accession number. Some larvae were identified by examining their polytene chromosomes, and these results are indicated alongside the corresponding sequence next to the vertical line. ■ Sequences obtained from GenBank (species name and GenBank accession number in parenthesis). ▲ Sequences obtained from cytologically identified reference *Chironomus* specimens (species name and GenBank accession number in parenthesis). □ *Chironomus gb7A* and *gb9* sequences obtained from GenBank were also added as outgroups (globin name, species name and GenBank accession number in parenthesis). △ *Chironomus gb7A* sequence obtained from cytologically identified reference specimens (globin name, species name and GenBank accession number in parenthesis).**

For visualisation purposes only, one representative of each unique sequence was used to illustrate the relationship between species in the *cox1* (Fig. 8) and *gb2β* ID-trees (Fig. 9). However, trees were also built using all sequences (including individuals that had identical gene sequences) which showed that using only unique sequences did not affect tree topology. Sequences were grouped into potential species according to molecular evidence (sequence clusters with bootstrap values >90% and sequence divergences of <4%) and larval morphology (see curly brackets in Figs. 8–9). Following this, species were identified through polytene chromosome analysis (71 larvae) and DNA barcoding. For cytological analyses, results of these identifications are given after the vertical line located to the right of the corresponding sequences in Figures 8 and 9. For DNA barcoding, Nearctic *Chironomus cox1*-sequences and *gb2β*-sequences from GenBank (http://www.ncbi.nlm.nih.gov) that grouped (bootstrap values >90% and sequence divergences <4%) with our sequences were added to our ID-trees. Moreover, we sequenced the *cox1* gene and/or the *gb2β* gene of voucher specimens (Table S2) and added these sequences to our ID-trees (Figs. 8–9). *C. acidophilus* (Keyl 1960), *C. calligraphus* (Goeldi 1905), *C. quinnitukqut* (Martin *et al*. 2010), *C*. sp. g (Martin 2013), *C*. sp. h (Martin 2013) and *C*. sp. u (Martin 2013) did not group with any of our collected species, so we could rule out these species as being any of our unrecognized species. Analysis of all the sequences together with simulation digests allowed us to identify the *Chironomus* species that had been previously separated using RFLP analysis. Simulation digests were performed on all of the *cox1* sequences obtained (see Table 4). Extra RFLP profiles were obtained from these simulation digests.

On the basis of the genetic, morphological and cytological information that we obtained, we conclude that the 404 *Chironomus* larvae that we collected represent 17 species, 14 of which were known while the status of three others remains uncertain. A detailed list of all the larvae analysed is presented in Table S1. In the following section, pertinent genetic, morphological and cytological information is presented for each of these species in the order that they are presented in Figure 8 (from top to bottom). Detailed genetic information, including *cox1*-RFLP sizes, the primers used to amplify the *gb2β* gene and whether or not the *gb2β* gene was amplified is given in Table 4. The *gb2β* gene of some *Chironomus* species includes an intron (type I or type II), whereas in others it is absent (Hankeln *et al*. 1997; Makarevich *et al*. 2000). This information is also given in Table 4.

Table 5. **Average (%) pairwise sequence divergence between species and within species (given diagonally in bold font) for the *cox1* and *gb2β* genes. For each species and each gene, the number of sequences analysed is given in parentheses. Interspecific sequence divergences within the intraspecific range of *Chironomus* species (*cox1*: 0-3% and *gb2β*: 0-2%; see Table S3) are highlighted in yellow.**

	C. anthracinus		*C. bifurcatus*		*C. cucini*		*C. decorus*-group sp. 2		*C. dilutus*		*C. entis*	
	***cox1* (16)**	***gb2β* (6)**	***cox1* (25)**	***gb2β* (10)**	***cox1* (7)**	***gb2β* (4)**	***cox1* (10)**	***gb2β* (0)**	***cox1* (11)**	***gb2β* (11)**	***cox1* (11)**	***gb2β* (11)**
C. anthracinus	**0.06**	**0.34**										
C. bifurcatus	15.21	30.34	**1.24**	**0.29**								
C. cucini	14.07	22.69	13.10	28.40	**0.21**	**0.21**						
C. decorus-group sp. 2	15.19		9.88		14.35		**0.26**					
C. dilutus	16.21	24.74	15.57	29.16	15.00	24.73	14.38		**1.34**	**0.00**		
C. entis	13.48	10.02	16.60	32.66	15.21	22.30	15.44		15.90	26.45	**1.30**	**0.25**
C. frommeri	13.26		13.20		11.89		10.90		15.80		12.91	
C. harpi	15.98	32.60	11.99	17.50	16.35	25.41	11.37		16.12	33.73	18.67	32.17
C. maturus	15.72	17.44	12.94	27.00	14.45	19.60	11.15		13.86	16.08	15.03	19.30
C. nr. *atroviridis* (sp. 2i)	19.04		15.93		16.17		14.85		14.44		17.70	
C. ochreatus	18.86		17.47		16.17		15.20		15.41		17.70	
C. plumosus	13.38	13.35	16.33	33.27	15.01	24.93	15.36		15.74	37.07	1.09	14.97
C. sp. NAI	4.48*	2.88*	15.47	32.39	14.73	26.05	15.47		17.79	28.72	14.01	13.09
C. sp. NAII	14.37		16.85		14.65		14.34		16.60		14.50	
C. sp. NAIII	16.43	35.27	18.03	31.82	15.54	18.00	15.55		18.87	28.64	19.45	32.61
C. staegeri	14.73		14.53		13.14		12.05		15.97		13.30	
C. 'tigris'	14.20	28.50	14.23	34.71	12.06	23.11	11.69		15.90	27.36	12.58	27.96

*Sequence interspecific divergences range from 1 to 5%.

Table 5. Cont.

	C. frommeri		*C. harpi*		*C. maturus*		*C.* nr. *atroviridis* (sp. 2i)		*C. ochreatus*		*C. plumosus*	
	***cox1* (3)**	***gb2β* (0)**	***cox1* (6)**	***gb2β* (6)**	***cox1* (3)**	***gb2β* (1)**	***cox1* (6)**	***gb2β* (0)**	***cox1* (3)**	***gb2β* (0)**	***cox1* (9)**	***gb2β* (29)**
C. frommeri	**0.41**											
C. harpi	14.83		**0.22**	**0.00**								
C. maturus	11.80		13.40	31.98	**0.21**							
C. nr. *atroviridis* (sp. 2i)	15.43		18.01		13.97		**1.33**					
C. ochreatus	15.12		19.55		16.04		12.14		**0.10**			
C. plumosus	12.70		18.48	28.69	14.73	27.49	17.54		17.59		**0.93**	**0.64**
C. sp. NAI	13.31		16.32	34.20	16.03	22.17	18.37		19.31		13.90	14.69
C. sp. NAII	12.34		14.89		13.35		17.88		17.26		14.58	
C. sp. NAIII	13.57		16.55	30.05	13.96	22.89	18.10		17.58		19.27	34.09
C. staegeri	3.40		14.36		12.55		16.68		15.85		13.19	
C. 'tigris'	2.68		14.75	35.83	12.38	18.48	16.47		15.66		12.44	33.11

Table 5. Cont.

	C. sp. NAI		*C.* sp. NAII		*C.* sp. NAIII		*C. staegeri*		*C. 'tigris'*	
	***cox1* (3)**	***gb2β* (3)**	***cox1* (2)**	***gb2β* (0)**	***cox1* (13)**	***gb2β* (4)**	***cox1* (16)**	***gb2β* (0)**	***cox1* (17)**	***gb2β* (4)**
C. sp. NAI	**0.82**	**0.00**								
C. sp. NAII	15.75		**0.77**							
C. sp. NAIII	16.54	37.28	14.40		**0.35**	**0.00**				
C. staegeri	14.79		13.49		13.94		**0.04**			
C. 'tigris'	14.24	30.69	12.76		13.33	15.01	1.49		**0.15**	**0.00**

Table 6. Larval morphology of fourth-instar larvae of the various *Chironomus* species collected in this study.

Species	n	Larval type (Table 3 and Fig. 1)	Mean tubule length in mm (range)			Mean larval length in mm (range)	Head width in mm (range)	Frontoclypeus color (Fig. 2)	Gula color (Fig. 3)	Mentum type		Mandible type	Pecten epipharyngis		Anterior margin of ventro-mental plates
			Lateral tubules	Anterior ventral tubules	Posterior ventral tubules					Central trifid tooth (Fig. 4)	4th lateral teeth (Fig. 6)	3rd inner tooth (Fig. 5)	Mean no. of teeth (range)	Type (Fig. 7)	
C. cucini	26-31	salinarius	absent	absent	absent	18 (15-21)	0.54 (0.49-0.62)	pale or slightly darkened with lobed dark spot anteriorly	posteriorly darkened	B	III	A	15 (12-19)	B	smooth
C. sp. NAII	4	salinarius	absent	absent	absent	12 (11-13)	not measured	dark longitudinal stripe with lobed dark spot medially	posteriorly darkened	C	II	B	13 (12-14)	A	smooth
C. sp. NAIII	47-52	salinarius	absent	absent	absent	14 (10-18)	0.48 (0.43-0.54)	pale	posteriorly to strongly darkened	B	II or III	A	12 (9-15)	A	smooth
C. bifurcatus	14-50	bathophilus	absent	1.5 (0.9-1.9)	1.2 (1.0-1.7)	13 (11-16)	0.49 (0.42-0.59)	pale	posteriorly to strongly darkened	B	II	A	13 (10-16)	A	smooth
C. decorus-group sp.2	5-17	bathophilus, fluviatilis melanotus	absent 0.18 (0.16-0.21)	0.9 (0.7-1.5)	0.9 (0.5-1.4)	14 (11-16)	0.56 (0.51-0.60)	pale	strongly to completely darkened	B or C	II	A	15 (13-20)	A	smooth
C. anthracinus	22-27	thummi	absent	1.3 (1.0-1.6)	1.0 (0.6-1.5)	18 (13-21)	0.64 (0.60-0.67)	pale	strongly to completely darkened	B or C	II	A or B	16 (13-18)	B	relatively smooth
C. nr. *atroviridis* (sp. 2i)	4	thummi	absent	1.0 (1.0-1.0)	0.9 (0.9-1.0)	19 (17-20)	0.57 (0.56-0.57)	pale	darkened posteriorly	A	I	C	16 (14-16)	D	smooth

Table 6. Cont.

Species	n	Larval type (Table 3 and Fig. 1)	Mean tubule length in mm (range)			Mean larval length in mm (range)	Head width in mm (range)	Frontoclypeus color (Fig. 2)	Gula color (Fig. 3)	Mentum type		Mandible type	Pecten epipharyngis		Anterior margin of ventro-mental plates
			Lateral tubules	Anterior ventral tubules	Posterior ventral tubules					Central trifid tooth (Fig. 4)	4th lateral teeth (Fig. 6)	3rd inner tooth (Fig. 5)	Mean no. of teeth (range)	Type (Fig. 7)	
C. ochreatus	2	thummi	absent	1.0 (1.0-1.1)	0.9 (0.7-1.2)	not measu-red	0.52 (0.51-0.52)	pale	slightly darkened	B	I	B	23 (22-24)	D	smooth
C. sp. NAI	9	thummi	absent	1.4 (1.2-1.8)	1.1 (0.9-1.7)	18 (16-21)	0.64 (0.64)	pale	strongly to completely darkened	B or C	II	A or B	15 (10-18)	B	relatively smooth
C. entis	5-8	semireduc-tus	0.26 (0.21-0.32)	0.5 (0.4-0.8)	0.6 (0.3-0.9)	25 (22-28)	0.83 (0.78-0.85)	pale	strongly to completely darkened	C or D	I	F	14 (13-17)	B	smooth to slightly crenula-ted
C. plumosus	14-33	semireduc-tus or plumosus	0.35 (0.18-0.49)	1.0 (0.5-1.7)	0.9 (0.4-1.8)	22 (13-26)	0.75 (0.65-0.89)	pale	strongly to completely darkened	C or D	I	F	16 (12-21)	B	smooth to slightly crenula-ted
C. dilutus	9	plumosus	0.47 (0.32-0.72)	1.8 (1.4-2.7)	1.8 (1.3-2.6)	24 (17-28)	0.67 (0.61-0.73)	dark, particularly in the center posteriorly	slightly to posteriorly darkened	C	I	B	13 (10-15)	A or C	smooth
C. frommeri	7	plumosus	0.52 (0.42-0.63)	3.4 (2.7-4.1)	3.7 (3.1-4.6)	22 (19-24)	0.64 (0.59-0.64)	pale	strongly to completely darkened	C	II	A or D	15 (13-18)	A	crenula-ted
C. harpi	2-6	plumosus	0.17 (0.15-0.22)	1.1 (0.9-1.3)	1.2 (0.9-1.6)	12 (10-14)	0.40 (0.38-0.42)	pale	slightly darkened	B	II	A	15 (14-15)	B	smooth
C. maturus	10	plumosus	0.40 (0.32-0.44)	2.9 (2.3-3.3)	2.8 (2.2-3.5)	18 (14-21)	0.52 (0.49-0.56)	pale	pale to slightly darkened	D	I	D	20 (18-20)	B	smooth
C. staegeri	30-44	plumosus	0.34 (0.23-0.55)	1.6 (0.9-3.7)	1.7 (1.0-3.8)	20 (14-28)	0.70 (0.62-0.76)	pale	strongly to completely darkened	C	II	A or D	17 (12-20)	A	crenula-ted
C. 'tigris'	42-66	plumosus	0.18 (0.11-0.25)	1.7 (0.9-2.5)	1.4 (0.8-2.1)	16 (9-22)	0.61 (0.54-0.68)	dark	strongly to completely darkened	C or D	II	A or D	15 (10-20)	B	relatively smooth

The intraspecific and interspecific divergences in *cox1* and *gb2β* sequences are summarized in Table 5, with more detail presented in Table S3. Overall, intraspecific divergences for *Chironomus* species characterized by *cox1* range between 0 and 3%, whereas intraspecific sequence divergences based on *gb2β* ranged between 0 and 2%. A detailed morphological description for each species is found in Table 6. Where pertinent, we also discuss the status of each species collected and its relationship to other closely-related species that we did not collect in our study. Following our species descriptions, we present a morphological key to discriminate among larvae of the 17 *Chironomus* species that we collected. Species from our study area (Ontario and Quebec) that are not included in the key are given in Martin (2013). Lastly, the distribution and ecology of each of the *Chironomus* species that we collected are summarized in Table 7.

Table 7. **Lakes in which the various *Chironomus* species were collected as well as lake characteristics. including sampling depth, pH and trophic status. Lake codes are given in Table 1. ND; not determined.**

Species	Depth (m)	pH	Trophic status	Water body
C. anthracinus	2-12	4.5-8.5	oligo to mesotrophic	AR, HA, OS, PI, RA, RM, SI (2011)
C. bifurcatus	1.5-24	2.7-7.8	oligo to eutrophic	AD, AR, DA, DP, KI, MC, OP, SJ, TI (2011)
C. cucini	9-35	6.2-7.5	oligo to mesotrophic	BO, CL, OP, SJ, VA
C. decorus-group sp.2	1-5	7.2-7.6	oligo to mesotrophic	AD, DF, DP, FO, OP, SC, SI (2011)
C. dilutus	1.5-5	7.5-8.4	eutrophic	KE
C. entis	1-9	7.1-8.3	meso to eutrophic	DA, DS, MN, OP, PE,
C. frommeri	1	ND	ND	PO
C. harpi	1-4	2.7-3.8	oligotrophic	AR
C. maturus	4-6	ND	ND	BE
C. nr. a*troviridis* (sp. 2i)	1	7.4	meso to eutrophic	MN
C. *ochreatus*	3	7.7	mesotrophic	OP
C. plumosus	1-8	6.8-8.5	oligo to eutrophic	AU, DA, DP, FO, KE, KI, MN, OS, PE, RO
C. sp. NAI	7.5	6.8	oligotrophic	KA
C. sp. NAII	4	5.9	oligotrophic	SI (2007)
C. sp. NAIII	5-12	7.1-7.9	oligo to mesotrophic	DA, HA, MC, RA, RM
C. staegeri	1-10	5.9-8.0	oligo to eutrophic	CR, DA, DP, KA, KI, MC, OP, PO, SI (2007), SJ, TI (2007 and 2011)
C. 'tigris'	2-10	5.9-8.0	oligo to mesotrophic	KA, MC, OP, SI (2007), SJ, TI (2007 and 2011)

Species descriptions and taxonomic status

Chironomus* (*Chironomus*) '*tigris'

(nomen nudum in Martin *et al*. (2008), for species *C*. sp. Am1 of Kiknadze *et al*. (1993)).

Material examined (Table S1) : 66 larvae from Kasten Lake, McFarlane Lake, Silver Lake and Tilton Lake in Ontario as well as from Lake Opasatica and Lake St. Joseph in Quebec.

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. Compared to the other *Chironomus* species (except *C*. sp. NAIII), the *gb2β* gene of *C. tigris* is 3 codons short immediately after the end of the 2nd intron. *C. 'tigris'* sequences form distinct clades in both the *cox1* and *gb2β* ID-trees. Consequently either gene can be used to accurately separate and identify *C. 'tigris'*. However, the range of interspecific divergence between the *cox1*-sequences of *C. 'tigris'* and *C. staegeri* (1–2%), as well as between *C. 'tigris'* and *C. frommeri* (2–3%), are within the intraspecific sequence divergence range of collected and reference *Chironomus* species (0–3%). Therefore, *cox1* sequence divergence values alone cannot be used to reliably separate *C. 'tigris'* from *C. staegeri* or *C. frommeri*. For the *gb2β* gene, we could not assess the interspecific sequence divergence between *C. 'tigris'*, *C. staegeri* and *C. frommeri* because we were unsuccessful in amplifying the *gb2β* gene for *C. staegeri* and *C. frommeri*. In the *cox1* ID-tree, sequences of collected larvae cluster with the reference sequence of *C. 'tigris'*, thus confirming the identification of this species. Chosen restriction enzymes for the *cox1* PCR-RFLP analysis correctly separated *C. 'tigris'* larvae from the other *Chironomus* species.

Morphology (Table 6). Our specimens are large sized plumosus-type larvae having their anterior ventral tubules longer than the posterior ventral tubules. The frontoclypeus of *C. 'tigris'* is dark-colored, which distinguishes it from larvae of the otherwise morphologically-similar *C. staegeri* and *C. frommeri*, which both have a pale frontoclypeus. Exceptionally, in larvae from some other regions, the frontoclypeus of *C. staegeri* is reported to be slightly darkened (Martin 2013). However, this criterion could prove to be less clear cut and other morphological features such as the length of the lateral tubules and the anterior margin of ventromental plates could be used to separate these species.

Cytology. The cytology of the two larvae analyzed clearly indicates that this species is *C. 'tigris'* since it is one of only two *Chironomus* species known to possess two polytene chromosomes. In this respect, it is clearly distinct from *C. staegeri* and *C. frommeri* which possess three and four chromosomes, respectively. The arm combination of *C. 'tigris'* chromosomes is GAB, FEDC and

its chromosomes are described in Martin *et al*. (1974), Butler *et al*. (1995, *C*. sp. r), Kiknadze *et al*. (1993, *C*. sp. Am1) and Martin (2013).

Distribution and ecology (Table 7). This species has been previously reported from lakes in Minnesota, Ontario, Quebec and Wisconsin (Butler *et al*. 1995; Martin *et al*. 2008; Martin 2013). We found *C. 'tigris'* in oligotrophic to mesotrophic lakes of pH 5.9–8.0. At all sites where *C. 'tigris'* was collected, *C. staegeri* was also present. However, the reverse was not necessarily the case. Lakes in which *C. staegeri* was present and *C. 'tigris'* was absent tended to be eutrophic (with the exception of Crooked Lake), which suggests that *C. 'tigris'* larvae prefer less productive systems. Their northerly distribution in North America may reflect this fact. We collected *C. 'tigris'* at water depths varying from 2–10 m, although it can live at greater depths (20 m; Butler *et al*. 1995).

Chironomus (*Chironomus*) *staegeri* Lundbeck (1898)

Material examined (Table S1): 44 larvae from Lake D'Alembert, Lake Duprat, Lake Kinojévis, Lake Opasatica, Lake St. Joseph and an unnamed pond in Quebec as well as from Crooked Lake, McFarlane Lake, Kasten Lake, Silver Lake, and Tilton Lake in Ontario.

DNA (Figs. 8–9 and Tables 4–5, S3). In the *cox1* ID-tree, *C. staegeri* sequences form a distinct clade. Sequences of collected larvae cluster with the reference sequence of *C. staegeri*, thereby confirming the identification of this species. We were not successful in amplifying the *gb2β* gene for this species. *Cox1* interspecific sequence divergences between *C. staegeri* and *C. 'tigris'* (1–2%) as well as *C. staegeri* and *C. frommeri* (3–4%) are within the intraspecific divergence range of *Chironomus* species assessed in this study (0– 3%). Therefore, *cox1* sequence divergence values cannot be used to separate *C. staegeri* from *C. 'tigris'* or from *C. frommeri*. Restriction enzymes for the *cox1* PCR-RFLP analysis correctly separated *C. staegeri* from the other *Chironomus* species.

Morphology (Table 6). Our specimens of *C. staegeri* are large sized plumosus-type larvae with a pale frontoclypeus. This latter feature clearly distinguishes them from *C. 'tigris'* larvae that have a dark frontoclypeus. Outside of our study area, some *C. staegeri* larvae are reported to have a slightly darkened frontoclypeus (Martin 2013). Thus other features such as the length of the lateral tubules and the outline of the anterior margin of ventromental plates could be examined to separate these two species. In our study area, *C. staegeri* could be distinguished from *C. frommeri* by the fact that the ventral and lateral tubules of the former were about half the

length of those of the latter (Table 6). However, tubule length is not likely a reliable character to separate these species because in the study by Sublette and Sublette (1971) tubule lengths overlapped between these species. Sublette and Sublette (1971) suggested that *C. frommeri* and *C. staegeri* larvae could be separated by the structure of the anterior margin and apex of their paralabial plates as well as the shape of the teeth of the pecten epipharyngis. However, these features did not reliably separate these species in our study area.

Cytology. The cytology of the single larva that we examined indicates that this species is *C. staegeri* since it has three chromosomes with a modified thummi arm combination of AB, CD, GEF (Wülker & Martin 1971; Kiknadze *et al*. 2004; Kiknadze *et al*. 2010). Thus it is distinct from *C. 'tigris'*, which possesses 2 chromosomes, and from *C. frommeri*, which has 4 chromosomes.

Distribution and ecology (Table 7). *C. staegeri* has been found in a variety of lentic habitats from deep lakes to shallow pools (Wülker *et al*. 1971) throughout Canada (British Columbia, Manitoba, Newfoundland, Northwest Territories, Ontario and Saskatchewan) and the United States (Alabama, California, Idaho, Illinois, Iowa, Kansas, Louisiana, Massachusetts, Michigan, Minnesota, Missouri, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Pennsylvania, South Carolina, South Dakota, Tennessee, Washington and Wisconsin) (Sublette & Sublette 1971; Oliver *et al*. 1990; Martin *et al*. 2008; Martin 2013). In our study, *C. staegeri* was found in oligotrophic to eutrophic lakes and in a pond at depths ranging from 1–10 m, and at pH values ranging from 5.9–8.0.

Taxonomic comment. Given the polymorphism of chromosomal inversions in populations of *C. staegeri* in Canada and the Unites States, Martin and Wülker (1971) speculated that *C. staegeri* might be in the process of splitting into three species based in part on their restriction to waters of different depths. Our DNA data do not support this idea since there is little variation in the *cox1* nucleotide sequences between *C. staegeri* that we collected from a pond, and over a range of depths in several lakes. In fact, the mean *cox1* intraspecific divergence among *C. staegeri* sequences is very low (0.04%). The different distributions of chromosomal inversions might therefore be due to populations with different inversion sequences adapting to different ecological niches, as has been suggested for species such as *C. plumosus* (Butler *et al*. 1999).

***Chironomus* (*Chironomus*) *frommeri* Sublette and Sublette (1971)**

Material examined (Table S1): 7 larvae collected from an unnamed pond on a military base near Trois-Rivières, Quebec.

DNA (Figs. 8–9 and Tables 4–5, S3). In the *cox1* ID-tree, *C. frommeri* nucleotide sequences form a distinct clade. However, as mentioned above, *cox1* sequence divergences between *C. frommeri* and *C. 'tigris'* (2–3%) as well as *C. frommeri* and *C. staegeri* (3–4%) are within the intraspecific sequence divergence range of *Chironomus* species assessed in this study (0–3%). Therefore, *cox1* sequence divergence values cannot be used to separate *C. frommeri* from *C. 'tigris'* or *C. staegeri*. We were not successful in amplifying the *gb2β* gene for this species. Chosen restriction enzymes for the *cox1* PCR-RFLP analysis correctly separated *C. frommeri* from the other *Chironomus* species.

Morphology (Table 6). Our specimens are large sized plumosus-type larvae with posterior ventral tubules longer than the anterior ones. Morphologically, larvae of *C. frommeri* from our study area can be distinguished from those of *C. 'tigris'* and *C. staegeri* (however, see comments above on the morphology of these species).

Cytology. The cytology of the larva analyzed permitted us to identify this species as *C. frommeri*. *C. frommeri* has four polytene chromosomes with the thummi arm combination of AB, CD, EF, G (Wülker & Martin 1971) as opposed to *C. 'tigris'* and *C. staegeri* that possess only 2 and 3 chromosomes, respectively. Arm G homologs of *C. frommeri* are closely paired, with a virtually terminal nucleolus, similar to the fused arm G of *C. crassicaudatus* (not collected in our study) (Wülker & Martin 1971). There is also another nucleolus proximal in arm B.

Distribution and ecology (Table 7). We were surprised to collect *C. frommeri* in eastern Canada because all previous collections of this species are from the western United States (California, Oregon, Utah and New Mexico) (Wülker *et al*. 1971; Oliver *et al*. 1990). A possible explanation for this apparent anomaly is that this species was transported from the west to the east via military equipment since it was collected in a pond located in a military base. This species is known to occur in lakes, oxbows and permanent ponds (Martin 2013).

***Chironomus* (*Chironomus*) *cucini* Webb (1969)**

Material examined (Table S1): 31 larvae collected in lakes in Quebec (Lake Bousquet, Lake Opasatica, Lake St. Joseph and Lake Vaudray) and in Ontario (Clearwater Lake).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. In both of these ID-trees, *C. cucini* forms a distinct cluster. Consequently both genes can be used to accurately separate and identify *C. cucini*. The *gb2β* gene sequence of *C. cucini* has no intron, but contains three extra base pairs at the 3' end of the sequence. Chosen restriction enzymes for the *cox1* PCR-RFLP analysis correctly separated *C. cucini* from the other *Chironomus* species.

Morphology (Table 6). Our specimens are large sized salinarius-type larvae lacking lateral and ventral tubules. In other study areas, larvae of *C. cucini* are reported to occasionally have small posterior ventral tubules (Wülker & Butler 1983). Morphologically, larvae of *C. cucini* are similar to those of *C*. sp. NAIII with two minor differences. First, the structure of the pecten epipharyngis differs slightly between the species (Table 6). Second, the mean (± 95% CI) ratio of the lengths of antennal segments 1 to 2–5 (AR) of *C*. sp. NAIII larvae (1.77 ± 0.08) was significantly lower than that of *C. cucini* (2.04 ± 0.08). Note however that there was overlap in the ranges of the ARs between the two species.

Cytology. The cytology of the 5 larvae analyzed permitted us to identify this species as *C. cucini*. *C. cucini* has four polytene chromosomes attached together by a chromocenter and with the thummi arm combination of AB, CD, EF, G (Martin 1979; Wülker & Butler 1983). In most populations, a single nucleolus is located in arm G, although in some California populations there is a second nucleolus in arm B.

Distribution and ecology (Table 7). *C. cucini* has been reported from across the Nearctic region (British Columbia, California, Indiana, Minnesota, New York and Ontario) (Oliver *et al*. 1990; Martin 2013). In our study area, *C. cucini* was found in the profundal zone of circum-neutral (pH 6.2–7.5), oligotrophic to mesotrophic, lakes.

Chironomus sp. NAII

Material examined (Table S1): 4 larvae from Silver Lake (Ontario).

DNA (Figs. 8–9 and Tables 4–5, S3). In the *cox1* ID-tree, *C*. sp. NAII nucleotide sequences clearly form a distinct clade. We were not successful in amplifying the *gb2β* gene for this species. Available DNA barcodes for *Chironomus* species did not cluster with those of *C*. sp. NAII. *Cox1* PCR-RFLP analysis correctly separated *C*. sp. NAII from the other *Chironomus* species.

Morphology (Table 6). Our specimens are medium sized salinarius-type larvae. Morphologically, larvae of *C*. sp. NAII closely resemble those of *C. cucini* and *C*. sp. NAIII, but differ by the lobed dark spot in the middle of its frontoclypeus, by the partial coloration of its 3rd mandibular tooth and by the type of mentum central trifid tooth.

Cytology. This species has four polytene chromosomes with two nucleoli, one of which is in arm G. The cytology does not correspond to any known salinarius-type specimens from North America or Europe. The main difference between *C*. sp. NAII and the other known salinarius-type species is its lack of heterochromatic centromeres and its banding sequence in arm G. The cytological preparations were generally too poor to determine further details. This may be the larva of a previously described northern *Chironomus* species, for which the larva is currently unknown, or it may be a completely new species.

Distribution and ecology (Table 7). This species was found in oligotrophic Silver Lake in 2007 at a depth of 4 m. In 2010 and 2011, we sampled the lake again in an effort to collect additional *C*. sp. NAII larvae. However, they were no longer present, perhaps because the pH of this lake had increased from 5.9 to 7.0 between 2007 and 2010.

Chironomus sp. NAIII (possibly *C. decumbens* (Malloch 1934))

Material examined (Table S1): 52 larvae collected in Lake D'Alembert (Quebec) and Hannah Lake, McFarlane Lake, Raft Lake and Ramsey Lake (Ontario).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. *C*. sp. NAIII sequences form a distinct clade in both the *cox1* and *gb2β* ID-trees. Like *C. cucini*, the *C*. sp. NAIII *gb2β* sequence contains 3 extra base pairs at the 3' end, but unlike *C. cucini*, whose *gb2β* sequence has no intron, the *C*. sp. NAIII *gb2β* sequence contains a type II intron. Additionally, unlike the other *Chironomus* species (except *C. 'tigris'*) the

gb2β of *C*. sp. NAIII is three codons short immediately after the end of the 2nd intron. *Cox1* PCR-RFLP analysis correctly separated *C*. sp. NAIII from the other *Chironomus* species. Sequences of *C*. sp. NAIII did not cluster with any of the available *Chironomus* species reference sequences.

Morphology (Table 6). Our specimens are medium sized salinarius-type larvae that are difficult to distinguish from those of *C. cucini* (see comments on the separation of these species under *C. cucini*). The morphology of *C*. sp. NAIII (see Table 6) is similar to that of *C. decumbens* (see Martin 2013).

Cytology. This species has three polytene chromosomes with heterochromatic centromeres. The arm combination is modified thummi-complex AB, CD, GEF. A nucleolus is located near the junction of arm G with arm E and a Balbiani ring is located towards the other end of arm G. Cytologically, this species fits the description of the North American cytospecies *C*. sp. 2x (Martin 2013) from Alaska, which is thought to be *C. decumbens* (Jim Sublette, personal communication). The only difference between our specimens and *C*. sp. 2x, is that our larvae possess a heavily heterochromatic centromere. This difference may or may not be significant since the presence of a heavily heterochromatic centromere can differ between populations and its detection can vary with the stain used. The voucher *C*. sp. 2x slide was stained with a brand of orcein that gave much paler staining.

Unfortunately, no *cox1* or *gb2β* sequences of *C. decumbens* voucher specimens were available for comparison with sequences for our study larvae. Further investigation on a possible relationship between *C. decumbens* and *C*. sp. NAIII is clearly warranted.

Distribution and ecology (Table 7). This species was found in oligotrophic to mesotrophic lakes at depths varying from 5–12 m and at pHs varying from 7.1–7.9.

Chironomus sp. NAI (*C. anthracinus*-group)

Material examined (Table S1): 9 larvae from Kasten Lake (Ontario).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. This species forms a distinct clade in the *cox1* ID-tree. However in the *gb2β* ID-tree, sequences of *C*. sp. NAI cluster with those of *C. anthracinus*. Chosen restriction enzymes for the *cox1* PCR-RFLP analysis correctly separated *C*. sp. NAI from the other

Chironomus species. Available DNA barcodes did not allow us to identify *C*. sp. NAI (see discussion at the end of this section).

Morphology (Table 6). Our specimens are large sized thummi-type larvae with the anterior ventral tubules slightly longer than the posterior ones. Morphologically, larvae of *C.* sp. NAI and *C. anthracinus* are indistinguishable.

Cytology. *C*. sp. NAI has four short polytene chromosomes with the thummi arm combination of AB, CD, EF, G. The most common sequence in each chromosome arm is similar to that in *C. anthracinus*.

Distribution and ecology (Table 7). This species was found in an oligotrophic, circum-neutral (pH 6.8), lake at a depth of 7.5 m.

Taxonomic comment. *C*. sp. NAI larval morphology and cytology strongly resemble those of *C. anthracinus*. Additionally, *gb2β* sequences of *C*. sp. NAI and *C. anthracinus* cluster together in the ID-tree (sequence divergence varies from 1 to 5%). In fact, there are no consistent base differences between these two *gb2β* sequences. Despite this lack of morphological, cytological or genetic difference, *cox1* sequences suggest that *C.* sp. NAI is a distinct species. The *cox1* sequence divergence between *C*. sp. NAI and *C. anthracinus* is relatively high (4–6%), and these sequences consistently differ by 22 bases (Table S4). For comparative purposes, other interspecific differences can be much lower, with *C. staegeri* and *C. 'tigris'* differing by only 9 specific bases, *C. frommeri* and *C. 'tigris'* by 13 specific bases, and *C. staegeri* and *C. frommeri* by specific 19 bases.

In the *cox1* ID-tree, the reference sequence for Palearctic *C. anthracinus* larvae from Lake Esrom (Denmark) clusters with larvae from our lakes that have been cytologically identified as *C. anthracinus*, not with the adjacent cluster formed by our *C.* sp. NAI sequences. We hypothesize that larvae of *C*. sp. NAI might be *C. rempelii* Thienemannn (1941). Currently, there are conflicting opinions as to the status of *C. rempelii*. Based on adult morphology, Townes (1945) concluded that *C. rempelii* was a synonym of *C. anthracinus* Zetterstedt (1860). Shobanov *et al*. (1996) and Kiknadze *et al*. (2005) reached the same conclusion when they compared the chromosomes of these species. However, the heterochromatin on arm F and the sequences A3, C3 and F3 have so far been found only in samples from western Canada that include the type locality of *C. rempelii* (British Columbia, Alberta, Saskatchewan and Manitoba; Kiknadze *et al*. 2005). The large hetrochromatic block in the original *C. rempelii* population occurred in all males. However, no other populations were sexed, and the smaller blocks and

the inversions are rare. Consequently, the absence of these in our material is not conclusive. Samples from the type locality of *C. rempelii* would be required to confirm our hypothesis. We amplified the *cox1* sequence of a *Chironomus* larva from British Columbia (Marion Lake) that was morphologically and cytologically indistinguishable from that of *C. anthracinus* (labelled as "*C*. (*anthracinus*-group.)" in Fig. 8) and found that its *cox1* sequence (Fig. 8) clusters with sequences of *C*. sp. NAI. Thus *cox1* sequences suggest that the currently recognized *C. anthracinus* in North America is not a single species, but a complex of at least two closely related species—the *C. anthracinus*-group. This may be the result of a recent speciation that occurred without hybridization, so that *cox1* has differentiated while the short and slower evolving *gb2β* sequence has not had time to accumulate significant changes. Further investigations of *C*. sp. NAI and the species status of *C. rempelii* are clearly warranted.

Chironomus (*Chironomus*) *anthracinus* Zetterstedt (1860)

Material examined (Table S1): 27 larvae from Hannah Lake, Pine Lake, Raft Lake, Ramsey Lake and Silver Lake in Ontario and from Lake Arnoux and Lake Osisko in Quebec.

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. *C. anthracinus* sequences form a distinct clade in the *cox1* ID-tree and cluster with the Palearctic *C. anthracinus* reference sequence, which confirms the identification of this species. In the *gb2β* ID-tree, *C. anthracinus* sequences cluster with sequences of *C*. sp. NAI, the identity of which is uncertain (see discussion in the *C*. sp. NAI section). Chosen restriction enzymes for the *cox1* PCR-RFLP analysis correctly separated *C. anthracinus* larvae from the other *Chironomus* species.

Morphology. Our specimens are large sized thummi-type larvae whose anterior ventral tubules are usually longer than their posterior ones. Morphologically, larvae of *C. anthracinus* and *C*. sp. NAI are identical.

Cytology. The cytology of the 5 larvae analyzed was consistent with *C. anthracinus*, but identical to *C*. sp. NAI. *C. anthracinus* has 4 relatively short chromosomes with the thummi arm combination of AB, CD, EF, G (Kiknadze *et al*. 2005). The short chromosomes mean that banding patterns are often difficult to see clearly. There are two nucleoli: one on arm G and the other on arm F.

Distribution and ecology (Table 7). *C. anthracinus* is widely distributed in the Holarctic region. In the Nearctic region, it occurs across Canada (Alberta, British Colombia, Manitoba, Ontario

and Saskatchewan) and the United States (California, Indiana, Massachusetts, New Hampshire New York and Wisconsin) (Sæther 1975; Oliver *et al*. 1990; Sæther 2012; Martin 2013). We found C. anthracinus at depths ranging from 2–12 m in highly acidic (pH 4.4) to circum-neutral (pH 8.5) lakes. The trophic status of these lakes was oligotrophic to mesotrophic, which is consistent with Sæther's (1975) suggestion that Nearctic *C. anthracinus* are more common in intermediate- to low-productivity lakes. In contrast, in the Palearctic region, *C. anthracinus* is known to be more frequently found in the profundal zone of moderately eutrophic lakes (Sæther 1975).

Chironomus (*Chironomus*) *entis* Shobanov (1989)

Material examined (Table S1): 8 larvae from Lake D'Alembert, Lake Dasserat, Lake Marlon, Lake Opasatica and Lake Pelletier (Quebec).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and the *gb2β* genes. In accordance with the findings of Makarevich *et al*. (2000), *C. entis gb2β* has a type II intron. In the *cox1* ID-tree, sequences of cytologically identified *C. plumosus* and *C. entis* larvae cluster together. In fact, some *C. plumosus* and *C. entis* sequences are identical. Similarities between the mitochondrial nucleotide sequences of these two species were also observed by Guryev and Blinov (2002), who found that trees based on the mitochondrial *cytb* gene did not group populations of *C. entis* and *C. plumosus* according to their species affiliation but rather according to their geographic occurrence. They attributed this phenomenon to mitochondrial gene flow that occurs when populations of sympatric sibling species produce fertile hybrids such that mitochondrial DNA appears in the progeny of the backcross. In contrast, in accordance with the findings of Guryev and Blinov (2002), our ID-tree based on nuclear *gb2β* gene sequences successfully groups *C. entis* and *C. plumosus* according to species. The intraspecific variability of the partial *gb2β* nucleotide sequences of our *C. entis* (0%) and *C. plumosus* (2.3%) larvae are considerably lower that their interspecific variability (15–16%). Thus *C. entis* and *C. plumosus* can be distinguished using the *gb2β* gene, but not the *cox1* gene. Lastly, since PCR-RFLP analysis was performed using the *cox1* gene, this result could not be used to separate *C. entis* and *C. plumosus*.

Morphology (Table 6). Our specimens are very large semireductus-type larvae. Morphologically, *C. entis* and *C. plumosus* are almost indistinguishable. Kiknadze *et al*. (1991) described the outer hooks on the anterior margin of the ventromental plates as being shorter

and blunter in *C. plumosus* than in *C. entis* in Palearctic populations; however, we did not observe such differences in our specimens. *C. entis* are semireductus-type larvae, whereas those of *C. plumosus* vary from being semireductus-type to plumosus-type. In fact, some *C. plumosus* larvae from our study lakes have ventral tubules that are intermediate between the plumosus-type and semireductus-type. Within a given lake, the ventral tubules of *C. plumosus* were always longer than those of *C. entis*, which allowed the larvae of these species to be separated. However, between lakes, there was considerable overlap in their lengths. Consequently, *C. plumosus* and *C. entis* cannot be distinguished based solely on morphology such that cytological and genetic techniques are needed to unambiguously separate them.

Cytology. All larvae were analyzed cytologically to confirm the identification of this species and to verify the accuracy of the DNA results. *C. entis* has four relatively short chromosomes with the thummi arm combination of AB, CD, EF, G (Kiknadze *et al*. 2000a; Kiknadze *et al*. 2000b; Gunderina *et al*. 2009), with only a single nucleolus in arm G. It shares two rare sequences with Nearctic populations of *C. plumosus* (Kiknadze *et al*. 2000a).

Distribution and ecology (Table 7). *C. entis* has previously been reported from lakes in Canada (British Columbia, Manitoba, Ontario and Saskatchewan) and in the United States (Colorado, Indiana, Minnesota, North Dakota, Oklahoma, South Dakota and Wisconsin) (Kiknadze *et al*. 2000a; Martin 2013). We found *C. entis* in mesotrophic to eutrophic, circum-neutral (pH 7.1–8.3) lakes at depths ranging from 1–9 m.

Chironomus (*Chironomus*) *plumosus* Linnaeus (1758)

Material examined (Table S1): 33 larvae from lakes in Quebec (Lake D'Alembert, Lake Duprat, Lake Fortune, Lake Kinojévis, Lake Marlon, Lake Osisko, Lake Pelletier, Lake Rouyn and Lake Saint Augustin) as well as Kelly Lake in Ontario.

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. In accordance with the findings of Makarevich *et al*. (2000), *C. plumosus gb2β* has a type II intron. *C. plumosus* cannot be distinguished from *C. entis* on the basis of *cox1* sequences. However, these species can be separated using *gb2β* sequences (see comments in the *C. entis* DNA section).

Morphology (Table 6). Our specimens are very large semireductus- (commonly referred to as "semi-reductus" type in reference to *C. plumosus*) to plumosus-type larvae (see comment in

section on *C. entis* morphology). Morphologically, larvae of *C. entis* and *C. plumosus* are indistinguishable (see comment in *C. entis* morphology section).

Cytology. All larvae were analyzed cytologically to confirm the identification of this species and to verify the accuracy of the DNA results. *C. plumosus* has four relatively short chromosomes with the thummi arm combination of AB, CD, EF, G (Butler *et al*. 1999) with only a single nucleolus in arm G.

Distribution and ecology (Table 7). This species was previously known from lakes in Canada (British Columbia, Manitoba, Ontario and Saskatchewan) and the United States (Alabama, California, Colorado, Indiana, Kentucky, Massachusetts, Minnesota, New Mexico, North Dakota, Oklahoma, South Dakota and Wisconsin) (Butler *et al*. 1999; Martin 2013) at depths up to 23m (Martin 2013). We found *C. plumosus* at depths ranging from 1–8 m, and in oligotrophic to eutrophic lakes ranging in pH from 6.8–8.5.

Chironomus (*Chironomus*) *maturus* Johannsen (1908)

Material examined (Table S1): 10 larvae from Lake Bédard (Quebec).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and the *gb2β* genes. In both ID-trees, *C. maturus* sequences form a distinct clade. In the *cox1* ID-tree, sequences of the larvae we collected cluster with the reference sequence of *C. maturus*, thereby confirming the identification of this species. *Cox1* PCR-RFLP analysis accurately separated *C. maturus* larvae from the other *Chironomus* species.

Morphology (Table 6). Our specimens are large sized plumosus-type larvae with long ventral and lateral tubules. The frontoclypeus is pale; however, some *C. maturus* from other regions are reported to have a dark frontoclypeus (Martin 2013). Larvae of *C. maturus* could be distinguished from the other collected plumosus-type larvae through a combination of morphological features (see morphological key at the end of this section).

Cytology. *C. maturus* larvae were not identified through cytology because their chromosomes were not in good enough condition. *C. maturus* is known to possess four polytene chromosomes with a maturus arm combination of AF, BE, CD, G. The cytology of *C. maturus* has been described by Wülker and Martin (1974) and Kiknadze *et al*. (2004).

Description and ecology. This species has been recorded previously from shallow pools and polluted water bodies (Martin 2013) in central Canada (Manitoba and Ontario) and the United

States (Alaska, California, Dakota, Indiana, Louisiana, New Mexico, New York, South Dakota and Wisconsin) (Oliver *et al*. 1990; Martin 2013). We collected *C. maturus* in a mesotrophic and circum-neutral (pH = 7.3) lake.

Chironomus (*Chironomus*) *decorus*-group sp. 2 Butler *et al*. (1995)

Material examined (Table S1): 17 larvae from Lake Adéline, Lake Dufault, Lake Duprat, Lake Fortune, Lake Opasatica and the St. Charles River in Quebec as well as Silver Lake in Ontario.

DNA (Figs. 8–9 and Tables 4–5, S3). In the *cox1* ID-tree, *C. decorus*-group sp. 2 nucleotide sequences form a distinct clade. Sequences of larvae we collected cluster with the reference sequence of *C. decorus*-group sp. 2 from GenBank, therefore confirming the identity of this species. The *cox1* interspecific sequence divergence between *C. decorus*-group sp. 2 and reference sequence *C. quinnitukqut* (3%) (data not shown) is within the intraspecific sequence divergence range (0–3%) of *Chironomus* species assessed in this study. Therefore, sequence divergence cannot be used to separate these species. *Cox1* PCR-RFLP analysis correctly separates *C. decorus*-group sp. 2 from the other *Chironomus* species. We were not successful in amplifying the *gb2β* gene of this species.

Morphology (Table 6). Our specimens are mostly medium sized bathophilus- or fluviatilis-type larvae (Table S1). However, whereas Quebec specimens had no lateral tubules, those from Silver Lake, Ontario, had small lateral tubules (melanotus-type). The 3rd inner tooth of the mandibles is pale and fused to the lower margin, however, in lakes from other regions, they are reported to be partially darkened (Martin 2013). From the morphological description of *C. quinnitukqut* in Martin (2013), larvae of *C. decorus*-group sp. 2 that do not possess lateral tubules could not be distinguished from those of *C. quinnitukqut*. Furthermore, larvae of *C. decorus*-group sp. 2 cannot be distinguished from those of *C. bifurcatus*.

Cytology. The cytology of the two larvae analyzed clearly indicates that they belong to a species currently referred to as *C. decorus*-group sp. 2. *C. decorus*-group sp. 2 has four polytene chromosomes with the thummi arm combination of AB, CD, EF, G (Butler *et al*. 1995). Typical of members of the *C. decorus*-group, it has only a single nucleolus, which is virtually terminal in arm G.

Description and ecology. This species has been collected previously in Canada (Saskatchewan) and the United States (Massachusetts, Minnesota, Mississippi, New Mexico, North Dakota, Vermont and Wisconsin) (Butler *et al*. 1995; Martin 2013). *C. decorus*-group sp. 2

larvae are reported from depths greater than 10 m (Martin 2013); however, in our study *C. decorus*-group sp. 2 larvae were found at depths of 1–5 m. *C. decorus*-group sp. 2 was collected in oligotrophic to mesotrophic lakes of circum-neutral pH (7.2–7.6). However, it was also found in the St. Charles River where sediments have been contaminated by untreated municipal waste waters.

***Chironomus* (*Chironomus*) *harpi* Sublette (in Wülker *et al*. 1991)**

Material examined (Table S1): 6 larvae from Lake Arnoux (Quebec).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. *C. harpi* sequences form distinct clades in both the *cox1* and *gb2β* ID-trees. *Cox1* sequences of collected larvae cluster with the reference sequence of *C. harpi*, thus confirming the identification of this species. *Cox1* PCR-RFLP profiles were not obtained for *C. harpi*. However, through simulation digests, we were able to demonstrate that the chosen restriction enzymes (*Ssp* I, *Hinf* I, *Rsa* I and *Taq* I) would have accurately separated *C. harpi* from the other *Chironomus* species.

Morphology (Table 6). Our specimens fit the morphological description of *C. harpi* (Martin 2013). They are medium sized plumosus-type larvae with posterior ventral tubules longer than the anterior ones. Morphologically, larvae of *C. harpi* strongly resemble those of the other plumosus-type larvae collected in our study by having a pale frontoclypeus (*C. frommeri*, *C. staegeri* and *C. maturus*). *C. harpi* larvae can be distinguished from larvae of these other species principally by the type of the middle trifid tooth on the mentum (see Table 6).

Cytology. We did not identify *C. harpi* through cytology. They are reported to possess four polytene chromosomes with the thummi arm combination of AB, CD, EF, G, with a large nucleolus near the centromere of arm D, and a second nucleolus sometimes developed medially in arm G. (Wülker *et al*. 1991).

Distribution and ecology (Table 7). In our study, we found *C. harpi* at depths of 1–4 m in a lake that has been heavily impacted by acid mine drainage (pH 2.7–3.8). This further supports the identification of this species, since *C. harpi* had been reported previously only from acidic pools (Arkansas, Illinois, New York and South Dakota) (Martin 2013).

***Chironomus* (*Chironomus*) *bifurcatus* Wuelker, Martin, Kiknadze, Sublette and Michiels (2009)**

Material examined (Table S1): 50 larvae collected in Quebec (Lake Adéline, Lake Arnoux, Lake D'Alembert, Lake Duprat, Lake Kinojévis, Lake Opasatica and Lake St. Joseph) and in Ontario (McFarlane Lake and Silver Lake).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both the *cox1* and *gb2β* genes. In the *cox1* ID-tree, sequences of collected larvae cluster with the reference sequences of *C. bifurcatus*, thereby confirming the identification of this species. However, in the *cox1* ID-tree, collected and reference *C. bifurcatus* nucleotide sequences form two distinct clades (labelled in Fig. 8 as group 1 and group 2) that differed by 7 specific bases (Table S5). There were also consistent cytological differences between these two groups (see cytology section). However, the sequence divergence between *cox1*-sequences from these two groups is low (2%) and, in the *gb2β* ID-tree, *C. bifurcatus* sequences from both groups cluster together.

Three different profiles were created through PCR-RFLP analysis of the *cox1* gene. One profile included all group 1 larvae and the other two profiles (obtained using the *Taq* I enzyme) included all group 2 larvae. Simulation digests demonstrate that the *Ssp* I, *Hinf* I, *Rsa* I and *Taq* I restriction enzymes would not have separated *C. bifurcatus* (gr. 2) larvae from *C. dilutus* because in some cases their restriction fragment lengths are so similar that it would be difficult to differentiate them on an agarose gel. However, simulation digests demonstrate that both species should be separable using the restriction enzyme *Alu* I.

Morphology (Table 6). Our specimens are medium sized bathophilus-type larvae with the anterior ventral tubules slightly longer than the posterior ones. All of our larvae lacked lateral tubules. In contrast, larvae from some populations of *C. bifurcatus* are reported to have either very small (180 µm) lateral tubules (about 180 µm; Wuelker *et al*. 2009) (melanotus-type) or be fluviatilis-type (Martin 2013). The frontoclypeus of our *C. bifurcatus* larvae is pale, but is reported to be slightly darkened in larvae from some other regions (Wuelker *et al*. 2009). There were no morphological differences between larvae belonging to groups 1 and 2. Morphologically, larvae of *C. bifurcatus* cannot be distinguished from those of *C. decorus*-group sp. 2.

Cytology. The cytology of the 5 specimens analyzed permitted us to identify them as *C. bifurcatus*. Larvae of this species have four polytene chromosomes with the thummi arm combination of AB, CD, EF, G, as well as a single, virtually terminal, nucleolus in arm G (Wuelker *et al*. 2009). However, we noted some cytological differences among our larvae and

among reference specimens that allowed all of the specimens to be separated into two groups that corresponded to those mentioned above for the *cox1* gene. Thus larvae from group 1 have cytological sequences B1 and F1 with no median Balbiani ring in the middle of arm G, whereas those from group 2 have cytological sequences B2 and F2, and a Balbiani ring in the middle of arm G.

Distribution and ecology (Table 7). This species has been collected previously in southern Canada (Manitoba, Ontario and Quebec) and the northern United States (Massachusetts, Michigan, Minnesota and Wisconsin) (Wuelker *et al*. 2009; Martin 2013). We collected larvae of *C. bifurcatus* gr. 1 only in Quebec lakes (Lake Arnoux, Lake D'Alembert, Lake Duprat, Lake Opasatica and Lake St. Joseph), whereas those in gr. 2 were found in lakes in both Quebec (Lake Kinojévis and Lake St. Joseph) and Ontario (McFarlane Lake and Silver Lake). Larvae belonging to the two genetic groups differed somewhat in their distribution with respect to lake water pH, trophic status and water depth. Specifically, larvae of *C. bifurcatus* (gr. 1) were collected in acidic to circum-neutral (pH 2.7–7.6) and oligotrophic to eutrophic lakes at depths ranging from 1.5–9 m. In contrast, larvae of *C. bifurcatus* (gr. 2) were collected in circum-neutral (pH 7.0–7.8) and oligotrophic to mesotrophic lakes over a wider range of depths (4–24m). We note that larvae of *C. bifurcatus* (gr. 2) were always found along with those of *C. staegeri* and *C. 'tigris'* whereas this was not the case for gr. 1 larvae.

Taxonomic comments. Although *cox1* sequences and cytological differences separate *C. bifurcatus* into two groups, we suggest that it is premature to recognize two species until further work has been completed.

Chironomus (*Chironomus*) *dilutus* Shobanov, Kiknadze and Butler (1999)

Material examined (Table S1): 9 larvae from Kelly Lake (Ontario).

DNA (Figs. 8–9 and Tables 4–5, S3). We were able to obtain PCR products for both *cox1* and *gb2β* genes. In accordance with the findings of Makarevich *et al*. (2000), *C. dilutus gb2β* has no intron. In the *cox1* ID-tree (Fig. 8), reference sequences of *C. pallidivittatus* (sensu Beermann 1955) cluster with reference sequences of *C. dilutus*. Similarities between the mitochondrial nucleotide sequences of these two species were also observed by Martin *et al*. (2002), who showed that trees based on mitochondrial sequences clustered populations of *C. dilutus* and *C. pallidivittatus* according to their geographic distribution, whereas those based on nuclear sequences clustered populations according to their species affiliation. The inability of the

mitochondrial *cox1* gene to separate *C. dilutus* and *C. pallidivittatus* is likely due to mitochondrial gene flow (Martin *et al*. 2002). To confirm the identification of larvae whose *cox1* sequences clustered with reference *C. dilutus* and *C. pallidivittatus* sequences, we amplified and sequenced the partial *gb2β* gene. Partial *gb2β* gene sequences of all larvae were identical to the *C. dilutus* reference sequence (Fig. 9), which suggests that our larvae are not *C. pallidivittatus* but more likely *C. dilutus*. *Cox1* PCR-RFLP profiles were not obtained for *C. dilutus*. However, simulation digests demonstrated that the *Ssp* I, *Hinf* I, *Rsa* I and *Taq* I restriction enzymes would not have separated *C. dilutus* from *C. bifurcatus* and *C*. nr. *atroviridis* (sp. 2i) because their restriction fragment lengths are so similar in some cases that it would be difficult to differentiate them on an agarose gel. However, simulation digests demonstrate that *C. dilutus* could be distinguished from *C. bifurcatus* and *C*. nr. *atroviridis* (sp. 2i) using the restriction enzyme *Alu* I.

Morphology (Table 6). Our specimens are large sized plumosus-type larvae with their posterior ventral tubules usually longer than their anterior ventral tubules. Although the teeth of the mentum and mandibles of *C. dilutus* are reported to be rounded (Martin 2013), in our larvae, they varied from being rounded to sharp, which suggests that this feature cannot be used to identify larvae of *C. dilutus* in our study area. Morphologically, larvae of *C. dilutus* cannot be distinguished from those of *C. pallidivittatus* (not collected in our study; see Martin 2013).

Cytology. *C. dilutus* larvae have four polytene chromosomes with the camptochironomus arm combination of AB, DE, CF, G. The cytology of *C. dilutus* has been described by several authors (see Martin 2013).

Distribution and ecology (Table 7). This species has been found previously in numerous localities across Canada (Alberta, British Columbia, Manitoba, Ontario and Saskatchewan) and the northern United States (Iowa, Massachusetts, Michigan, Minnesota, New York, North Dakota, South Dakota, Utah, Wisconsin and Wyoming) (Shobanov *et al*. 1999; Martin 2013). *C. dilutus* is known to thrive in organically-enriched eutrophic water bodies (Townes 1945, referred to as Tendipes (Tendipes) tentans (Fabricius)). We collected large numbers of *C. dilutus* in eutrophic Kelly Lake (at 5 m, pH 7.5) where sediments have been highly contaminated by untreated sewage from the city of Sudbury (1880s–1972) along with discharges from mining, milling and smelting operations (1880s–present) (City of Greater Sudbury 2013). Most of the larvae we collected had deformed mouthparts, likely due to the contaminants to which they were exposed (Hare & Carter 1976).

Chironomus (*Chaetolabis*) *ochreatus* Townes (1945)

Material examined (Table S1): 2 larvae from Lake Opasatica (Quebec).

DNA (Figs. 8–9 and Tables 4–5, S3). In the *cox1* ID-tree, *C. ochreatus* nucleotide sequences clearly form a distinct clade. Sequences of collected species cluster with the reference sequence of *C. ochreatus* thereby confirming the identification of this species. We were not successful in amplifying the *gb2β* gene for this species and *cox1* PCR-RFLP profiles were not obtained for this species. However, simulation digests using the restriction endonucleases *Ssp* I, *Hinf* I, *Rsa* I and *Taq* I demonstrate that *cox1* PCR-RFLP analysis would have separated *C. ochreatus* from the other *Chironomus* species.

Morphology (Table 6). Our specimens are medium sized thummi-type larvae. The 3rd inner teeth of the mandibles are partially darkened and fused to the lower margin (type B; Fig. 5). In contrast, in specimens studied by Martin (2013), the 3rd inner tooth was pale and well separated from the lower margin.

Cytology. The cytology of the two larvae analyzed clearly indicates that this species is *C. ochreatus*. *C. ochreatus* has three polytene chromosomes that are thought to have a modified thummi arm combination of AB, CD, GEF (Martin 2012). Arm G is generally unpaired with a nucleolus near the junction with arm E.

Distribution and ecology (Table 7). This species was previously known from the eastern United States (Arkansas, Maine, Massachusetts, Michigan, New Jersey, New York, Rhode Island, South Carolina, Virginia and Wisconsin) (Townes 1945; Oliver *et al*. 1990; Martin 2013). In our study, we collected *C. ochreatus* in a single mesotrophic Quebec lake at a depth of 2 m (pH 7.7).

Chironomus (*Chaetolabis*) nr. *atroviridis* (sp. 2i) Martin (2013)

C. atroviridis Townes has been found to comprise two species in North America, one with four polytene chromosomes (sp. 2i of Martin 2013), and the other with only three polytene chromosomes (sp. 2h of Martin 2013). Only the former species occurred in our samples.

Material examined (Table S1): 4 larvae from Lake Marlon (Quebec).

DNA (Figs. 8–9 and Tables 4–5, S3). In the *cox1* ID-tree, *C*. nr. *atroviridis* (sp. 2i) nucleotide sequences form a distinct clade. Sequences of collected larvae cluster with the reference sequence of *C*. nr. *atroviridis* (sp. 2i), which confirms the identification of this species. We were

not successful in amplifying the *gb2β* gene of this species. *Cox1* PCR-RFLP profiles were not obtained for this species. However, simulation digests demonstrate that *Ssp* I, *Hinf* I, *Rsa* I and *Taq* I could not be used to separate *C*. nr. *atroviridis* (sp. 2i) from all *C. dilutus* specimens because their restriction fragment lengths are so similar that it would be difficult to differentiate them on an agarose gel. However, simulation digests demonstrate that both species could be separated using the restriction enzyme *Alu* I.

Morphology (Table 6). Our specimens are large sized thummi-type larvae. They are morphologically very similar to those of *C. ochreatus*, in that both species have the teeth of the pecten epipharyngis flattened, however, the gula of *C*. nr. *atroviridis* (sp. 2i) is darkened posteriorly whereas that of *C. ochreatus* is pale or only slightly darkened.

Cytology. The cytology of the 2 larvae analyzed clearly indicates that this species is *C*. nr. *atroviridis* (sp. 2i). *C*. nr. *atroviridis* (sp. 2i) has four polytene chromosomes with some indication of a thummi arm combination (Martin 2013). Arm G is generally unpaired with a virtually terminal nucleolus. There are no nucleoli in the other chromosomes.

Distribution and ecology (Table 7). This species has been collected previously in Manitoba and Ontario in shallow water near macrophytes (Martin 2013). Likewise, we collected *C*. nr. *atroviridis* (sp. 2i) in the vegetated littoral zone (1 m depth) of a mesotrophic to eutrophic lake (pH 7.4).

Taxonomic comment. Wiederholm (1979) considered *C. ochreatus* to be a synonym of *C. atroviridis*, but mentioned that further study was needed. Because Wiederholm (1979) was not aware that there were two forms of *C. atroviridis* (2i and 2h; Martin 2013), we do not know which form would correspond to the material he was comparing. In any case, our results clearly indicate that *C*. nr. *atroviridis* (sp. 2i) is distinct from *C. ochreatus*. First, the *cox1* sequences of *C*. nr. *atroviridis* (sp. 2i) and of *C. ochreatus* are strikingly different such that their average interspecific divergence (12%; Table 5) is much higher than their intraspecific divergences (<3%; Table S3). Second, *C*. nr. *atroviridis* (sp. 2i) has 4 chromosomes whereas *C. ochreatus* has only 3. *C. ochreatus* also differs from the three chromosome forms of *C.* nr. *atroviridis* (sp. 2h) by the position of the nucleolus, which is subterminal in the latter species.

Morphological key to larvae of the *Chironomus* species collected in our study

The following key for identifying fourth-instar larvae of the *Chironomus* species collected in our study is based on the morphology of the tubules on the 10th and 11th body segments and features of the head capsule. Illustrations of these features are given in Figures 1–7.

Although the majority of our study species can be separated using the following key, we acknowledge that, as with most morphological classifications, the characters used are likely to show some variability due to genetic or environmental factors. In this key, larvae are first separated based on the absence/presence, length and shape of tubules, which was effective in separating larvae of all of our collected species with the exception of *C. decorus*-group sp. 2 since some larvae of this species had lateral tubules whereas others did not. The ventral tubules of *C. decorus*-group sp. 2 larvae also varied from being straight (bathophilus-type) to slightly curved (fluviatilis-type). Our *C. bifurcatus* larvae were all bathophilus-type, but in other geographical regions have been ascribed to several larval types (bathophilus, fluviatilis or melanotus; Martin 2013) and these differences are reported to be related to the depth or the type of substrate on which larvae occur (Martin 2013). At an extreme, the presence or absence of lateral tubules among *C. bifurcatus* larvae is reported to vary among larvae hatched from the single egg mass from which the type was reared. (J. Martin, unpublished).

Note that this key is based on morphological features of the *Chironomus* species that we collected, such that other species in the Nearctic could fit these descriptions (see notes at the end of the key).

1	11th segment without ventral tubules; 10th segment without lateral tubules (salinarius-type larvae)	2
-	11th segment with one or two pairs of ventral tubules	3
2	Frontoclypeus with a dark longitudinal stripe and a lobed dark spot in the middle; central trifid tooth of mentum with outer teeth almost completely separated from middle tooth (type C); 3rd inner tooth of mandibles partially darkened and fused to lower margin (type B)	*C.* sp. NAII[1]
-	Frontoclypeus pale or slightly darkened with a lobed dark spot in anterior portion; central trifid tooth of mentum with outer teeth partially separated from middle tooth (type B); 3rd inner tooth of mandibles pale and fused to lower margin (type A) ……..	*C. cucini* (in part) or *C.* sp. NAIII[1 and 2]
3	11th segment with only one pair of short ventral tubules (located posteriorly); 10th segment without lateral tubules (halophilus-type) ……	*C. cucini* (in part)
-	11th segment with two pairs of ventral tubules	4
4	10th segment without lateral tubules …….....	5
-	10th segment with a pair of lateral tubules …….....	8
5	Ventral tubules straight (bathophilus-type) or slightly curved (fluviatilis-type) ……..…….. ……	*C. bifurcatus* (in part) or *C. decorus*-group sp. 2 (in part)[3]
-	Anterior ventral tubules with an elbow; posterior ventral tubules coiled (thummi-type) ……..…..	6
6	Pecten epipharyngis teeth flattened (type C or D) …….	7
-	Pecten epipharyngis teeth elongated (type A or B) ……..……	*C. anthracinus* or *C*. sp. NAI

7 Gular region almost completely pale or at most slightly darkened ……..…… *C. ochreatus*

- Gular region darkened posteriorly ……...…… *C*. nr. *atroviridis* (2i)

8 Ventral tubules straight or slightly curved ……..……. 9

- Anterior pair of ventral tubules with an elbow; posterior pair of ventral tubules coiled (plumosus-type larvae) . …… 10

9 Ventral tubules equal to or greater than the width of the 11th segment (melanotus type); 3rd inner tooth of mandible pale ……..…... *C. decorus*-group sp. 2 (in part) or *C. bifurcatus* (in part)

- Ventral tubules less than the width of the 11th segment (semireductus-type larvae); 3rd inner tooth of mandible dark ……... *C. entis* or *C. plumosus* (in part)

10 3rd inner tooth of mandible partially dark to dark ……..……. 11

- 3rd inner tooth of mandible pale ……. 12

11 Frontoclypeus pale and gula strongly to completely darkened ……..…….. *C. plumosus* (in part)

- Frontoclypeus dark and gula slightly to posteriorly darkened ……..…… *C. dilutus*[4]

12 Anterior margin of ventromental plates crenulated ……..……. *C. frommeri* or *C. staegeri*[5]

- Anterior margin of ventromental plate smooth to relatively smooth ……..… 13

13 Frontoclypeus pale ……..… . 14

- Frontoclypeus dark ……..… 15

14 3rd inner tooth of mandible fused to lower margin, central trifid tooth of mentum with outer teeth only partially separated from middle tooth (type B) and 4th lateral teeth reduced to the height of the 5th lateral teeth (type II); found in highly acidic waters ……..…… *C. harpi*

- 3rd inner tooth of mandible separated from lower margin, central trifid tooth of mentum with outer teeth distinctly separated from middle tooth (type D) and 4th lateral teeth only slightly reduced (type I) ..... ……... *C. maturus* (in part)

15 Gula pale to slightly darkened and 4th lateral teeth of mentum only slightly reduced (type I) …..……..……..... ……..….. *C. maturus* (in part)

- Gula strongly to completely darkened and 4th lateral teeth of mentum about the same height as the 5th lateral teeth (type II) ……..……... *C. 'tigris'*

Notes:

[1] It is likely that *C. atritibia* (Malloch 1934) would also key here, as it is reported to have a salinarius-type larva (Wülker & Butler 1983). Although *C. atritibia* is thought to have a more northerly distribution, we cannot rule out the possibility that it corresponds to either *C.* sp. NAII or *C*. sp. NAIII.

[2] In our specimens, the mean AR of *C. cucini* (2.08) was significantly greater than that of *C.* sp. NAIII (1.77). Although there was some overlap in the range of ARs between the two species, 4 of 5 *C. cucini* larvae had an AR >2.0, whereas 10 of 11 specimens of *C.* sp. NAIII had an AR <1.95.The more southern *C. major* (Wülker & Butler 1983) would also key here, but is much larger (30-55 mm; Epler 2001).

[3] Other Nearctic species of the *C. decorus*-group (Wuelker 2010; Sæther 2012) are also likely to key out here (ex. *C. quinnitukqut*)

[4] *C. pallidivittatus* would also key out here.

[5] *C. crassicaudatus* (Malloch 1915) would also key out here.

Approaches used to delimit *Chironomus* species

No single approach (morphology, cytology, genetics) was adequate for delimiting and identifying larvae of all of the *Chironomus* species that we collected. Thus larval morphology alone could not be used to separate five pairs of *Chironomus* species that we collected, i.e., *C. cucini* and *C*. sp. NAIII, *C. bifurcatus* and *C. decorus*-group sp. 2, *C. anthracinus* and *C*. sp. NAI, *C. staegeri* and *C. frommeri*, as well as *C. entis* and *C. plumosus*. Furthermore, some Nearctic species that

we did not collect are reported to be morphologically identical to our study species (see notes at the end of the morphological key). In addition, for some of our species, specimens from other regions are reported to differ morphologically from those that we collected, and it is known that some larval characters are affected by wear, environmental conditions and genetic variation (Martin 2013). This said, larval morphology was undeniably important when used in combination with other methods of species delimitation. Differences in larval cytology, based on the structure of salivary-gland polytene chromosomes, allowed the definitive identification of many of the species that we collected. Indeed, the cytology of most North American *Chironomus* species has been described (Martin 2013). However, one must bear in mind that cytology is faced with the same challenges as the other identification methods; that is, it is not always possible to determine whether or not differences in chromosome banding patterns and other structures are attributable to species differences or to regional or individual differences within a given species (Martin 2011). Indeed, only a handful of taxonomists worldwide have the necessary expertise to identify *Chironomus* species through cytology, which is a major drawback for non-cytological experts wishing to identify *Chironomus* species. Genetic techniques, namely PCR-RFLP analysis and DNA barcoding of the *cox1* gene, successfully separated and identified most of the *Chironomus* species that we collected. We present the first *cox1* sequences for many of the known Nearctic *Chironomus* species (*C. acidophilus*, *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. frommeri*, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C. plumosus*, *C. quinnitukqut*, *C*. sp. g, *C*. sp. h and *C. 'tigris'*). However, DNA barcoding failed to distinguish between two species pairs (*C. entis* and *C. plumosus*; *C. dilutus* and *C. pallidivittatus*) because each pair has identical *cox1* nucleotides sequences. Such sequence similarities are likely the result of mitochondrial gene flow, and have been found in a number of closely related species groups around the world (e.g. Martin 2011).

Using the *gb2β* gene, we successfully separated *C. entis* from *C. plumosus* and *C. dilutus* from *C. pallidivittatus* and confirmed the species statuses of *C. cucini*, *C. bifurcatus*, *C. harpi*, *C. maturus*, *C*. sp. NAIII and *C. 'tigris'*. We present the first published *gb2β* sequences for *C. anthracinus*, *C. bifurcatus*, *C. calligraphus*, *C. cucini*, *C. harpi*, *C. maturus*, *C*. sp. u and *C. 'tigris'*. One downside of using the *gb2β* gene was that we were not able to obtain PCR products for all species. In fact, no primer combination was able to amplify all *Chironomus* species, which is a limitation when using this gene for DNA barcoding. For these species we sometimes obtained two PCR products or sequences belonging to the *globin 7A* or *9* genes. Our results confirm those of Hankeln *et al*. (1997) who found that this gene is highly variable and that the only conserved regions are also conserved in the *gb7A* and *gb9* genes. This is a major

drawback for using the *gb2β* gene for identifying *Chironomus* species. Thus, the use of another nuclear gene might be more appropriate for *Chironomus* species identifications. Other studies have used the nuclear *internal transcribed spacer* (*ITS*) region to separate *C. plumosus* from *C. entis* as well as other *Chironomus* species (Gunderina & Katokhin 2011; Martin 2011; Gunderina 2012). The nuclear *carbamoylphosphate synthetase* (*CAD*) region has also been successfully used to identify chironomid species (Carew *et al*. 2011). Another disadvantage of using the *gb2β* gene is that nuclear genes evolve more slowly than do mitochondrial genes. Thus, relatively recent speciation might not always be detected when using the *gb2β* gene. This might be the reason why sequences of *C*. sp. NAI and *C. anthracinus*, as well as those of *C. bifurcatus* (gr. 1) and *C. bifurcatus* (gr. 2), differ for the *cox1* gene but are identical for the *gb2β* gene. Thus, incorporation of both mitochondrial and nuclear genes, whose modes of inheritance and mutation rate differ, clearly provides better resolution for *Chironomus* species identification. Several studies have advocated the use of sequence divergence thresholds to separate species (ex. Hebert *et al*. 2004b). However, our results demonstrate that sequence divergence thresholds cannot be used to separate all *Chironomus* species. Thus we recorded overlap between intra- and inter-specific sequence divergences for both of the genes that we studied (Table 5); similar overlaps have been reported for other *Chironomus* species (Martin 2011), as well as for species of other chironomid genera (Carew *et al*. 2005; Ekrem *et al*. 2007) and other types of dipterans (Meier *et al*. 2006). In our study, *Chironomus cox1* intraspecific sequence divergences were < 3% (Table S3). For most species, *cox1* interspecific sequence divergences ranged from 9% to 20%, but between some of our study species the interspecific divergences ranged from 1 to 4% (Table 5). This overlap was due either to some of our study species sharing identical sequences (i.e., *C. entis*/*C. plumosus* and *C. dilutus*/*C. pallidivittatus*) or to interspecific divergences being so low that they fell within the intraspecific range for the genus (i.e., *C. staegeri*/*C. 'tigris'*/*C. frommeri* (Table 5) and *C. decorus*-group sp. 2/*C. quinnitukqut* (data not shown)). With respect to the *gb2β* gene, *Chironomus* species intraspecific sequence divergences were <2%. The interspecific divergences between most species ranged from 5 to 46% (Table 5), but sequence divergences between *C*. sp. NAI and *C. anthracinus* ranged from 1 to 5% (Table 5), which is within the intraspecific range for species. In light of our results, the calculated intraspecific sequence divergences of 3% for the *cox1* gene and 2% for the *gb2β* gene can be used as a guide to help sort *Chironomus* species, but should not be used in isolation. DeSalle *et al*. (2005) have suggested that, rather than looking at sequence divergences, specific base differences that characterize related species should be sought. We used this approach to determine whether or not *C.* sp. NAI and *C. anthracinus*, as well as *C.*

bifurcatus (gr. 1) and *C. bifurcatus* (gr. 2), are distinct species. However, even when using specific base differences, we are still faced with the same challenge inherent to other species-delimitating methods; that is, how much of a difference is needed for species to be considered different. DNA barcoding is more precise than PCR-RFLP because it allows the exact determination of base pair differences between individuals. Nevertheless, when a large number of individuals need to be identified, PCR-RFLP has been advocated as a cost-effective technique to assess molecular variation (Pfrender *et al*. 2010). However, as the cost of sequencing continues to fall, sequencing is becoming the most effective and economical approach, even for determining large numbers of individuals. The disadvantage of the PCR-RFLP approach is that if the right enzymes are not chosen for analysis, sequence nucleotide differences can go unnoticed. In our study, the first chosen restriction enzymes (*Ssp* I, *Hinf* I, *Rsa* I and *Taq* I) were not able to discern differences in the *cox1* nucleotide sequences of *C. bifurcatus*, *C. dilutus* and *C*. nr. *atroviridis* (sp. 2i) as well as those of *C. staegeri*, *C. 'tigris'* and *C. frommeri*. Additional restriction enzymes were necessary to separate these species. Likewise, PCR-RFLP did not discriminate between *C. entis* and *C. plumosus* and it would not likely be able to separate *C. dilutus* from *C. pallidivittatus* because both of these species pairs share identical *cox1* sequences. Overall, for non-cytological experts, we recommend the use of combined genetic and morphological techniques to identify *Chironomus* larvae to species since this combination was much more effective than either of these techniques alone.

Overall conclusions and recommendations for identifying *Chironomus* species

Overall, using morphology, cytology and genetics we conclude that our 404 *Chironomus* larvae represent 17 species, 14 of which have been identified as *C*. (*Chaetolabis*) nr. *atroviridis* (sp. 2i), *C*. (*Chaetolabis*) *ochreatus*, *C*. (*Chironomus*) *anthracinus*, *C*. (*Chironomus*) *bifurcatus*, *C*. (*Chironomus*) *cucini*, *C*. (*Chironomus*) *decorus*-group sp. 2, *C*. (*Chironomus*) *dilutus*, *C*. (*Chironomus*) *entis*, *C*. (*Chironomus*) *frommeri*, *C*. (*Chironomus*) *harpi*, *C*. (*Chironomus*) *maturus*, *C*. (*Chironomus*) *plumosus*, *C*. (*Chironomus*) *staegeri* and *C*. (*Chironomus*) '*tigris'* while the identification of three others remains uncertain (*C*. sp. NAI-III). The species status of *C*. sp. NAI requires further investigation and additional studies are necessary to determine whether are not *C. bifurcatus* is a single species or a complex of at least two closely related species. Of the 14 identified *Chironomus* species, two belong to the subgenus *Chaetolabis* whereas 12 belong to the subgenus *Chironomus*. We collected and identified 11 (*C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. entis*, *C. maturus*, *C*.

nr. *atroviridis* (sp. 2i), *C. plumosus*, *C. staegeri* and *C. 'tigris'*) of the 20 *Chironomus* species currently known from the Canadian provinces east of the Rocky Mountains (that is, from Canada excluding British Columbia and the three northern territories; Martin 2013). Since all but three of the 31 water bodies that we sampled are located in the same ecozone (the Boreal Shield), some of the nine species that we did not find could be restricted to other ecozones, such as the prairies, where water chemistry and other factors are likely to differ from those in Boreal Shield lakes. In fact, it is surprising that we were able to collected so many *Chironomus* species from a single ecozone in which lake waters are generally nutrient poor, circum-neutral and soft (low concentrations of calcium and magnesium), which is likely to limit the range of habitats available for *Chironomus* species. This large proportion of known species is likely explained by the fact that our study lakes in this ecozone encompass wide ranges in these variables because some of them have been altered by discharges from mining, milling and smelting operations, or sewage treatment plants, or by the addition of lime to counter lake acidification (Lakes Arnoux, Osisko, Pelletier, Rouyn and Kelly). The range of chemical conditions under which some of the *Chironomus* species were collected was quite wide. For example, *C. anthracinus* and *C. bifurcatus* were found in waters that were highly acidic to circum-neutral and *C. entis*, *C. bifurcatus*, *C. plumosus* and *C. staegeri* were found in water bodies that were oligotrophic to eutrophic. In contrast, *C. harpi* was restricted to a highly acidic lake and *C. dilutus* was collected only in a lake that had been organically enriched by sewage. We found three *Chironomus* species (*C. frommeri*, *C. harpi* and *C. ochreatus*) that were previously known from the Nearctic (Martin 2013), but had not been reported from eastern Canada. The identification of another three species remains unclear (*C*. sp. NAI-III). We note that other species are likely to exist in eastern Canada, since 19 cytologically-defined but as yet unidentified or unassigned *Chironomus* species have been reported from this region (Martin 2013). Applying the combination of morphological and genetic techniques used in our study would likely resolve many of these taxonomic gaps in the Canadian and Nearctic fauna.

ACKNOWLEDGEMENTS

Funding was provided by the Metals In the Human Environment - Strategic Network and the Natural Sciences and Engineering Research Council of Canada. We thank Victor Thomasson, Julien Lacharité, Hugo Lavoie, Nicolas Fabien-Ouellet and Anaïs Nanou Clercq for their assistance in collecting samples, Dominic Ponton and Maikel Rosabal-Rodriguez for their assistance in collecting samples and for providing water quality measurements for 2009 and

2011 and Jean Christian Auclair for providing water quality measurements for Lake Bédard. Special thanks goes to Veronika Golygina, who karyosystematically identified *C. entis* and *C. plumosus* larvae, and to Malcolm G. Butler, who kindly reviewed the manuscript.

REFERENCES

Andersen, F. S. (1949) On the subgenus *Chironomus*. Studies on the systematics and biology of Chironomidae III. *Videnskabelige Meddelser fra Dansk Naturhistorisk Forening*, III, 1-66.

Ashe, P. & Cranston, P. S. (1990) Family Chironomidae. *In:* A. Soos & L. Papp (Eds.), *Catalogue of Palearctic Diptera. Vol. 2: Psychodidae - Chironomidae*. Elsevier, Amsterdam, pp. 113-355.

Ball, S. L., Hebert, P. D. N., Burian, S. K. & Webb, J. M. (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society*, 24, 508-524.

Beermann, W. (1955) Cytologische Analyse eines *Camptochironomus*-Artbastards. I. Kreuzungsergebnisse und die Evolution des Karyotypus. *Chromosoma*, 7, 198-259.

Brooks, S. J., Langdon, P. G. & Heiri, O. (2007) *The identification and use of Palearctic Chironomidae larvae in palaeoecology*. QRA Technical Guide No. 10, Quaternary Research Association, London, 276 pp.

Butler, M. G. (1982) Production dynamics of some artic *Chironomus* larvae. *Limnology and Oceanography*, 27, 728-736.

Butler, M. G., Kiknadze, I. I., Cooper, J. K. & Siirin, M. T. (1995) Cytologically identified *Chironomus* species from lakes in North Dakota and Minnesota, USA. *In:* P. S. Cranston (Ed.), *Chironomids, from Gene to Ecosystems - Proceedings of the 12th International Symposium on Chironomidae (Canberra, January 23-26, 1994)*. CSIRO, Canberra, pp. 31-37.

Butler, M. G., Kiknadze, I. I., Golygina, V. V., Martin, J., Istomina, A. G., Wülker, W. F., Sublette, J. E. & Sublette, M. F. (1999) Cytogenetic differentiation between Palearctic and Nearctic populations of *Chironomus plumosus* L. (Diptera, Chironomidae). *Genome*, 42, 797-815.

Carew, M. E., Marshall, S. E. & Hoffmann, A. A. (2011) A combination of molecular and morphological approaches resolves species in the taxonomically difficult genus *Procladius* Skuse (Diptera: Chironomidae) despite high intra-specific morphological variation. *Bulletin of Entomological Research*, 101, 505-519.

Carew, M. E., Pettigrove, V., Cox, R. L. & Hoffmann, A. A. (2007) DNA identification of urban Tanytarsini chironomids (Diptera:Chironomidae). *Journal of the North American Benthological Society*, 26, 587-600.

Carew, M. E., Pettigrove, V. & Hoffmann, A. A. (2003) Identifying chironomids (Diptera: Chironomidae) for biological monitoring with PCR-RFLP. *Bulletin of Entomological Research*, 93, 483-490.

Carew, M. E., Pettigrove, V. & Hoffmann, A. A. (2005) The utility of DNA markers in classical taxonomy: Using cytochrome oxidase I markers to differentiate Australian *Cladopelma* (Diptera: Chironomidae) midges. *Annals of the Entomological Society of America*, 98, 587-594.

City of Greater Sudbury (2013) Individual Lake Information. Available from http://www.greatersudbury.ca/living/lakes-facts/local-lake-descriptions/ (accessed 17 September 2013).

Davis, G. A., Havill, N. P., Adelman, Z. N., Caccone, A., Kok, L. T. & Salom, S. M. (2011) DNA barcodes and molecular diagnostics to distinguish an introduced and native *Laricobius* (Coleoptera: Derodontidae) species in eastern North America. *Biological Control*, 58, 53-59.

DeSalle, R., Egan, M. G. & Siddall, M. (2005) The unholy trinity: taxonomy, species delimitation and DNA barcoding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 1905-1916.

Ekrem, T., Stur, E. & Hebert, P. D. N. (2010) Females do count: Documenting Chironomidae (Diptera) species diversity using DNA barcoding. *Organisms Diversity and Evolution*, 10, 397-408.

Ekrem, T., Willassen, E. & Stur, E. (2007) A comprehensive DNA sequence library is essential for identification with DNA barcodes. *Molecular Phylogenetics and Evolution*, 43, 530-542.

Elderkin, C. L., Corkum, L. D., Bustos, C., Cunningham, E. L. & Berg, D. J. (2012) DNA barcoding to confirm morphological traits and determine relative abundance of burrowing mayfly species in western Lake Erie. *Journal of Great Lakes Research*, 38, 180-186.

Epler, J. H. (2001) *Identification manual for the larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida*. Special Publication SJ2001-SP13 North Carolina Department of Environmental and Natural Resources, Raleigh, NC, and St. John's River Water Management District, Palatka, FL, 526 pp.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294-299.

Fortin, C., Couillard, Y., Vigneault, B. & Campbell, P. G. C. (2010) Determination of free Cd, Cu and Zn concentrations in lake waters by in situ diffusion followed by column equilibration ion-exchange. *Aquatic Geochemistry*, 16, 151-172.

Goeldi, E. A. (1905) Os mosquito no Pará. Reunião de quatro trabalhos sobre os Mosquitos indigenas, principalmente as especies que molestam o homem. *Memorias do Meseu Goeldi (Museu Paraense) de Historia Natural e Ethnographia*, 4, 134-137.

Gunderina, L. I. (2012) Species-specific PCR primers for identification of the sibling species *Chironomus plumosus* (Linnaeus, 1758) and *Chironomus balatonicus* (Devai, Wuelker et Scholl, 1983) (Chironomidae, Diptera). *Fauna Norvegica*, 31, 151-157.

Gunderina, L. I. & Katokhin, A. V. (2011) Variation and divergence of the rDNA ITS-1 region in species of the genus *Chironomus* (Diptera: Chironomidae). *In:* X. Wang & W. Liu (Eds.), *Contemporary Chironomid Studies - Proceedings of the 17th International Symposium on Chironomidae (July 6-9, 2009 Nankai University, China)*. Nankai University Press, Tianjin, pp. 22-35.

Gunderina, L. I., Kiknadze, I. I., Istomina, A. G. & Butler, M. (2009) Geographic differentiation of genomic DNA of *Chironomus plumosus* (Diptera, Chironomidae) in natural Holarctic populations. *Russian Journal of Genetics*, 45, 54-62.

Guryev, V., Makarevitch, I., Blinov, A. & Martin, J. (2001) Phylogeny of the genus *Chironomus* (Diptera) inferred from DNA sequences of mitochondrial *Cytochrome b* and *Cytochrome oxidase I*. *Molecular Phylogenetics and Evolution*, 19, 9-21.

Guryev, V. P. & Blinov, A. G. (2002) Phylogenetic relationships among Holarctic populations of *Chironomus entis* and *Chironomus plumosus* in view of possible horisontal transfer of mitochondrial genes. *Russian Journal of Genetics*, 38, 239-243.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95-98.

Hankeln, T., Friedl, H., Ebersberger, I., Martin, J. & Schmidt, E. R. (1997) A variable intron distribution in globin genes of *Chironomus*: Evidence for recent intron gain. *Gene*, 205, 151-160.

Hare, L. & Carter, J. C. H. (1976) The distribution of *Chironomus* (*s.s.*) ?*cucini* (*salinarius* group) larvae (Diptera: Chironomidae) in Parry Sound, Georgian Bay, with particular reference to structural deformities. *Canadian Journal of Zoology*, 54, 2129-2134.

Hare, L. & Carter, J. C. H. (1986) The benthos of a natural West African lake, with emphasis on the diel migrations and lunar and seasonal periodicities of the *Chaoborus* populations (Diptera, Chaoboridae). *Freshwater Biology*, 16, 759-780.

Harnisch, O. (1942) Die sogenannten "Blutkiemen" der Larven der Gattung *Chironomus* Mg. *Biologia Generalis*, 16, 593-609.

Hebert, P. D. N., Cywinska, A., Ball, S. L. & DeWaard, J. R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, 270, 313-321.

Hebert, P. D. N., Penton, E. H., Burns, J. M., Janzen, D. H. & Hallwachs, W. (2004a) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812-14817.

Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S. & Francis, C. M. (2004b) Identification of birds through DNA barcodes. *PLoS Biology*, 2, 1657-1663.
Hogg, I. D. & Hebert, P. D. N. (2004) Biological identification of springtails (Hexapoda: Collembola) from the Canadian Arctic, using mitochondrial DNA barcodes. *Canadian Journal of Zoology*, 82, 749-754.
Ineichen, H., Meyer, B. & Lezzi, M. (1983) Determination of the developmental stage of living fourth instar larvae of *Chironomus tentans*. *Developmental Biology*, 98, 278-286.
Johannsen, O. A. (1908) New North American Chironomidae. *New York State Museum Bulletin*, 124, 264-285.
Johannsen, O. A. (1937) Part IV. Chironomidae: Subfamily Chironomidae. *In:* O. A. Johannsen (Ed.), *Aquatic Diptera - Eggs, Larvae, and Pupae of Aquatic Flies*. Entomological Reprint Specialists, Los Angeles, pp. 18-26.
Jónasson, P. M. (1972) Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. *Oikos Supplement*, 14, 1-148.
Kao, W. Y., Trewitt, P. M. & Bergtrom, G. (1994) Intron-containing globin genes in the insect *Chironomus thummi*. *Journal of Molecular Evolution*, 38, 241-249.
Keyl, H. G. (1960) Die cytologische Diagnostik der Chironomiden. II. Diiagnosen der Geschwisterarten *Chironomus acidophilus* n. sp. und *Ch. uliginosus* n. sp. *Archiv für Hydrobiologie*, 57, 187-195.
Keyl, H. G. (1962) Chromosomenevolution bei *Chironomus* II. Chromosomenumbauten und phylogenetische Beziehungen der Arten. *Chromosoma*, 13, 464-514.
Kiknadze, I. I., Aimanova, K. G., Butler, M. & Cooper, K. (1993) The pattern of the reduction of the chromosome number in the chironomid evolution (In Russian). *Tsitologiya*, 35, 96-104.
Kiknadze, I. I., Butler, M. G., Golygina, V. V., Martin, J., Wulker, W. F., Sublette, J. E. & Sublette, M. F. (2000a) Intercontinental karyotypic differentiation of *Chironomus entis* Shobanov, a Holarctic member of the *C. plumosus* group (Diptera, Chironomidae). *Genome*, 43, 857-873.
Kiknadze, I. I., Butler, M. G., Golygina, V. V., Wuelker, W. F., Martin, J., Sublette, J. E. & Sublette, M. F. (2000b) Macrogeographic patterns of banding sequences in Holarctic *Chironomus entis* Shobanov. *In:* O. Hoffrichter (Ed.), *Late 20th Century Research on Chironomidae: an Anthology from the 13th International Symposium on Chironomidae*, Shaker Verlag, Aachen, p. 621.
Kiknadze, I. I., Butler, M. G., Gunderina, L. I., Istomina, A. G., Gusev, V. D. & Nemytikova, L. A. (2010) Chromosomal evolution of Nearctic and Palearctic *Chironomus* species (Diptera: Chironomidae). *In:* L. C. Ferrington (Ed.), *Proceedings of the XV International Symposium on Chironomidae*, Chironomidae Research Group, University of Minnesota, Saint Paul, Minnesota, pp. 203-221.
Kiknadze, I. I., Golygina, V. V., Istomina, A. G. & Gunderina, L. I. (2004) Pattern of chromosomal polymorphism during population and species divergence in *Chironomus* (Diptera, Chironomidae) (In Russian, English summary). *Sibirskiy Ekologicheskiy Zhurnal*, 11, 635-651.
Kiknadze, I. I., Shilova, A. I., Kerkis, I. E., Shobanov, N. A., Zelenkov, N. I., Grebenchov, L. P., Istomina, A. G. & Prasolov, B. A. (1991) *Karyotype and Morphology of Larvae of the Tribe Chironomini*. ATLAS, Novosibirsk, 114 pp.
Kiknadze, I. I., Wuelker, W. F., Istomina, A. G. & Andreeva, E. N. (2005) Banding sequence pool of *Chironomus anthracinus* Zett. (Diptera, Chironomidae) in Palearctic and Nearctic. *Euroasian Entomological Journal*, 4, 13-27.
Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16, 111-120.
Lindeberg, B. & Wiederholm, T. (1979) Notes on the taxonomy of European species of *Chironomus* (Diptera Chironomidae). *Entomologica Scandinavica Supplements*, 10, 99-116.
Linnaeus, C. (1758) *Systema naturae per regna tria naturae (Vol. 1, 10th ed.)*. Laurentii Salvii, Stockholm.
Lundbeck, W. (1898) Diptera groenlandica. *Videnskabelige Meddelelser fra den Naturhistorisk Forening i Kjøbenhavn*, 1898, 236-314.
Makarevich, I. F., Berezikov, E. V., Guryev, V. P. & Blinov, A. G. (2000) Molecular phylogeny of the *Chironomus* genus deduced from nucleotide sequences of two nuclear genes, *ssp160* and the Globin 2b gene. *Molecular Biology*, 34, 606-612.
Malloch, J. R. (1915) The Chironomidae, or midges, of Illinois, with particular reference to the species occuring in the Illinois River. *Bulletin of the Illinois State Laboratory of Natural History*, 10, 275-543.

Malloch, J. R. (1934) III. Chironomidae, Sciaridae, Phoridae, Syrphidae, Oestridae, Piophilidae, Helomyzidae, Calliphoridae and Tachinidae. *In:* W. J. Holland (Ed.), *The Exploration of Southampton Island, Hudson Bay, by G.M. Sutton Supposed by J.B. Semple 1929-1930*. Memoirs of the Carnegie Museum, pp. 13-32.

Martin, J. (1979) Chromosomes as tools in taxonomy and phylogeny of Chironomidae (Diptera). *Entomologica Scandinavica Supplements*, 10, 67-74.

Martin, J. (2011) From bands to base pairs: Problems in the identification of species using the example of *Chironomus oppositus* Walker (Honorary Thienemann Lecture). *In:* X. Wang & W. Liu (Eds.), *Contemporary Chironomid Studies - Proceedings of the 17th International Symposium on Chironomidae (July 6-9, 2009 Nankai University, China)*. Nankai University Press, Tianjin, pp. 126-143.

Martin, J. (2013) North American species of the genus *Chironomus*. Available from http://genetics.unimelb.edu.au/Martin/NACytfiles/NAChiron.html (accessed 17 September 2013).

Martin, J., Andreeva, E. N., Kiknadze, I. I. & Wülker, W. F. (2006) Polytene chromosomes and phylogenetic relationships of *Chironomus atrella* (Diptera: Chironomidae) in North America. *Genome*, 49, 1384-1392.

Martin, J., Guryev, V. & Blinov, A. (2002) Population variability in *Chironomus* (*Camptochironomus*) species (Diptera, Nematocera) with a Holarctic distribution: Evidence of mitochondrial gene flow. *Insect Molecular Biology*, 11, 387-397.

Martin, J., Sublette, J. E. & Caldwell, B. A. (2010) Description of *Chironomus quinnitukqut*, n. sp., closely related to the *C. decorus* group in North America, with characterization of an additional larval form from halobiontic habitats. *Zootaxa*, 2716, 29-41; & 2743, 2768.

Martin, J. & Wülker, W. (1971) Inversion polymorphism in *Chironomus staegeri* Lundbeck. *Canadian Journal of Genetics and Cytology*, 13, 306-321.

Martin, J., Wülker, W. & Sublette, J. E. (1974) Evolutionary cytology of the genus *Chironomus* (Diptara: Chironomidae). *Studies in Natural Sciences (Portales, N.M.)*, 1, 1-12.

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

Meier, R., Shiyang, K., Vaidya, G. & Ng, P. K. (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology*, 55, 715-728.

Ministère du Développement durable de l'Environnement et des Parcs (2012) Le réseau de surveillance volontaire des lacs. Available from http://www.mddep.gouv.qc.ca/eau/rsvl (accessed 20 August 2012).

Nei, M. & Kumar, S. (2000) *Molecular Evolution and Phylogenetics*. Oxford University Press, New York, 352 pp.

Nyman, M., Korhola, A. & Brooks, S. J. (2005) The distribution and diversity of Chironomidae (Insecta: Diptera) in western Finnish Lapland, with special emphasis on shallow lakes. *Global Ecology and Biogeography*, 14, 137-153.

Oliver, D. R., Dillon, M. E. & Cranston, P. S. (1990) *A catalog of Nearctic Chironomidae* (Vol. 1857/B). Research Branch Agriculture Canada Publication, 89 pp.

Pfenninger, M., Nowak, C., Kley, C., Steinke, D. & Streit, B. (2007) Utility of DNA taxonomy and barcoding for the inference of larval community structure in morphologically cryptic *Chironomus* (Diptera) species. *Molecular Ecology*, 16, 1957-1968.

Pfrender, M. E., Ferrington Jr, L. C., Hawkins, C. P., Hartzell, P. L., Bagley, M., Jackson, S., Courtney, G. W., Larsen, D. P., Creutzburg, B. R., Lévesque, C. A., Epler, J. H., Morse, J. C., Fend, S., Petersen, M. J., Ruiter, D., Schindel, D. & Whiting, M. (2010) Assessing macroinvertebrate biodiversity in freshwater ecosystems: Advances and challenges in DNA-based approaches. *Quarterly Review of Biology*, 85, 319-340.

Ponton, D. E. & Hare, L. (2009) Assessment of nickel contamination in lakes using the phantom midge *Chaoborus* as a biomonitor. *Environmental Science and Technology*, 43, 6529-6534.

Proulx, I. & Hare, L. (2008) Why bother to identify animals used for contaminant monitoring? *Integrated Environmental Assessment and Management*, 4, 125-126.

Proulx, I. & Hare, L. (2013) Differences in feeding behaviours among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae. *Freshwater Biology*, in press.

Ryser, H. M., Wuelker, W. & Scholl, A. (1985) Revision der Gattung *Chironomus* Meigen (Diptera) X: *Lobochironomus* n. subgen. (*C. montuosus* n. sp., *C. storai* Goetgh., *C. mendax* Stora). *Revue Suisse de Zoologie*, 92, 385-404.

Sæther, O. A. (1975) Nearctic chironomids as indicators of lake typology. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 19, 3127-3133.

Sæther, O. A. (2012) The *Chironomus* group (Diptera: Chironomidae) in Lake Winnipeg, Canada. *Zootaxa*, 3275, 1-19.

Saitou, N. & Nei, M. (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4, 406-425.

Sharley, D. J., Pettigrove, V. & Parsons, Y. M. (2004) Molecular identification of *Chironomus* spp. (Diptera) for biomonitoring of aquatic ecosystems. *Australian Journal of Entomology*, 43, 359-365.

Shobanov, N. A. (1989) The morphological differentiation of *Chironomus* species of *plumosus* group (Diptera, Chironomidae). Larvae. *Acta Biologica Debrecina, Supplementum Oecologica Hungarica*, 2, 335-344.

Shobanov, N. A. (2002) Evolution of the genus *Chironomus* (Diptera, Chironomidae). 1. Ancestral form and major lines of phylogenesis. *Entomological Review*, 82, 487-493.

Shobanov, N. A., Kiknadze, I. I. & Butler, M. G. (1999) Palearctic and Nearctic *Chironomus* (*Camptochironomus*) *tentans* (Fabricius) are different species (Diptera: Chironomidae). *Insect Systematics and Evolution*, 30, 311-322.

Shobanov, N. A., Shilova, A. I. & Belyanina, S. I. (1996) Extent and content of the genus *Chironomus* Meig. (Diptera, Chironomidae): review of world fauna (In Russian). *In:* N. A. Shobanov & T. D. Zinchenko (Eds.), *Ecology, Evolution and Systematics of Chironomids*, Inst. Biol. Inland Waters & Inst. Ecol. Volga Basin, Russian Academy of Sciences, Tolyatti, Boruk, Russia, pp. 44-96.

Sinclair, C. S. & Gresens, S. E. (2008) Discrimination of *Cricotopus* species (Diptera: Chironomidae) by DNA barcoding. *Bulletin of Entomological Research*, 98, 555-563.

Stur, E. & Ekrem, T. (2011) Exploring unknown life stages of Arctic Tanytarsini (Diptera: Chironomidae) with DNA barcoding. *Zootaxa*, 2743, 27-39.

Sublette, J. E. & Sublette, M. F. (1971) A review of the genus *Chironomus* (Diptera, Chironomidae) B. Description of the immature stages and adults of the *Chironomus staegeri* group. *Studies in Natural Sciences (Portales, N.M.)*, 1 (1), 6-21.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28, 2731-2739.

Thienemannn, A. (1941) Lappländische Chironomiden und ihre Wohngewasser. (Ergebnisse von Untersuchungen im Abiskogebeit in Schwedisch-Lappland). *Archiv für Hydrobiologie Supplement*, 17, 1-253.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673-4680.

Townes, H. K., Jr. (1945) The Nearctic species of Tendipedini [Diptera, Tendipedidae (= Chironomidae)]. *American Midland Naturalist*, 34, 1-206.

Vallenduuk, H. J. & Moller Pillot, H. K. M. (1997) Key to the larvae of *Chironomus* in Western Europe. *In: RIZA Rapport 97.053, 1-13 + appendix*.

Webb, C. J. & Scholl, A. (1985) Identification of larvae of European species of *Chironomus* Meigen (Diptera: Chironomidae) by morphological characters. *Systematic Entomology*, 10, 353-372.

Webb, C. J., Scholl, A. & Ryser, H. M. (1985) Comparative morphology of the larval ventromental plates of European species of *Chironomus* Meigen (Diptera: Chironomidae). *Systematic Entomology*, 10, 373-385.

Webb, D. W. (1969) New species of chironomids from Costello Lake, Ontario (Diptera: Chironomidae). *Journal of the Kansas Entomological Society*, 42, 91-108.

Wiederholm, T. (1979) Morphology of *Chironomus macani* Freeman, with notes on the taxonomic status of subg. *Chaetolabis* Town. (Diptera: Chironomidae). *Entomologica Scandinavica Supplements*, 10, 145-150.

Wuelker, W. (2010) The role of chromosomes in chironomid systematics, ecology and phylogeny. *In:* L. C. Ferrington (Ed.), *Proceedings of the XV International Symposium on Chironomidae*. Chironomidae Research Group, University of Minnesota, Saint Paul, Minnesota, pp. 1-13.

Wuelker, W., Martin, J., Kiknadze, I. I., Sublette, J. E. & Michiels, S. (2009) *Chironomus blaylocki* sp. n. and *C. bifurcatus* sp. n., North American species near the base of the decorus-group (Diptera: Chironomidae). *Zootaxa*, 28-46.

Wülker, W., Dévai, G. & Dévai, I. (1989) Computer assisted studies of chromosomes evolution in the genus *Chironomus* (Dipt.). Comparative and integrated analysis of chromosome arms A, E and F. *Acta biologica debrecina, supplement oecologica Hungarica*, 2, 373-387.

Wülker, W. & Götz, P. (1968) Die Verwendung der Imaginalscheiben zur Bestimmung des Entwicklungszustandes von *Chironomus*-Larven (Dipt.). *Zeitschrift für Morphologie und Okologie der Tiere*, 62, 363-388.

Wülker, W. & Martin, J. (1974) A review of the genus *Chironomus* (Diptera, Chironomidae) VI. Cytology of the maturus-complex. *Studies in Natural Sciences (Portales, N.M.)*, 1 (9), 1-21.

Wülker, W., Sublette, J. E. & Martin, J. (1991) *Chironomus utahensis* Malloch and *Chironomus harpi* new species and their karyosystematic relationships to other species in the *decorus*-group of *Chironomus*. *Spixiana*, 14, 71-94.

Wülker, W. F. & Butler, M. G. (1983) Karyosystematics and morphology of Northern *Chironomus* (Diptera: Chironomidae): Freshwater species with larvae of the *salinarius*-type. *Entomologica Scandinavica*, 14, 121-136.

Wülker, W. F. & Martin, J. (1971) A review of the genus *Chironomus* (Diptera, Chironomidae) C. Karyosystematics of the *Chironomus staegeri* group. *Studies in Natural Sciences (Portales, N.M.)*, 1 (1), 22-30.

Wülker, W. F., Sublette, J. E., Sublette, M. F. & Martin, J. (1971) A review of the genus *Chironomus* (Diptera, Chironomidae) I. The *staegeri* group. *Studies in Natural Sciences (Portales, N.M.)*, 1 (1), 1-89.

Zetterstedt, J. W. (1860) *Diptera Scandinaviae disposita et descripta. Tomus decimus quartus seu ultimus, continens addenda, corrigena & emendada tomis prioribus, una cum indice alphabetico navarum specierum hujus & praecedentis tomi, atque generico omnium tomorum*. *Lundae*, 14, 6497-6512.

Table S1. **Individual *Chironomus* larvae indicating their associated: voucher code, location and year of collection, and species name. Performed *cox1* PCR-RFLP, morphological and cytological analyses are marked (x). For *cox1* and *gb2β* gene sequencing, GenBank accession numbers are given.**

Voucher code	Larvae type	Locations	Year	Performed analysis					Species
				cox1 PCR-RFLP	*cox1* sequencing (GenBank assession #)	*gb2β* sequencing (GenBank assession #)	Morphology	Cytology	
AR10-TH1	thummi	AR	2010		KF278225	KF278426	x		*C. anthracinus*
AR10-TH2	thummi	AR	2010		KF278226	KF278427	x		*C. anthracinus*
HA07-TH1	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH2	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH3	thummi	HA	2007	x	KF278221		x		*C. anthracinus*
HA07-TH4	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH5	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH6	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH7	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH8	thummi	HA	2007	x	KF278222		x	x	*C. anthracinus*
HA07-TH9	thummi	HA	2007	x			x		*C. anthracinus*
HA07-TH10	thummi	HA	2007	x			x		*C. anthracinus*
OS10-TH1	thummi	OS	2010		KF278227	KF278393	x		*C. anthracinus*
OS10-TH2	thummi	OS	2010		KF278223		x		*C. anthracinus*
OS10-TH3	thummi	OS	2010		KF278228		x		*C. anthracinus*
PI10-TH1	thummi	PI	2010		KF278229	KF278395	x	x	*C. anthracinus*
PI10-TH2	thummi	PI	2010		KF278224		x		*C. anthracinus*
AnthRL21m	thummi	RA	2010		KF278232		x	x	*C. anthracinus*
RA10-TH1	thummi	RA	2010		KF278230	KF278394	x		*C. anthracinus*
RA10-TH4	thummi	RA	2010		KF278231	KF278425	x		*C. anthracinus*
RAM07-TH1	thummi	RM	2007	x			x		*C. anthracinus*

RAM07-TH2	thummi	RM	2007	x			x		*C. anthracinus*
RAM07-TH3	thummi	RM	2007	x	KF278233		x		*C. anthracinus*
RAM07-TH4	thummi	RM	2007	x			x	x	*C. anthracinus*
RAM07-TH5	thummi	RM	2007	x			x		*C. anthracinus*
RAM07-TH6	thummi	RM	2007	x			x		*C. anthracinus*
SI11-TH1	thummi	SI	2011		KF278234		x		*C. anthracinus*
AD10-BA2	bathophilus	AD	2010		KF278316		x		*C. bifurcatus* (gr. 1)
AR10-BA1	bathophilus	AR	2010		KF278319	KF278435	x		*C. bifurcatus* (gr. 1)
AR10-BA2	bathophilus	AR	2010				x	x	*C. bifurcatus* (gr. 1)
AR10-BA3	bathophilus	AR	2010		KF278317	KF278436	x		*C. bifurcatus* (gr. 1)
AR10-BA4	bathophilus	AR	2010		KF278318		x		*C. bifurcatus* (gr. 1)
AL06-BA1	bathophilus	DA	2006	x	KF278315		x		*C. bifurcatus* (gr. 1)
AL06-BA2	bathophilus	DA	2006	x			x		*C. bifurcatus* (gr. 1)
AL06-BA3	bathophilus	DA	2006	x			x		*C. bifurcatus* (gr. 1)
AL06-BA4	bathophilus	DA	2006	x			x		*C. bifurcatus* (gr. 1)
AL06-BA5	bathophilus	DA	2006	x			x		*C. bifurcatus* (gr. 1)
AL06-BA6	bathophilus	DA	2006				x	x	*C. bifurcatus* (gr. 1)
DU06-BA1	bathophilus	DP	2006	x			x		*C. bifurcatus* (gr. 1)
DU06-BA2	bathophilus	DP	2006	x			x		*C. bifurcatus* (gr. 1)
DU06-BA3	bathophilus	DP	2006	x			x		*C. bifurcatus* (gr. 1)
DU06-BA4	bathophilus	DP	2006	x			x		*C. bifurcatus* (gr. 1)
DU06-BA5	bathophilus	DP	2006	x			x		*C. bifurcatus* (gr. 1)
DU07-BA1	bathophilus	DP	2007	x	KF278320		x		*C. bifurcatus* (gr. 1)
DU07-BA2	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)
DU07-BA3	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)
DU07-BA4	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)
DU07-BA5	bathophilus	DP	2007	x			x	x	*C. bifurcatus* (gr. 1)
DU07-BA6	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)
DU07-BA7	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)

DU07-BA8	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)
DU07-BA9	bathophilus	DP	2007	x			x		*C. bifurcatus* (gr. 1)
DU07-BA10	bathophilus	DP	2007	x	KF278321		x		*C. bifurcatus* (gr. 1)
DU10-BA1	bathophilus	DP	2010		KF278322	KF278437	x		*C. bifurcatus* (gr. 1)
DU10-BA2	bathophilus	DP	2010		KF278323	KF278440	x		*C. bifurcatus* (gr. 1)
OP07-BA1	bathophilus	OP	2007	x			x		*C. bifurcatus* (gr. 1)
OP07-BA2	bathophilus	OP	2007	x	KF278324		x		*C. bifurcatus* (gr. 1)
OP07-BA5	bathophilus	OP	2007		KF278325	KF278438	x		*C. bifurcatus* (gr. 1)
OP09-BA2	bathophilus	OP	2009		KF278326	KF278441	x		*C. bifurcatus* (gr. 1)
SJ07-BA4	bathophilus	SJ	2007	x	KF278352		x		*C. bifurcatus* (gr. 1)
SJ07-BA5	bathophilus	SJ	2007	x			x		*C. bifurcatus* (gr. 1)
KI06-BA1	bathophilus	KI	2006	x	KF278307		x		*C. bifurcatus* (gr. 2)
KI06-BA2	bathophilus	KI	2006	x			x		*C. bifurcatus* (gr. 2)
FA07-BA1	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
FA07-BA2	bathophilus	MC	2007	x	KF278310		x		*C. bifurcatus* (gr. 2)
FA07-BA3	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
FA07-BA4	bathophilus	MC	2007	x	KF278308		x		*C. bifurcatus* (gr. 2)
FA07-BA5	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
FA07-BA6	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
FA07-BA7	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
FA07-BA8	bathophilus	MC	2007	x	KF278311		x	x	*C. bifurcatus* (gr. 2)
FA07-BA9	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
FA07-BA10	bathophilus	MC	2007	x			x		*C. bifurcatus* (gr. 2)
SJ07-BA1	bathophilus	SJ	2007		KF278312		x		*C. bifurcatus* (gr. 2)
SJ07-BA2	bathophilus	SJ	2007		KF278313	KF278442	x		*C. bifurcatus* (gr. 2)
SJ07-BA3	bathophilus	SJ	2007		KF278314	KF278443	x		*C. bifurcatus* (gr. 2)
TI11-BA1	bathophilus	TI	2011		KF278309	KF278439	x	x	*C. bifurcatus* (gr. 2)
BO06-SA1	salinarius	BO	2006	x			x		*C. cucini*
BO06-SA2	salinarius	BO	2006	x			x		*C. cucini*

BO06-SA3	salinarius	BO	2006	x			x		*C. cucini*
BO06-SA4	salinarius	BO	2006	x			x		*C. cucini*
BO06-SA5	salinarius	BO	2006	x			x		*C. cucini*
BO06-SA6	salinarius	BO	2006				x	x	*C. cucini*
BO06-SA7	salinarius	BO	2006				x	x	*C. cucini*
CW07-SA1	salinarius	CL	2007	x	KF278288		x		*C. cucini*
CW07-SA2	salinarius	CL	2007	x	KF278289		x		*C. cucini*
CW07-SA3	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA4	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA5	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA6	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA7	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA8	salinarius	CL	2007	x	KF278290		x	x	*C. cucini*
CW07-SA9	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA10	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA11	salinarius	CL	2007	x	KF278286		x		*C. cucini*
CW07-SA12	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA13	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA14	salinarius	CL	2007	x			x		*C. cucini*
CW07-SA15	salinarius	CL	2007	x			x		*C. cucini*
OP07-SA1	salinarius	OP	2007	x	KF278287		x		*C. cucini*
SJ06-SA1	salinarius	SJ	2006	x			x		*C. cucini*
SJ06-SA2	salinarius	SJ	2006	x			x		*C. cucini*
SJ06-SA3	salinarius	SJ	2006	x			x		*C. cucini*
SJ06-SA4	salinarius	SJ	2006	x		KF278417	x		*C. cucini*
SJ06-SA5	salinarius	SJ	2006	x		KF278418	x		*C. cucini*
VA10-SA1	salinarius	VA	2010		KF278284	KF278419	x		*C. cucini*
VA10-SA2	salinarius	VA	2010		KF278285	KF278420	x	x	*C. cucini*
VA10-SA3	salinarius	VA	2010				x	x	*C. cucini*

AD10-BAFL1	fluviatilis	AD	2010		KF278298		x		*C. decorus*-group sp. 2
AD10-BAFL2	fluviatilis	AD	2010		KF278295		x	x	*C. decorus*-group sp. 2
DUF06-BAFL1	bathophilus	DF	2006	x			x		*C. decorus*-group sp. 2
DUF06-BAFL2	bathophilus	DF	2006	x			x		*C. decorus*-group sp. 2
DUF06-BAFL3	bathophilus	DF	2006	x			x		*C. decorus*-group sp. 2
DUF06-BAFL4	bathophilus	DF	2006	x	KF278293		x		*C. decorus*-group sp. 2
DUF06-BAFL5	bathophilus	DF	2006	x			x		*C. decorus*-group sp. 2
DP06-BAFL1	bathophilus	DP	2006	x			x		*C. decorus*-group sp. 2
DP06-BAFL3	bathophilus	DP	2006	x	KF278299		x		*C. decorus*-group sp. 2
DP06-BAFL4	bathophilus	DP	2006	x			x		*C. decorus*-group sp. 2
DP06-BAFL5	bathophilus	DP	2006	x			x		*C. decorus*-group sp. 2
FO06-BAFL1	bathophilus	FO	2006	x	KF278300		x		*C. decorus*-group sp. 2
OP09-BAFL1	bathophilus	OP	2009		KF278296		x		*C. decorus*-group sp. 2
OP09-BAFL2	bathophilus	OP	2009		KF278297		x		*C. decorus*-group sp. 2
SC10-BAFL1	bathophilus	SC	2010		KF278294		x		*C. decorus*-group sp. 2
SC10-BAFL3	bathophilus	SC	2010				x	x	*C. decorus*-group sp. 2
SI11-BAFL1	melanotus	SI	2011		KF278301		x		*C. decorus*-group sp. 2
KE10-PL1	plumosus	KE	2010		KF278333	KF278401	x		*C. dilutus*
KE10-PL3	plumosus	KE	2010		KF278337	KF278402	x		*C. dilutus*
KE10-PL7	plumosus	KE	2010		KF278338	KF278403	x		*C. dilutus*
KE11-PL1	plumosus	KE	2011		KF278334	KF278404	x		*C. dilutus*
KE11-PL2	plumosus	KE	2011		KF278339	KF278405	x		*C. dilutus*
KE11-PL3	plumosus	KE	2011		KF278340	KF278406	x		*C. dilutus*
KE11-PL4	plumosus	KE	2011		KF278335	KF278407	x		*C. dilutus*
KE11-PL5	plumosus	KE	2011		KF278341	KF278408	x		*C. dilutus*
KE11-PL6	plumosus	KE	2011		KF278336	KF278409	x		*C. dilutus*
AL06-SRPL10	semireductus	DA	2006	x		KF278410	x	x	*C. entis*
DAS10-SRPL1	semireductus	DS	2010		KF278208	KF278411	x	x	*C. entis*
M-585	semireductus	MN	2007					x	*C. entis*

OP09-SRPL1	semireductus	OP	2009		KF278213	KF278412	x	x	*C. entis*
OP09-SRPL2	semireductus	OP	2009			KF278413	x	x	*C. entis*
PE10-SRPL1	semireductus	PE	2010		KF278212	KF278414	x	x	*C. entis*
PE10-SRPL2	semireductus	PE	2010			KF278415	x	x	*C. entis*
PE10-SRPL3	semireductus	PE	2010			KF278416	x	x	*C. entis*
AL06-SRPL3	plumosus	AL	2006	x					*C. entis* or *C. plumosus*
AL06-SRPL5	plumosus	AL	2006	x					*C. entis* or *C. plumosus*
AL06-SRPL8	plumosus	AL	2006	x					*C. entis* or *C. plumosus*
AL06-SRPL9	plumosus	AL	2006	x					*C. entis* or *C. plumosus*
AL06-SRPL6	plumosus	DA	2006	x	not published				*C. entis* or *C. plumosus*
AL06-SRPL7	plumosus	DA	2006	x	not published				*C. entis* or *C. plumosus*
DU07-SRPL2	semireductus to plumosus	DU	2007	x					*C. entis* or *C. plumosus*
DU07-SRPL6	semireductus to plumosus	DU	2007	x					*C. entis* or *C. plumosus*
DU07-SRPL7	semireductus to plumosus	DU	2007	x					*C. entis* or *C. plumosus*
DU07-SRPL8	semireductus to plumosus	DU	2007	x					*C. entis* or *C. plumosus*
DU07-SRPL9	semireductus to plumosus	DU	2007	x					*C. entis* or *C. plumosus*
DU07-SRPL10	semireductus to plumosus	DU	2007	x					*C. entis* or *C. plumosus*
FO06-SRPL3	semireductus to plumosus	FO	2006	x					*C. entis* or *C. plumosus*
FO06-SRPL4	semireductus	FO	2006	x					*C. entis* or *C. plumosus*
FO06-SRPL5	semireductus	FO	2006	x					*C. entis* or *C. plumosus*
FO06-SRPL6	semireductus to plumosus	FO	2006	x					*C. entis* or *C. plumosus*
FO06-SRPL7	semireductus to plumosus	FO	2006	x					*C. entis* or *C. plumosus*
KI06-SRPL2	plumosus	KI	2006	x					*C. entis* or *C. plumosus*
KI06-SRPL3	plumosus	KI	2006	x					*C. entis* or *C. plumosus*
KI06-SRPL4	plumosus	KI	2006	x					*C. entis* or *C. plumosus*
KI06-SRPL5	plumosus	KI	2006	x					*C. entis* or *C. plumosus*
MA06-SRPL1	semireductus	MA	2006	x					*C. entis* or *C. plumosus*
MA07-SRPL2	semireductus to plumosus	MA	2007	x					*C. entis* or *C. plumosus*
MA07-SRPL3	semireductus	MA	2007	x					*C. entis* or *C. plumosus*

OP06-SRPL1	semireductus to plumosus	OP	2006	x					*C. entis* or *C. plumosus*
OS06-SRPL1	semireductus	OS	2006	x					*C. entis* or *C. plumosus*
PO07-PL1	plumosus	PO	2007	x			x		*C. frommeri*
PO07-PL3	plumosus	PO	2007	x			x		*C. frommeri*
PO07-PL5	plumosus	PO	2007	x			x		*C. frommeri*
PO07-PL6	plumosus	PO	2007	x	KF278235		x	x	*C. frommeri*
PO07-PL7	plumosus	PO	2007	x	KF278236		x		*C. frommeri*
PO07-PL8	plumosus	PO	2007	x			x		*C. frommeri*
PO07-PL9	plumosus	PO	2007	x	KF278237		x		*C. frommeri*
AR10-PL1	plumosus	AR	2010		KF278304	KF278430	x		*C. harpi*
AR10-PL2	plumosus	AR	2010		KF278306	KF278433	x		*C. harpi*
AR10-PL3	plumosus	AR	2010		KF278302	KF278428	x		*C. harpi*
AR10-PL4	plumosus	AR	2010		KF278305	KF278431	x		*C. harpi*
AR10-PL5	plumosus	AR	2010		KF278303	KF278429	x		*C. harpi*
AR10-PL6	plumosus	AR	2010			KF278432	x		*C. harpi*
BD07-PL1	plumosus	BE	2007	x	KF278292		x		*C. maturus*
BD07-PL2	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL3	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL4	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL5	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL6	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL7	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL8	plumosus	BE	2007	x			x		*C. maturus*
BD07-PL9	plumosus	BE	2007	x	KF278291	KF278400	x		*C. maturus*
BD07-PL10	plumosus	BE	2007	x			x		*C. maturus*
MA09-TH1	thummi	MN	2009		KF278330		x	x	*C.* nr. *atroviridis* (sp. 2i)
MA09-TH2	thummi	MN	2009		KF278329		x	x	*C.* nr. *atroviridis* (sp. 2i)
MA09-TH3	thummi	MN	2009		KF278331		x		*C.* nr. *atroviridis* (sp. 2i)
MA09-TH4	thummi	MN	2009		KF278332		x		*C.* nr. *atroviridis* (sp. 2i)

OP09-TH1	thummi	OP	2009		KF278327		x	x	*C. ochreatus*
OP09-TH2	thummi	OP	2009		KF278328		x	x	*C. ochreatus*
AU10-SRPL1	plumosus	AU	2010		KF278214	KF278389	x	x	*C. plumosus*
AU10-SRPL2	semireductus to plumosus	AU	2010				x	x	*C. plumosus*
AU10-SRPL4	plumosus	AU	2010			KF278376	x	x	*C. plumosus*
AL06-SRPL1	plumosus	DA	2006	x		KF278384	x	x	*C. plumosus*
AL06-SRPL2	plumosus	DA	2006	x		KF278362	x	x	*C. plumosus*
AL06-SRPL4	plumosus	DA	2006	x			x	x	*C. plumosus*
DU07-SRPL1	semireductus to plumosus	DP	2007	x			x	x	*C. plumosus*
DU10-SRPL1	semireductus to plumosus	DP	2010		KF278209	KF278363	x	x	*C. plumosus*
DU10-SRPL2	semireductus to plumosus	DP	2010			KF278377	x	x	*C. plumosus*
DU10-SRPL3	semireductus to plumosus	DP	2010			KF278364	x	x	*C. plumosus*
FO06-SRPL1	plumosus	FO	2006	x		KF278378	x	x	*C. plumosus*
FO06-SRPL2	plumosus	FO	2006	x		KF278380	x	x	*C. plumosus*
KE10-SRPL2	plumosus	KE	2010			KF278372	x	x	*C. plumosus*
KE10-SRPL3	plumosus	KE	2010			KF278365	x	x	*C. plumosus*
KE10-SRPL6	plumosus	KE	2010			KF278373	x	x	*C. plumosus*
KE10-SRPL8	plumosus	KE	2010			KF278379	x	x	*C. plumosus*
KI06-SRPL1	plumosus	KI	2006	x	KF278210		x	x	*C. plumosus*
MA07-SRPL1	semireductus	MN	2007	x	KF278216		x	x	*C. plumosus*
MA10-SRPL1	semireductus	MN	2010		KF278218	KF278385	x	x	*C. plumosus*
OS09-SRPL1	plumosus	OS	2009		KF278211	KF278366	x	x	*C. plumosus*
OS09-SRPL2	plumosus	OS	2009			KF278367	x	x	*C. plumosus*
OS09-SRPL3	plumosus	OS	2009			KF278375	x	x	*C. plumosus*
PE10-SRPL4	semireductus	PE	2010			KF278374	x	x	*C. plumosus*
PE10-SRPL5	semireductus to plumosus	PE	2010			KF278386	x	x	*C. plumosus*
PE10-SRPL6	plumosus	PE	2010		KF278217	KF278368	x	x	*C. plumosus*
PE10-SRPL7	semireductus to plumosus	PE	2010			KF278369	x	x	*C. plumosus*
PE10-SRPL8	plumosus	PE	2010			KF278381	x	x	*C. plumosus*

PE10-SRPL9	semireductus to plumosus	PE	2010			KF278382	x	x	*C. plumosus*
PE10-SRPL10	plumosus	PE	2010			KF278387	x	x	*C. plumosus*
PE10-SRPL11	semireductus to plumosus	PE	2010			KF278370	x	x	*C. plumosus*
PE10-SRPL13	plumosus	PE	2010			KF278388	x	x	*C. plumosus*
RO10-SRPL1	plumosus	RO	2010		KF278215			x	*C. plumosus*
RO10-SRPL3	plumosus	RO	2010			KF278371	x	x	*C. plumosus*
KA07-TH1	thummi	KA	2007	x			x		*C.* sp. NAI
KA07-TH2	thummi	KA	2007	x			x		*C.* sp. NAI
KA07-TH3	thummi	KA	2007	x	KF278220		x		*C.* sp. NAI
KA07-TH5	thummi	KA	2007	x			x		*C.* sp. NAI
KA07-TH6	thummi	KA	2007	x		KF278390	x	x	*C.* sp. NAI
KA07-TH7	thummi	KA	2007	x	KF278219		x	x	*C.* sp. NAI
KA07-TH8	thummi	KA	2007	x			x	x	*C.* sp. NAI
KA07-TH9	thummi	KA	2007	x		KF278391	x		*C.* sp. NAI
KA07-TH10	thummi	KA	2007	x		KF278392	x		*C.* sp. NAI
SI07-SA1	salinarius	SI	2007	x			x		*C.* sp. NAII
SI07-SA2	salinarius	SI	2007	x	KF278270		x		*C.* sp. NAII
SI07-SA3	salinarius	SI	2007	x			x		*C.* sp. NAII
SI07-SA4	salinarius	SI	2007	x	KF278269		x		*C.* sp. NAII
AL06-SA1	salinarius	DA	2006	x	KF278276		x		*C.* sp. NAIII
AL06-SA2	salinarius	DA	2006	x			x		*C.* sp. NAIII
AL06-SA3	salinarius	DA	2006	x			x		*C.* sp. NAIII
AL06-SA4	salinarius	DA	2006	x			x		*C.* sp. NAIII
AL06-SA5	salinarius	DA	2006	x			x		*C.* sp. NAIII
AL06-SA6	salinarius	DA	2006	x			x		*C.* sp. NAIII
HA07-SA1	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA2	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA3	salinarius	HA	2007	x	KF278271		x		*C.* sp. NAIII
HA07-SA4	salinarius	HA	2007	x			x	x	*C.* sp. NAIII

HA07-SA5	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA6	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA7	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA8	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA9	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA10	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA11	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA12	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA13	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA14	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA15	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA16	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA17	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA18	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA19	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA07-SA20	salinarius	HA	2007	x			x		*C.* sp. NAIII
HA10-SA1	salinarius	HA	2010		KF278274	KF278423	x		*C.* sp. NAIII
FA07-SA1	salinarius	MC	2007	x	KF278281		x		*C.* sp. NAIII
FA07-SA2	salinarius	MC	2007	x			x		*C.* sp. NAIII
FA07-SA3	salinarius	MC	2007	x			x		*C.* sp. NAIII
FA07-SA4	salinarius	MC	2007	x			x	x	*C.* sp. NAIII
FA07-SA5	salinarius	MC	2007	x			x		*C.* sp. NAIII
FA07-SA6	salinarius	MC	2007	x			x		*C.* sp. NAIII
FA07-SA7	salinarius	MC	2007	x			x		*C.* sp. NAIII
FA07-SA8	salinarius	MC	2007	x			x		*C.* sp. NAIII
FA07-SA9	salinarius	MC	2007	x			x		*C.* sp. NAIII
RA10-SA1	salinarius	RA	2010		KF278277		x		*C.* sp. NAIII
RA10-SA3	salinarius	RA	2010		KF278272	KF278422	x		*C.* sp. NAIII
RA10-SA4	salinarius	RA	2010		KF278273	KF278424	x		*C.* sp. NAIII

RA10-SA5	salinarius	RA	2010		KF278283		x		*C.* sp. NAIII
RA10-SA6	salinarius	RA	2010		KF278278		x		*C.* sp. NAIII
RA10-SA7	salinarius	RA	2010		KF278279		x		*C.* sp. NAIII
RAM07-SA1	salinarius	RM	2007	x	KF278275		x		*C.* sp. NAIII
RAM07-SA2	salinarius	RM	2007	x			x		*C.* sp. NAIII
RAM07-SA3	salinarius	RM	2007	x			x		*C.* sp. NAIII
RAM07-SA4	salinarius	RM	2007	x	KF278282	KF278421	x		*C.* sp. NAIII
RAM07-SA5	salinarius	RM	2007	x	KF278280		x		*C.* sp. NAIII
RAM07-SA6	salinarius	RM	2007	x			x	x	*C.* sp. NAIII
RAM07-SA7	salinarius	RM	2007	x			x		*C.* sp. NAIII
RAM07-SA8	salinarius	RM	2007	x			x		*C.* sp. NAIII
RAM07-SA9	salinarius	RM	2007	x			x		*C.* sp. NAIII
RAM07-SA10	salinarius	RM	2007	x			x		*C.* sp. NAIII
CR10-PL1	plumosus	CR	2010		KF278257		x		*C. staegeri*
AL06-PL1	plumosus	DA	2006	x	KF278256		x		*C. staegeri*
AL06-PL2	plumosus	DA	2006	x			x		*C. staegeri*
AL06-PL3	plumosus	DA	2006	x			x		*C. staegeri*
AL06-PL4	plumosus	DA	2006	x			x		*C. staegeri*
AL06-PL5	plumosus	DA	2006	x			x		*C. staegeri*
DU06-PL2	plumosus	DP	2006	x			x		*C. staegeri*
DU07-PL3	plumosus	DP	2007	x			x		*C. staegeri*
DU07-PL4	plumosus	DP	2007	x	KF278258		x		*C. staegeri*
DU07-PL5	plumosus	DP	2007	x			x		*C. staegeri*
DU10-PL1	plumosus	DP	2010		KF278259		x		*C. staegeri*
KA07-PL21	plumosus	KA	2007	x			x		*C. staegeri*
KI06-PL1	plumosus	KI	2006	x	KF278261		x		*C. staegeri*
FA07-PL1	plumosus	MC	2007	x	KF278260		x		*C. staegeri*
FA07-PL2	plumosus	MC	2007	x			x		*C. staegeri*
FA07-PL3	plumosus	MC	2007	x			x		*C. staegeri*

FA07-PL4	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL5	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL6	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL7	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL8	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL9	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL10	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL11	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL12	plumosus	MC	2007	x	KF278254	x		*C. staegeri*
FA07-PL13	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL14	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL15	plumosus	MC	2007	x		x		*C. staegeri*
FA07-PL16	plumosus	MC	2007	x		x		*C. staegeri*
OP09-PL1	plumosus	OP	2009		KF278262	x		*C. staegeri*
PO07-PL2	plumosus	PO	2007	x	KF278255	x	x	*C. staegeri*
PO07-PL4	plumosus	PO	2007	x		x		*C. staegeri*
SI07-PL1	plumosus	SI	2007	x		x		*C. staegeri*
SI07-PL2	plumosus	SI	2007	x		x		*C. staegeri*
SI07-PL3	plumosus	SI	2007	x		x		*C. staegeri*
SI07-PL4	plumosus	SI	2007	x		x		*C. staegeri*
SI07-PL5	plumosus	SI	2007	x	KF278263	x		*C. staegeri*
SI07-PL6	plumosus	SI	2007	x		x		*C. staegeri*
SI07-PL7	plumosus	SI	2007	x		x		*C. staegeri*
SJ07-PL1	plumosus	SJ	2007		KF278264	x		*C. staegeri*
SJ07-PL2	plumosus	SJ	2007		KF278265	x		*C. staegeri*
SJ07-PL3	plumosus	SJ	2007		KF278268	x		*C. staegeri*
TI07-PL11	plumosus	TI	2007	x	KF278266	x		*C. staegeri*
TI11-PL1	plumosus	TI	2011		KF278267	x		*C. staegeri*
KA07-PL1	plumosus	KA	2007	x		x		*C. 'tigris'*

KA07-PL2	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL3	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL4	plumosus	KA	2007	x	KF278242	x		*C. 'tigris'*
KA07-PL5	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL6	plumosus	KA	2007	x	KF278243	x	x	*C. 'tigris'*
KA07-PL7	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL8	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL9	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL10	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL11	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL12	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL13	plumosus	KA	2007	x	KF278244	x		*C. 'tigris'*
KA07-PL14	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL15	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL16	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL17	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL18	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL19	plumosus	KA	2007	x		x		*C. 'tigris'*
KA07-PL20	plumosus	KA	2007	x		x		*C. 'tigris'*
FA07-PL17	plumosus	MC	2007	x	KF278245	x		*C. 'tigris'*
FA07-PL18	plumosus	MC	2007	x		x		*C. 'tigris'*
FA07-PL19	plumosus	MC	2007	x		x		*C. 'tigris'*
FA07-PL20	plumosus	MC	2007	x	KF278253	x		*C. 'tigris'*
FA07-PL21	plumosus	MC	2007	x		x		*C. 'tigris'*
FA07-PL22	plumosus	MC	2007	x	KF278246	x		*C. 'tigris'*
FA07-PL23	plumosus	MC	2007	x		x		*C. 'tigris'*
FA07-PL24	plumosus	MC	2007	x		x		*C. 'tigris'*
FA07-PL25	plumosus	MC	2007	x		x		*C. 'tigris'*
FA07-PL26	plumosus	MC	2007	x		x		*C. 'tigris'*

FA07-PL27	plumosus	MC	2007		KF278247	KF278396	x			*C. 'tigris'*
OP06-PL1	plumosus	OP	2006		KF278238	KF278397	x			*C. 'tigris'*
OP07-PL1	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL2	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL3	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL4	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL5	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL6	plumosus	OP	2007	x	KF278239		x		x	*C. 'tigris'*
OP07-PL7	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL8	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL9	plumosus	OP	2007	x			x			*C. 'tigris'*
OP07-PL10	plumosus	OP	2007	x			x			*C. 'tigris'*
OP09-PL2	plumosus	OP	2009		KF278240	KF278398	x			*C. 'tigris'*
SI07-PL8	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL9	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL10	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL11	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL12	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL13	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL14	plumosus	SI	2007	x			x			*C. 'tigris'*
SI07-PL15	plumosus	SI	2007	x	KF278248		x			*C. 'tigris'*
SI07-PL16	plumosus	SI	2007	x			x			*C. 'tigris'*
SJ07-PL4	plumosus	SJ	2007	x	KF278249		x			*C. 'tigris'*
SJ07-PL5	plumosus	SJ	2007	x			x			*C. 'tigris'*
TI07-PL1	plumosus	TI	2007	x			x			*C. 'tigris'*
TI07-PL2	plumosus	TI	2007	x	KF278250		x			*C. 'tigris'*
TI07-PL3	plumosus	TI	2007	x			x			*C. 'tigris'*
TI07-PL4	plumosus	TI	2007	x	KF278241		x			*C. 'tigris'*
TI07-PL5	plumosus	TI	2007	x			x			*C. 'tigris'*

TI07-PL6	plumosus	TI	2007	x			x	*C. 'tigris'*
TI07-PL7	plumosus	TI	2007	x			x	*C. 'tigris'*
TI07-PL8	plumosus	TI	2007	x			x	*C. 'tigris'*
TI07-PL9	plumosus	TI	2007	x			x	*C. 'tigris'*
TI07-PL10	plumosus	TI	2007	x	KF278252		x	*C. 'tigris'*
TI11-PL2	plumosus	TI	2011		KF278251	KF278399	x	*C. 'tigris'*

Table S2. List of voucher *Chironomus* species sequenced.

***Chironomus* species**	**Location**	**Collector**	**Identified by**	**GenBank Accession #**		**Voucher number**
				cox1	***gb2B***	
C. (*Chaetolabis*) nr. *atroviridis* (sp.2i)	White Lake, Three Mile Bay, ON, Canada	Don R. Oliver	Jon Martin	KF278342		Ch.sp2i15m
C. (*Chaetolabis*) nr. *atroviridis* (sp.2i)	White Lake, Three Mile Bay, ON, Canada	Don R. Oliver	Jon Martin	KF278360		Chaet.2i16m
C. (*Chaetolabis*) nr. *atroviridis* (sp.2i)	White Lake, Three Mile Bay, ON, Canada	Don R. Oliver	Jon Martin		KF278450	DRO.14.6 16M
C. (*Chaetolabis*) *ochreatus*	Little John Jr. Lake, WI, United States	Jon Martin	Jon Martin	KF278351		Ch.ochr13F
C. (*Chironomus*) *acidophilus*	Potters Marsh, Anchorage Co., AK, United States	Dave Wartenbee	Jon Martin	KF278358		UAK.1.14F or acidUAK1*
C. (*Chironomus*) *anthracinus*	Lake Esrom, Denmark	Henk Vallendduuk	Claus Lindegaard	KF278343		ES(DAN)95-BA3
C. (*Chironomus*) *bifurcatus*	Arboretum, Madison, Dane Co., WI, United States	Jon Martin	Jon Martin	KF278345		AAW4003*
C. (*Chironomus*) *bifurcatus*	Lake Pleasant, Franklin Co., MA, United States	Sean Werle	Jon Martin	KF278361		bifMa21
C. (*Chironomus*) *bifurcatus*	Arboretum, Madison, Dane Co., WI, United States	Jon Martin	Jon Martin	KF278353		bifMad7
C. (*Chironomus*) *calligraphus*	Gainsville, Alachua Co., FL, United States	Pauline O. Lawrence	Jon Martin	KF278357	KF278449	ABZ9507* or UFL.2.1 male4.1
C. (*Chironomus*) *dilutus*	Stevens Pond, Madison, Dane Co., WI, United States	Barry T.O. Lee	Jon Martin	KF278359		Stevens Pond_Madison WI (Eastern)
C. (*Chironomus*) *entis*	Saginaw Bay, Lake Michigan, MI, United States	Michael H. Winnell	Jon Martin	KF278355	KF278446	C.entisMI22 or UM1.3.1 22
C. (*Chironomus*) *entis*	Brewer Lake, Cass Co., ND, United States	Malcolm G. Butler	Jon Martin		KF278445	UND.2.1 2
C. (*Chironomus*) *harpi*	Bradleys Acid Pit, Jackson Co., IL, United States	Ken D. Yamamoto	Jon Martin	KF278346		AAJ4275*
C. (*Chironomus*) *plumosus*	Saginaw Bay, Lake Michigan, MI, United States	Michael H. Winnell	Jon Martin	KF278354	KF278444	C.plumMI21 or UM1.3.1 21
C. (*Chironomus*) *quinnitukqut*	Truro, Cape Cod, Barnstable Co., MA, United States	Jon Martin	Jon Martin	KF278347		AAB7030*
C. sp. g	Lake Bat, Algonquin Park, ON, Canada	Jon Martin	Jon Martin	KF278348		C.spgBatLk
C. sp. h	Lake Bat, Algonquin Park, ON, Canada	Jon Martin	Jon Martin	KF278349		C.sphBatLk
C. sp. u	Calgary, AL, Canada	Jon Martin	Jon Martin		KF278447	CAL.2.4 egg mass #3, 3.2f
C. (*Chironomus*) *staegeri*	Lake Pleasant, Franklin Co., MA, United States	Jon Martin	Jon Martin	KF278356		AAW3999*
C. (*Chironomus*) '*tigris*'	Turtle Lake, Becker Co., MN, United States	Malcolm G. Butler	Jon Martin	KF278350		C.tigris_TurtleLk_MN_USA
C. (*Chironomus*) *anthracinus*-gr.	Marion Lake, Garibaldi Prov.Pk., BC, Canada	Andrew L. Hamilton	Andrew L. Hamilton	KF278344		CBC.1.1 14f(1)

*Published in BOLD (BIN #)

Table S3. Mean and range of intraspecific sequence divergences of the *cox1* and *gb2β* genes for collected and reference *Chironomus* species.

Chironomus species	*cox1*			*gb2β*		
	No. of specimens	K2P divergence (%)		No. of specimens	K2P divergence (%)	
		Mean	Range		Mean	Range
C. anthracinus	16	0.06	0.00-0.15	6	0.34	0.00-0.86
C. bifurcatus	25	1.24	0.00-2.60	10	0.29	0.00-0.97
C. cucini	7	0.21	0.00-0.48	4	0.21	0.00-0.32
C. decorus-group sp.2	10	0.26	0.00-0.61	0		
C. dilutus	11	1.34	0.00-3.26	11	0.00	0.00-0.00
C. entis	5	1.30	0.31-2.16	9	0.25	0.00-1.10
C. frommeri	3	0.41	0.00-0.61	0		
C. harpi	6	0.22	0.00-0.66	6	0.00	0.00-0.00
C. maturus	3	0.21	0.15-0.31	1		
C. nr. *atroviridis* (sp. 2i)	6	1.33	0.00-2.65	0		
C. ochreatus	3	0.10	0.00-0.15	0		
C. plumosus	9	0.93	0.00-1.54	29	0.64	0.00-2.23
C. sp. NAI	3	0.82	0.15-1.23	3	0.00	0.00-0.00
C. sp. NAII	2	0.77		0		
C. sp. NAIII	13	0.35	0.00-0.77	4	0.00	0.00-0.00
C. staegeri	16	0.04	0.00-0.31	0		
C. 'tigris'	17	0.15	0.00-0.61	4	0.00	0.00-0.00

Table S4. Bases that differ between *cox1* sequences of *C.* sp. NAI and *C. anthracinus*. Refer to Fig. 8 for sequence label.

Species	Sequences	Base position																				
		7	1	1	2	2	2	3	3	4	4	4	4	4	4	5	5	5	5	5	6	6
		6	1	6	2	8	8	2	6	0	1	3	7	7	9	0	0	2	4	4	6	2	4
		6	2	6	3	0	4	8	4	9	5	3	5	8	0	0	8	6	1	2	8	2	6
C. sp. NAI	thummi (n=1 / KA / KF2782219)	A	C	G	C	C	T	A	C	G	A	A	C	G	T	C	A	C	A	T	T	C	T
C. sp. NAI	thummi (n=1 / KA / KF278220)	A	C	G	C	C	T	A	C	G	A	A	C	G	T	C	A	C	A	T	T	C	T
C. sp. NAI	*C. anthracinus*-group (KF278344)	A	C	G	C	C	T	A	C	G	A	A	C	G	T	C	A	C	A	T	T	C	T
C. anthracinus	thummi (n=4 / HA, OS, PI / KF278221-KF278224)	G	T	A	T	T	C	G	T	A	G	G	T	A	C	T	G	T	G	C	C	T	C
C. anthracinus	thummi (n=10 /AR, OS, PI, RA, RM, SI / KF278225-KF278234)	G	T	A	T	T	C	G	T	A	G	G	T	A	C	T	G	T	G	C	C	T	C
C. anthracinus	*C. anthracinus* (KF278343)	G	T	A	T	T	C	G	T	A	G	G	T	A	C	T	G	T	G	C	C	T	C

Table S5. Bases that differ between *cox1* sequences of *C. bifurcatus* groups 1 and 2. Refer to Fig. 8 for sequence label.

Species	Sequences	Base position									
		1	2	3	3	3	4	4	4	5	6
		9	7	3	4	8	3	7	9	1	2
		9	1	7	3	2	3	8	9	7	2
C. bifurcatus (gr. 1)	bathophilus (n=1 / DA / KF278315)	C	C	T	T	A	G	T	C	C	A
C. bifurcatus (gr. 1)	bathophilus (n=11 / AD, AR, DP, OP / KF278316-KF278326)	C	C	T	T	A	G	T	C	C	A
C. bifurcatus (gr. 1)	*C. bifurcatus* (KF278361)	C	C	T	T	A	G	T	A	C	A
C. bifurcatus (gr. 1)	bathophilus (n=1 / SJ / KF278352)	C	C	T	T	A	G	T	C	C	A
C. bifurcatus (gr. 2)	*C. bifurcatus* (KF278353)	T	A	A	C	T	A	A	T	T	C
C. bifurcatus (gr. 2)	*C. bifurcatus* (KF278345)	T	A	A	C	T	A	A	T	T	C
C. bifurcatus (gr. 2)	bathophilus (n=1 / KI / KF278307)	T	G	A	C	T	A	A	T	T	C
C. bifurcatus (gr. 2)	bathophilus (n=3 / SJ / KF278312-KF278314)	T	G	A	C	T	A	A	T	T	C
C. bifurcatus (gr. 2)	bathophilus (n=2 / MC / KF278310-KF278311)	T	G	A	C	T	A	A	T	T	T
C. bifurcatus (gr. 2)	bathophilus (n=2 / MC, TI / KF278308-KF278309)	T	G	A	C	T	A	A	T	T	T

ADDENDA - ARTICLE 2

p. 89 La phrase «The standard gene used for DNA barcoding is the **3' end** of the mitochondrial *cytochrome oxidase subunit I* (*cox1*: Hebert *et al.* 2003)» devrait plutôt être «The standard gene used for DNA barcoding is the **5' end** of the mitochondrial *cytochrome oxidase subunit I* (*cox1*: Hebert *et al.* 2003)».

p. 94 Les amorces 911 et 912 devraient plutôt être étiquetées LCO1490 et HCO2198 respectivement.

p. 95 La phrase suivante «All ambiguous positions were removed for each sequence pair.» devrait être ajoutée entre les deux phrases « Bootstrap analysis was performed with 1000 replicates. » et « For the *cox1* identification (ID) tree […] ».

p. 95 L'alignement des séquences *gb2β* est présenté ci-dessous. Idéalement, l'alignement aurait dû être présenté en information supplémentaire dans l'article pour faciliter la tâche à ceux et celles qui voudraient aligner ces séquences dans le futur.

```
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                          10         20         30         40         50
salinarius_(n-2_/_HA_RA_/_26)    CAAGGCTAAC CCAGACATCA TGGCTAAATT TCCACAATTT GCCGGCAAGA
salinarius_(n-2_/_RA_RM_/_25)    CAAGGCTAAC CCAGACATCA TGGCTAAATT TCCACAATTT GCCGGCAAGA
plumosus_(n-4_/_MC_OP_TI_/_18)   CAAGGCTAAC CCAGACATCA TGGCTAAGTT TCCACAATTC GCAGGCAAGA
thummi_(n-3_/_KA_/_14)           CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAGTTT GCTGGAAAGG
thummi_(n-1_/_RA_/_16)           CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
thummi_(n-1_/_PI_/_17)_|_C._an   CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
thummi_(n-1_/_OS_/_15)           CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACARTTT GCTGGAAAGG
thummi_(n-1_/_RA_/_28)           CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAGTTT GCTGGAAAGG
thummi_(n-1_/_AR_/_29)           CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
thummi_(n-1_/_AR_/_30)           CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACARTTT GCTGGAAAGG
C._annularius_(AJ003784)         CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAATTT GCCGGAAAGG
semireductus/plumosus_(n-1_/_P   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumousus_(n-10_/   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-2_/_K   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-1_/_O   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-7_/_D   CGATGCCAAT CCAGACATCA TGGCTAAATT CCCACAATTT GCTGGAAAGG
salinarius_(n-2_/_VA_/_23)_|_C   CAAGGCCAAC CCAGACATCA TGGCTAAGTT CCCACAATTC GCTGGCAAAA
salinarius_(n-2_/_SJ_/_22)       CAAGGCCAAC CCAGACATCA TGGCTAAGTT CCCACAATTC GCTGGCAAAA
plumosus_(n-6_/_AR_/_31)         CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGCAAGG
C._entis_(40)                    CGATGCCAAT CCAGACATCA TGGCTAAATT CCCACAATTT GCTGGAAAGG
C._entis_(39)                    CGATGCCAAT CCAGACATCA TGGCTAAATT CCCACAATTT GCTGGAAAGG
C._acidophilus_(42)              CAAGGCTCAT CCAGACATCA TGGCTAAATT CCCACAGTTT GCCGGCAAGG
C._calligraphus_(43)             CAAGGCAAAC CCAGACATCA TGGCTAAATT CCCACAATTC GCTGGCAAGA
C._sp._u_(41)                    CAAGGCCAAT CCAGACATCA TGGCTAAGTT CCCACAATTC GCTGGAAAGG
C._plumosus_(38)                 CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-4_/_A   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-1_/_F   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-2_/_P   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-1_/_A   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-5_/_D   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
semireductus/plumosus_(n-1_/_A   CAAGGCTAAT CCTGATATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGG
plumosus_(n-1_/_BD_/_19)         CAAGGCTCAC CCAGACATCA TGGCTAAGTT CCCACAATTT GCTGGAAAGA
plumosus_(n-9_/_KE_/_20)         CAAGGCCAAC CCAGACATCA TGGCTAAGTT CCCACAATTC GTTGGCAAGG
```

```
bathophilus_(n-3_/_DP_OP_TI_/_   CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGAAAGG
bathophilus_(n-2_/_AR_/_32)      CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGAAAGG
bathophilus_(n-1_/_AR_/_33)      CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGAAAGG
bathophilus_(n-1_/_DP_/_34)      CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGAAAGG
bathophilus_(n-1_/_OP_/_35)      CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGAAAGG
bathophilus_(n-2_/_SJ_/_37)      CAAGGCCAAT CCAGACATCA TGGCAAAGTT CCCACAATTT GCTGGAAAGG
C._pallidivittatus_(AF110170)    NNNNNNNAAT CCAGACATCA TGGCTAAGTT CCCACAATTC GCTGGAAAGG
C._pallidivittatus_(AF110169)    NNNNNNNAAT CCAGACATCA TGGCTAAGTT CCCACAATTC GCTGGAAAGG
C._pallidivittatus_(AF110168)    NNNNNNNAAT CCAGACATCA TGGCTAAGTT CCCACAATTC GCCGGAAAGG
C._pallidivittatus_(AF110167)    NNNNNNNAAT CCAGACATCA TGGCTAAGTT CCCACAATTC GCTGGAAAGG
C._dilutus_(AF110173)            NNNNNNNAAC CCAGACATCA TGGCTAAGTT CCCACAATTC GTTGGCAAGG
C._dilutus_(AF110174)            NNNNNNNAAC CCAGACATCA TGGCTAAGTT CCCACAATTC GTTGGCAAGG
gb7A_-_C._thummi_(AJ003812)      CACTGCTTAC CCAGACATTC AAGCCCGTTT CCCACAATTC GCTGGAAAGG
gb7A_-_C._nr._atroviridis_(sp.   CACAGCCAAC CCAGACATCC AAGCCCATTT CCCACAATTC GCCGGAAAGG
gb7A_-_C._melanotus_(AJ003797)   CACTGCCTAC CCAGACATCC AAGCYCGTTT CCCACAATTC GCTGGAAAGG
gb9_-_C._tentans_(AJ003809)      NNNNNNNNNN CCAGATATCC AAGCCCGTTT CCCACAATTC GCCGGAAAGG
gb9_-_C._zealandicus_(AJ003814   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG
gb9_-_C._aprilinus_(AJ003787)    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCTGGCAAGG
gb9_-_C._luridus_(AJ003794)      NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG
gb9_-_C._pallidivittatus_(AJ00   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG
gb9_-_C._thummi_piger_(AJ00380   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG
gb9_-_C._cingulatus_(AJ003788)   NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCTGGAAAGG
gb9_-_C._oppositus_(AJ003800)    NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG
gb9_-_C._duplex_(AJ003790)       NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG
gb9_-_C._jacksoni_(AJ003793)     NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN NNCACAATTC GCCGGAAAGG


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                       60         70         80         90        100
salinarius_(n-2_/_HA_RA_/_26)    ACCTCGATTC ATTAAAGGAC ACTGCTTCAT TTGCAACCCA TGCCG-----
salinarius_(n-2_/_RA_RM_/_25)    ACCTCGATTC ATTAAAGGAC ACTGCTTCAT TTGCAACCCA TGCCG-----
plumosus_(n-4_/_MC_OP_TI_/_18)   ACCTCGATTC ACTTAAGGAC ACTGCTTCAT TTGCCACCCA TGCAG-----
thummi_(n-3_/_KA_/_14)           ACCTCGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
thummi_(n-1_/_RA_/_16)           ACCTCGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
thummi_(n-1_/_PI_/_17)_|_C._an   ACCTCGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
thummi_(n-1_/_OS_/_15)           ACCTYGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
thummi_(n-1_/_RA_/_28)           ACCTYGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
thummi_(n-1_/_AR_/_29)           ACCTTGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
thummi_(n-1_/_AR_/_30)           ACCTCGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACCCA TGCCG-----
C._annularius_(AJ003784)         ACCTCGATTC ATTGAAAAAT ACTGCTTCAT TTGCCACTCA TGCCG-----
semireductus/plumosus_(n-1_/_P   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA CGCTG-----
semireductus/plumousus_(n-10_/   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA CGCTG-----
semireductus/plumosus_(n-2_/_K   AYCTCRATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA CGCTG-----
semireductus/plumosus_(n-1_/_O   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA CGCTG-----
semireductus/plumosus_(n-7_/_D   ACCTCGCTTC ATTAAAAAAT ACTGCTTCAT TTGCCACCCA TGCTG-----
salinarius_(n-2_/_VA_/_23)_|_C   ATCTCGACTC ATTAAAGGAA TCTGCTTCAT TTGCAACTCA TGCCG-----
salinarius_(n-2_/_SJ_/_22)       ATCTCGACTC ATTAAAGGAA TCTGCTTCAT TTGCAACTCA TGCCG-----
plumosus_(n-6_/_AR_/_31)         ATCTTGACAC AATAAAATCA AGCGCATCAT TCGCAACTCA TGCTG-----
C._entis_(40)                    ACCTCGCTTC ATTAAAAAAT ACTGCTTCAT TTGCCACCCA TGCTG-----
C._entis_(39)                    ACCTCGCTTC ATTAAAAAAT ACTGCTTCAT TTGCCACCCA TGCTG-----
C._acidophilus_(42)              ATCTCGATAC AATTAAGACG AGTGGACAGT TCGCAACTCA TGCTG-----
C._calligraphus_(43)             ACATTGAATC ATTGAAGGAC ACAGCTGCTT TTGCCACACA CGCTG-----
C._sp._u_(41)                    AACTCGATTC aCTTAAGGAT ACCGCTTCAT TTGCAACGCA TGCCG-----
C._plumosus_(38)                 ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA CGCTG-----
semireductus/plumosus_(n-4_/_A   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA YGCTG-----
semireductus/plumosus_(n-1_/_F   ATCTCAATTC ATTAAAAAAT ACTGCCWCAT TTGCAACACA YGCTG-----
semireductus/plumosus_(n-2_/_P   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA CGCTG-----
semireductus/plumosus_(n-1_/_A   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA YGCTG-----
semireductus/plumosus_(n-5_/_D   ATCTCAATTC ATTAAAAAAT ACTGCCACAT TTGCAACACA TGCTG-----
semireductus/plumosus_(n-1_/_A   ATCTCAATTC ATTAAAAAAT ACTGCCTCAT TTGCAACACA YGCTG-----
plumosus_(n-1_/_BD_/_19)         ACCTCGACTC ACTTAAGGAT ACTGCTTCAT TTGCCACCCA TGCAG-----
plumosus_(n-9_/_KE_/_20)         ATCTCGACTC ATTGAAAGAC ACAGCTGCAT TCGCCACACA TGCCG-----
bathophilus_(n-3_/_DP_OP_TI_/_   ATATTGAAAC AATGAAGTCA AGTGCATCTT TTGCTACACA TGCTG-----
bathophilus_(n-2_/_AR_/_32)      ATATTGAAAC AATGAAGTCA AGTGCATCTT TTGCTACACA TGCTG-----
bathophilus_(n-1_/_AR_/_33)      ATATTGAAAC AATGAAGTCA AGTGCATCTT TTGCTACACA TGCTG-----
bathophilus_(n-1_/_DP_/_34)      ATATTGAAAC AATGAAGTCA AGTGCATCTT TTGCTACACA TGCTG-----
bathophilus_(n-1_/_OP_/_35)      ATATTGAAAC AATGAAGTCA AGTGCATCTT TTGCTACACA TGCTG-----
bathophilus_(n-2_/_SJ_/_37)      ATATTGAAAC AATGAAGTCA AGTGCATCTT TTGCTACACA TGCTG-----
C._pallidivittatus_(AF110170)    ATCTCGAGTC ATTGAAGGGC ACAGCTGCAT TCGCCACACA TGCCG-----
C._pallidivittatus_(AF110169)    ATCTCGAGTC ATTGAAGGGC ACAGCTGCAT TCGCCACACA TGCCG-----
C._pallidivittatus_(AF110168)    ATCTCGAGTC ATTGAAGGGC ACAGCTGCAT TCGCCACACA TGCCG-----
```

```
C._pallidivittatus_(AF110167)    ATCTCGAGTC ATTGAAGGGC ACAGCTGCAT TCGCCACACA TGCCG-----
C._dilutus_(AF110173)            ATCTCGACTC ATTGAAAGAC ACAGCTGCAT TCGCCACACA TGCCG-----
C._dilutus_(AF110174)            ATCTCGACTC ATTGAAAGAC ACAGCTGCAT TCGCCACACA TGCCG-----
gb7A_-_C._thummi_(AJ003812)      ACGTCGCTTC AATCAAGGAT ACTGGTGCAT TCGCCACACA CGCCGGTAAG
gb7A_-_C._nr._atroviridis_(sp.   ACGTCGCTGC MTTGAGAGAC ACAGCTGCCT TCGCCACACA CGCCG-----
gb7A_-_C._melanotus_(AJ003797)   ATGTTGCTTC ACTTAAGGAC ACCGCTGCCT TTGCCACCCA CGCCG-----
gb9_-_C._tentans_(AJ003809)      ACGTCGACTC ATTGAAGGAC ACAGCTGCTT TCGCCACACA CGCCG-----
gb9_-_C._zealandicus_(AJ003814   ACCTCGAATC ACTTGTTGAC ACAGCTGCTT TTGCCACACA CGCCG-----
gb9_-_C._aprilinus_(AJ003787)    ACCTCGCTTC AATCAAGGAT ACTGGTGCAT TCGCTACTCA TGCTGGTGAG
gb9_-_C._luridus_(AJ003794)      ACCTCGCTTC AATCAAGGAT ACTGGTGCAT TTGCCACTCA TGCTGGTAAG
gb9_-_C._pallidivittatus_(AJ00   ACGTCGACTC ATTGAAGGAC ACAGCTGCTT TCGCCACACA CGCCG-----
gb9_-_C._thummi_piger_(AJ00380   ACCTCGCCTC AATCAAGGAT ACTGGTGCAT TCGCCACACA CGCTGGTGAG
gb9_-_C._cingulatus_(AJ003788)   ACCTCGCTGC TCTTAAGGAC ACCGCTGCCT TCGCCACCCA CGCCG-----
gb9_-_C._oppositus_(AJ003800)    ACCTCGAATC ACTTGTTGAC ACAGCTGCTT TCGCCACACA CGCCG-----
gb9_-_C._duplex_(AJ003790)       ACCTCGAATC ATTGGTAGAC ACAGCTGCTT TCGCCACACA CGCCG-----
gb9_-_C._jacksoni_(AJ003793)     ACCTCGCATC ACTTGTTGAC ACAGCTGCTT TCGCCACACA CGCCG-----


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                        110        120        130        140        150
salinarius_(n-2_/_HA_RA_/_26)    ---------- ---------- ---------- ---------- ----------
salinarius_(n-2_/_RA_RM_/_25)    ---------- ---------- ---------- ---------- ----------
plumosus_(n-4_/_MC_OP_TI_/_18)   ---------- ---------- ---------- ---------- ----------
thummi_(n-3_/_KA_/_14)           ---------- ---------- ---------- ---------- ----------
thummi_(n-1_/_RA_/_16)           ---------- ---------- ---------- ---------- ----------
thummi_(n-1_/_PI_/_17)_|_C._an   ---------- ---------- ---------- ---------- ----------
thummi_(n-1_/_OS_/_15)           ---------- ---------- ---------- ---------- ----------
thummi_(n-1_/_RA_/_28)           ---------- ---------- ---------- ---------- ----------
thummi_(n-1_/_AR_/_29)           ---------- ---------- ---------- ---------- ----------
thummi_(n-1_/_AR_/_30)           ---------- ---------- ---------- ---------- ----------
C._annularius_(AJ003784)         ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-1_/_P   ---------- ---------- ---------- ---------- ----------
semireductus/plumousus_(n-10_/   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-2_/_K   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-1_/_O   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-7_/_D   ---------- ---------- ---------- ---------- ----------
salinarius_(n-2_/_VA_/_23)_|_C   ---------- ---------- ---------- ---------- ----------
salinarius_(n-2_/_SJ_/_22)       ---------- ---------- ---------- ---------- ----------
plumosus_(n-6_/_AR_/_31)         ---------- ---------- ---------- ---------- ----------
C._entis_(40)                    ---------- ---------- ---------- ---------- ----------
C._entis_(39)                    ---------- ---------- ---------- ---------- ----------
C._acidophilus_(42)              ---------- ---------- ---------- ---------- ----------
C._calligraphus_(43)             ---------- ---------- ---------- ---------- ----------
C._sp._u_(41)                    ---------- ---------- ---------- ---------- ----------
C._plumosus_(38)                 ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-4_/_A   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-1_/_F   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-2_/_P   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-1_/_A   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-5_/_D   ---------- ---------- ---------- ---------- ----------
semireductus/plumosus_(n-1_/_A   ---------- ---------- ---------- ---------- ----------
plumosus_(n-1_/_BD_/_19)         ---------- ---------- ---------- ---------- ----------
plumosus_(n-9_/_KE_/_20)         ---------- ---------- ---------- ---------- ----------
bathophilus_(n-3_/_DP_OP_TI_/_   ---------- ---------- ---------- ---------- ----------
bathophilus_(n-2_/_AR_/_32)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-1_/_AR_/_33)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-1_/_DP_/_34)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-1_/_OP_/_35)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-2_/_SJ_/_37)      ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110170)    ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110169)    ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110168)    ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110167)    ---------- ---------- ---------- ---------- ----------
C._dilutus_(AF110173)            ---------- ---------- ---------- ---------- ----------
C._dilutus_(AF110174)            ---------- ---------- ---------- ---------- ----------
gb7A_-_C._thummi_(AJ003812)      TT-AAATAGA TAAAAAGGAT TAAGTRCAAT TTAAGGTATT TRTTATAATT
gb7A_-_C._nr._atroviridis_(sp.   ---------- ---------- ---------- ---------- ----------
gb7A_-_C._melanotus_(AJ003797)   ---------- ---------- ---------- ---------- ----------
gb9_-_C._tentans_(AJ003809)      ---------- ---------- ---------- ---------- ----------
gb9_-_C._zealandicus_(AJ003814   ---------- ---------- ---------- ---------- ----------
gb9_-_C._aprilinus_(AJ003787)    AACATATTGG TGCTATATTG ACTA--TACT TAGGTA--CT AATTATAATT
```

```
gb9_-_C._luridus_(AJ003794)      GACATATTGG TGCTATATTG ACTA--TATT AAGGTA--CT AATTATAATT
gb9_-_C._pallidivittatus_(AJ00   ---------- ---------- ---------- ---------- ----------
gb9_-_C._thummi_piger_(AJ00380   TAGCTATTGA TGCTGGATTG ACTT--TATT AAGGTA--CT CATTATAATT
gb9_-_C._cingulatus_(AJ003788)   ---------- ---------- ---------- ---------- ----------
gb9_-_C._oppositus_(AJ003800)    ---------- ---------- ---------- ---------- ----------
gb9_-_C._duplex_(AJ003790)       ---------- ---------- ---------- ---------- ----------
gb9_-_C._jacksoni_(AJ003793)     ---------- ---------- ---------- ---------- ----------


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                        160        170        180        190        200
salinarius_(n-2_/_HA_RA_/_26)    ---------- -------GTA GAATCGTCGG ATTTGTATCA GAGGTTGTTT
salinarius_(n-2_/_RA_RM_/_25)    ---------- -------GTA GAATCGTCGG ATTTGTATCA GAGGTTGTTT
plumosus_(n-4_/_MC_OP_TI_/_18)   ---------- -------GTA GAATCGTCGG ATTTGTATCG GAGGTTTGTT
thummi_(n-3_/_KA_/_14)           ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
thummi_(n-1_/_RA_/_16)           ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
thummi_(n-1_/_PI_/_17)_|_C._an   ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
thummi_(n-1_/_OS_/_15)           ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
thummi_(n-1_/_RA_/_28)           ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
thummi_(n-1_/_AR_/_29)           ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
thummi_(n-1_/_AR_/_30)           ---------- -------GTA GAATCGTCGG ATTC------ -----GTAAG
C._annularius_(AJ003784)         ---------- -------GTA GAATTGTCGG ATTC-----G TAAGTTTTCA
semireductus/plumosus_(n-1_/_P   ---------- -------GAA GAATCGTTGG ATTC------ -----GTAAG
semireductus/plumousus_(n-10_/   ---------- -------GAA GAATCGTTGG ATTC------ -----GTAAG
semireductus/plumosus_(n-2_/_K   ---------- -------GAA GAATCGTTGG ATTC------ -----GTAAG
semireductus/plumosus_(n-1_/_O   ---------- -------GAA GAATYGTYGG ATTC------ -----GTAAG
semireductus/plumosus_(n-7_/_D   ---------- -------GTA GAATTGTCGG ATTC------ -----GTAAG
salinarius_(n-2_/_VA_/_23)_|_C   ---------- -------GAA GAATCGTCGG ATTT------ ----------
salinarius_(n-2_/_SJ_/_22)       ---------- -------GTA GAATCGTCGG ATTT------ ----------
plumosus_(n-6_/_AR_/_31)         ---------- -------GAC GTATCGTAGG CTTT------ ----------
C._entis_(40)                    ---------- -------GTA GAATTGTCGG ATTC------ -----GTAAG
C._entis_(39)                    ---------- -------GTA GAATTGTCGG ATTC------ -----gtaag
C._acidophilus_(42)              ---------- -------GCA CGTTTATAGA TGCT-----G TATTTAGTCA
C._calligraphus_(43)             ---------- -------GAA GAATCGTTGG ATTT------ ----------
C._sp._u_(41)                    ---------- -------GTA GAATTGTCGG ATTT------ ----------
C._plumosus_(38)                 ---------- -------GAA GAATCGTTGG ATTC------ -----GTAAG
semireductus/plumosus_(n-4_/_A   ---------- -------GAA GAATYGTYGG ATTC------ -----GTAAG
semireductus/plumosus_(n-1_/_F   ---------- -------GWA GAATYGTYGG ATTC------ -----GTAAG
semireductus/plumosus_(n-2_/_P   ---------- -------GAA GAATCGTTGG ATTC------ -----GTAAG
semireductus/plumosus_(n-1_/_A   ---------- -------GAA GAATYGTYGG ATTC------ -----GTAAG
semireductus/plumosus_(n-5_/_D   ---------- -------GTA GAATTGTCGG ATTC------ -----GTAAG
semireductus/plumosus_(n-1_/_A   ---------- -------GWA GAATYGTYGG ATTC------ -----GTAAG
plumosus_(n-1_/_BD_/_19)         ---------- -------GWA GAATTGTTGG ATTC------ ----------
plumosus_(n-9_/_KE_/_20)         ---------- -------GAA GAATCGTCGG ATTC------ ----------
bathophilus_(n-3_/_DP_OP_TI_/_   ---------- -------GAC GTATCGTAGG ATTT------ ----------
bathophilus_(n-2_/_AR_/_32)      ---------- -------GAC GTATCGTAGG ATTT------ ----------
bathophilus_(n-1_/_AR_/_33)      ---------- -------GAC GTATCGTAGG ATTT------ ----------
bathophilus_(n-1_/_DP_/_34)      ---------- -------GAC GTATCGTAGG ATTT------ ----------
bathophilus_(n-1_/_OP_/_35)      ---------- -------GAC GTATCGTAGG ATTT------ ----------
bathophilus_(n-2_/_SJ_/_37)      ---------- -------GAC GTATCGTAGG ATTT------ ----------
C._pallidivittatus_(AF110170)    ---------- -------GAA GAATCGTTGG ATTC------ ----------
C._pallidivittatus_(AF110169)    ---------- -------GAA GAATCGTTGG ATTC------ ----------
C._pallidivittatus_(AF110168)    ---------- -------GAA GAATCGTTGG ATTC------ ----------
C._pallidivittatus_(AF110167)    ---------- -------GAA GAATCGTTGG ATTC------ ----------
C._dilutus_(AF110173)            ---------- -------GAA GAATCGTCGG ATTC------ ----------
C._dilutus_(AF110174)            ---------- -------GAA GAATCGTCGG ATTC------ ----------
gb7A_-_C._thummi_(AJ003812)      TGT----AAA TTTACAGGAA GAATCGTCGG ATTC------ ----------
gb7A_-_C._nr._atroviridis_(sp.   ---------- -------GAA GAATCGTTGG ATTC------ ----------
gb7A_-_C._melanotus_(AJ003797)   ---------- -------GAA GAATCGTCGG ATTT------ ----------
gb9_-_C._tentans_(AJ003809)      ---------- -------GAA GAATCGTCGG ATTC------ ----------
gb9_-_C._zealandicus_(AJ003814   ---------- -------GAA GAATCGTCGG ATTC------ ----------
gb9_-_C._aprilinus_(AJ003787)    TAT----TAA TTTACAGGAA GAATCGTCGG ATTC------ ----------
gb9_-_C._luridus_(AJ003794)      TAT----AAA TTTATAGGAA GAATCGTCGG ATTC------ ----------
gb9_-_C._pallidivittatus_(AJ00   ---------- -------GAA GAATCGTCGG ATTC------ ----------
gb9_-_C._thummi_piger_(AJ00380   TATATTTAAA TTTACAGGAA GAATCGTCGG ATTC------ ----------
gb9_-_C._cingulatus_(AJ003788)   ---------- -------GAA GAATCGTCGG ATTC------ ----------
gb9_-_C._oppositus_(AJ003800)    ---------- -------GAA GAATCGTCGG ATTC------ ----------
gb9_-_C._duplex_(AJ003790)       ---------- -------GAA GAATTGTCGG ATTC------ ----------
gb9_-_C._jacksoni_(AJ003793)     ---------- -------GAA GAATCGTCGG ATTC------ ----------
```

```
                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                         210        220        230        240        250
salinarius_(n-2_/_HA_RA_/_26)    GTTTGTTTGT TTATTTATAG CAATTCTCAT TGAATATTTT TTTAAATTCA
salinarius_(n-2_/_RA_RM_/_25)    GTTTGTTTGT TTATTTATAG CAATTCTCAT TGAATATTTT TTTAAATTCA
plumosus_(n-4_/_MC_OP_TI_/_18)   GTTTATTTGT TTATTTATAG CAATTATCAT TGAAC----- TTTAAATTCA
thummi_(n-3_/_KA_/_14)           TTTTTAATTT TTATTATAT- AAAAATTTAT TAAAATGAAA CGTTTTTTCA
thummi_(n-1_/_RA_/_16)           TTTTTAATTT TTATTATAT- AAAAATTTAT TAAAATGAAA CGTTTTTTCA
thummi_(n-1_/_PI_/_17)_|_C._an   TTTTTAATTT TTATTATAT- AAAAATTTAT TAAAATGAAA CGTTTTTTCA
thummi_(n-1_/_OS_/_15)           TTTTTAATTT TTATTATAT- AAAAATTTAT TAAAATGAAA CGTTTTTTCA
thummi_(n-1_/_RA_/_28)           TTTTTAATTT TTATTATAT- AAAAATTTAT TAAAATGAAA CGTTTTTTCA
thummi_(n-1_/_AR_/_29)           TTTTTAATTT TTATTATAT- AAAAATTTAT TGAAATGAAA CGTTTTTTCA
thummi_(n-1_/_AR_/_30)           TTTTTAATTT TTATTATAT- AAAAATTTAT TAAAATGAAA CGTTTTTTCA
C._annularius_(AJ003784)         GTTTTTATTA TGTAAAAAAT ACTGATTAAA AATTTATTGA AATGAATTTA
semireductus/plumosus_(n-1_/_P   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumousus_(n-10_/   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-2_/_K   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-1_/_O   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-7_/_D   TTTTTAATTT TTATTGTGT- AAAAATTTAT TGAAATGAAT GGTTTTTTCA
salinarius_(n-2_/_VA_/_23)_|_C   ---------- ---------- ---------- ---------- ----------
salinarius_(n-2_/_SJ_/_22)       ---------- ---------- ---------- ---------- ----------
plumosus_(n-6_/_AR_/_31)         ---------- ---------- ---------- ---------- ----------
C._entis_(40)                    TTTTTAATTT TTATTGTGT- AAAAATTTAT TGAAATGAAT GGTTTTTTCA
C._entis_(39)                    tttttaattt ttactgtgt- aaaaatttat tgaaatgaat ggtttttca
C._acidophilus_(42)              TTGAAGGTAC TAATTATAAT TTATAAATTT ACAGGTAGAA TCGTAGGATT
C._calligraphus_(43)             ---------- ---------- ---------- ---------- ----------
C._sp._u_(41)                    ---------- ---------- ---------- ---------- ----------
C._plumosus_(38)                 TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-4_/_A   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-1_/_F   TTTTTAATTT TTATTATTTT AAAAATTTAT TRAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-2_/_P   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-1_/_A   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-5_/_D   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
semireductus/plumosus_(n-1_/_A   TTTTTAATTT TTATTATTTT AAAAATTTAT TGAAATGAAT GGTTTTTTCA
plumosus_(n-1_/_BD_/_19)         ---------- ---------- ---------- ---------- ----------
plumosus_(n-9_/_KE_/_20)         ---------- ---------- ---------- ---------- ----------
bathophilus_(n-3_/_DP_OP_TI_/_   ---------- ---------- ---------- ---------- ----------
bathophilus_(n-2_/_AR_/_32)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-1_/_AR_/_33)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-1_/_DP_/_34)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-1_/_OP_/_35)      ---------- ---------- ---------- ---------- ----------
bathophilus_(n-2_/_SJ_/_37)      ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110170)    ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110169)    ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110168)    ---------- ---------- ---------- ---------- ----------
C._pallidivittatus_(AF110167)    ---------- ---------- ---------- ---------- ----------
C._dilutus_(AF110173)            ---------- ---------- ---------- ---------- ----------
C._dilutus_(AF110174)            ---------- ---------- ---------- ---------- ----------
gb7A_-_C._thummi_(AJ003812)      ---------- ---------- ---------- ---------- ----------
gb7A_-_C._nr._atroviridis_(sp.   ---------- ---------- ---------- ---------- ----------
gb7A_-_C._melanotus_(AJ003797)   ---------- ---------- ---------- ---------- ----------
gb9_-_C._tentans_(AJ003809)      ---------- ---------- ---------- ---------- ----------
gb9_-_C._zealandicus_(AJ003814   ---------- ---------- ---------- ---------- ----------
gb9_-_C._aprilinus_(AJ003787)    ---------- ---------- ---------- ---------- ----------
gb9_-_C._luridus_(AJ003794)      ---------- ---------- ---------- ---------- ----------
gb9_-_C._pallidivittatus_(AJ00   ---------- ---------- ---------- ---------- ----------
gb9_-_C._thummi_piger_(AJ00380   ---------- ---------- ---------- ---------- ----------
gb9_-_C._cingulatus_(AJ003788)   ---------- ---------- ---------- ---------- ----------
gb9_-_C._oppositus_(AJ003800)    ---------- ---------- ---------- ---------- ----------
gb9_-_C._duplex_(AJ003790)       ---------- ---------- ---------- ---------- ----------
gb9_-_C._jacksoni_(AJ003793)     ---------- ---------- ---------- ---------- ----------


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                         260        270        280        290        300
salinarius_(n-2_/_HA_RA_/_26)    TTATTTACAG ATTGTTGCCC TTATGGGTAG TTCTGCTAAC ATGCCTGCAA
salinarius_(n-2_/_RA_RM_/_25)    TTATTTACAG ATTGTTGCCC TTATGGGTAG TTCTGCTAAC ATGCCTGCAA
plumosus_(n-4_/_MC_OP_TI_/_18)   TTTCAAATAG ATTGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCTGCAA
thummi_(n-3_/_KA_/_14)           GATGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
thummi_(n-1_/_RA_/_16)           GGTGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
thummi_(n-1_/_PI_/_17)_|_C._an   GGTGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
thummi_(n-1_/_OS_/_15)           GGTGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
```

```
thummi_(n-1_/_RA_/_28)           GRTGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
thummi_(n-1_/_AR_/_29)           GGTGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
thummi_(n-1_/_AR_/_30)           GRTGAGTGAG ATTGTTGCTC TTATGGGCAA TTCTGCTAAC ATGCCAGCAA
C._annularius_(AJ003784)         GGTTTCTGAG ATTGTTGCTC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-1_/_P   GGTGAGTGAG ATSGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumousus_(n-10_/   GGTGAGTGAG ATCGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-2_/_K   GGTGAGTGAG ATCGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-1_/_O   GGTGAGTGAG ATSGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-7_/_D   GGTGAGTGAG ATTGTTGCCC TTATGGGTAA TGCTGCCAAC ATGCCAGCAA
salinarius_(n-2_/_VA_/_23)_|_C   -GTGAGTGAG ATTGTTGCAC TTATGGGTAA TTCTGCCAAC ATGCCAGCAA
salinarius_(n-2_/_SJ_/_22)       -GTGAGTGAG ATTGTTGCAC TTATGGGTAA TTCTGCCAAC ATGCCAGCAA
plumosus_(n-6_/_AR_/_31)         -GTATCTGAG GTCATCGGAC TTATGGGAAA TGCTGCTAAC ATGCCTGCAA
C._entis_(40)                    GGTGAGTGAG ATTGTTGCCC TTATGGGTAA TGCTGCCAAC ATGCCAGCAA
C._entis_(39)                    gGTGAGTGAG ATTGTTGCCC TTATGGGTAA TGCTGCCAAC ATGCCAGCAA
C._acidophilus_(42)              TGTCTCAGAA ATTGTTGCTC TTATGGGTAA TGCTGCTAAC ATGCCTGCAA
C._calligraphus_(43)             -GTCTCAGAA ATTGTTGCTC TTATGGGAAA TTCAGCTAAC ATGCCAGCTA
C._sp._u_(41)                    -ATTTCTGAG ATTGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCTGCAA
C._plumosus_(38)                 GGTGAGTGAG ATGGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-4_/_A   GGTGAGTGAG ATSGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-1_/_F   GGTGAGTGAG ATSGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-2_/_P   GGTGAGTGAG ATGGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-1_/_A   GGTGAGTGAG ATGGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-5_/_D   GGTGAGTGAG ATGGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
semireductus/plumosus_(n-1_/_A   GGTGAGTGAG ATSGTTGCCC TTATGGGTAA TTCTGCTAAC ATGCCAGCAA
plumosus_(n-1_/_BD_/_19)         -GTCAGCGAA ATTGTTGCTC TTATGGGCAA YTCTGCTAAC ATGCCAGCCA
plumosus_(n-9_/_KE_/_20)         -ATGTCACAA ATTATTGCTC TTATGGGAAA TGAATCCAAC AGACCAGCCA
bathophilus_(n-3_/_DP_OP_TI_/_   -GTTTCAGAA ATTGTTGCTC TTATGGGAAA TGATGCTAAC ATGCCTGCAA
bathophilus_(n-2_/_AR_/_32)      -GTTTCAGAA ATTGTTGCTC TTATGGGAAA TGATGCTAAC ATGCCTGCAA
bathophilus_(n-1_/_AR_/_33)      -GTTTCAGAA ATTGTTGCTC TTATGGGAAA TGATGCTAAC ATGCCTGCAA
bathophilus_(n-1_/_DP_/_34)      -GTTTCAGAA ATTGTTGCTC TTATGGGAAA TGATGCTAAC ATGCCTGCAA
bathophilus_(n-1_/_OP_/_35)      -GTTTCAGAA ATTGTTGCTC TTATGGGAAA TGATGCTAAC ATGCCTGCAA
bathophilus_(n-2_/_SJ_/_37)      -GTTTCAGAA ATTGTTGCTC TTATGGGAAA TGATGCTAAC ATGCCTGCAA
C._pallidivittatus_(AF110170)    -ATGTCAGAA ATTATTGCTC TTATGGGAAA TGAAGCCAAC AGACCTGCCA
C._pallidivittatus_(AF110169)    -ATGTCAGAA ATTATTGCTC TTATGGGAAA TGAAGCCAAC AGACCAGCCA
C._pallidivittatus_(AF110168)    -ATGTCAGAA ATTATTGCTC TTATGGGAAA TGAAGCCAAC AGACCAGCCA
C._pallidivittatus_(AF110167)    -ATGTCAGAA ATTATTGCTC TTATGGGAAA TGAAGCCAAC AGACCAGCCA
C._dilutus_(AF110173)            -ATGTCACAA ATTATTGCTC TTATGGGAAA TGAATCCAAC AGACCAGCCA
C._dilutus_(AF110174)            -ATGTCACAA ATTATTGCTC TTATGGGAAA TGAATCCAAC AGACCAGCCA
gb7A_-_C._thummi_(AJ003812)      -GTCTCAGAA ATCATTGCCC TCATCGGAAA CGAATCAAAT GCCCCAGCAG
gb7A_-_C._nr._atroviridis_(sp.   -GTCTCAGAA ATCATTGCCC TCGTCGGAAA CGAATCAAAT GCTCCAGCTG
gb7A_-_C._melanotus_(AJ003797)   -GTTTCAGAA ATCATTGCCC TCATCGGAAA TGAAGCCAAC GCCCCAGCTG
gb9_-_C._tentans_(AJ003809)      -ATCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAAGCTAAC CGCCCAGCCA
gb9_-_C._zealandicus_(AJ003814   -ATCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAAGCTAAT GCCCCAGCCA
gb9_-_C._aprilinus_(AJ003787)    -GTCTCAGAA ATTGTTGCTC TTGTTGGAAA TGAAGCAAAC GCTCCAGCTA
gb9_-_C._luridus_(AJ003794)      -GTCTCAGAA ATTGTTGCTC TTGTTGGAAA TGAATCAAAC GCTCCAGCCA
gb9_-_C._pallidivittatus_(AJ00   -GTCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAAGCTAAC CGCCCAGCCA
gb9_-_C._thummi_piger_(AJ00380   -ATCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAATCAAAC GCCCCAGCCA
gb9_-_C._cingulatus_(AJ003788)   -GTTTCAGAA ATTGTTGCTC TTGTTGGAAA TGAAGCCAAC GCCCCAGCCA
gb9_-_C._oppositus_(AJ003800)    -ATCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAAGCTAAT GCCCCAGCTA
gb9_-_C._duplex_(AJ003790)       -ATCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAAGCTAAT GCCCCAGCCA
gb9_-_C._jacksoni_(AJ003793)     -ATCTCAGAA ATTGTTGCTC TTGTTGGAAA CGAAGCTAAT GCCCCAGCTA


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                        310        320        330        340        350
salinarius_(n-2_/_HA_RA_/_26)    TGGAGTCATT GATTAAAGAA ATGGCCGGCA ACCACAAAGC TCGTGGAATT
salinarius_(n-2_/_RA_RM_/_25)    TGGAGTCATT GATTAAAGAA ATGGCCGGCA ACCACAAAGC TCGTGGAATT
plumosus_(n-4_/_MC_OP_TI_/_18)   TGGAGTCATT AATTAAAGAA ATGGCCGGCA ACCACAAGGC CCGTGGAATC
thummi_(n-3_/_KA_/_14)           TGGAGACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
thummi_(n-1_/_RA_/_16)           TGGAAACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
thummi_(n-1_/_PI_/_17)_|_C._an   TGGAAACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
thummi_(n-1_/_OS_/_15)           TGGAAACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
thummi_(n-1_/_RA_/_28)           TGGARACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
thummi_(n-1_/_AR_/_29)           TGGAAACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
thummi_(n-1_/_AR_/_30)           TGGAAACTTT GATCACTGAA ATGGCCGGTA ACCACAAAGC TCGTGGAATC
C._annularius_(AJ003784)         TGGAGACTTT GATCACTGAA ATGGCTGGTA ATCACAAAAC TCGTGGAATC
semireductus/plumosus_(n-1_/_P   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumousus_(n-10_/   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-2_/_K   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-1_/_O   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-7_/_D   TGGAGACTTT GATCACTGAA ATGGCCGGAA ACCACAAAGC TCGTGGAATC
```

```
salinarius_(n-2_/_VA_/_23)_|_C   TGGAATATTT GATTAAAGAT ATGGCTGGTA ACCATAAAGC TCGTGGAATT
salinarius_(n-2_/_SJ_/_22)       TGGAATATTT GATTAAAGAT ATGGCTGGTA ACCATAAAGC TCGTGGAATT
plumosus_(n-6_/_AR_/_31)         TGGAAACTCT CATCAAGGAC ATGGCCAGCA ACCACAAATC TCGTGGAATT
C._entis_(40)                    TGGAGACTTT GATCACTGAA ATGGCCGGAA ACCACAAAGC TCGTGGAATC
C._entis_(39)                    TGGAGACTTT GATCACTGAA ATGGCCGGAA ACCACAAAGC TCGTGGAATC
C._acidophilus_(42)              TGGAAACACT CATCAAGGAA ATGGCTGCCA ACCACAAAGC TCGTGGAATT
C._calligraphus_(43)             TGGAAACACT CGTCAACGAA ATGGCTGCTA ACCACAAAGC TCGTGGAATC
C._sp._u_(41)                    TGGAGACATT AATCAACGAA ATGGCCAGCA ACCACAAAAC TCGTGGAATC
C._plumosus_(38)                 TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-4_/_A   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-1_/_F   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-2_/_P   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-1_/_A   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-5_/_D   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
semireductus/plumosus_(n-1_/_A   TGGAGACTTT GATCACTGAA ATGGCTGGAA ACCACAAAGC TCGTGGAATC
plumosus_(n-1_/_BD_/_19)         TGGAAACACT CATCAAGGAA ATGGCTGCTA ACCACAAAGC TCGTGGCATT
plumosus_(n-9_/_KE_/_20)         TGGAAACACT TATCAACGAA ATGGCTGCCA GCCACAAAGC TCGTAAAATT
bathophilus_(n-3_/_DP_OP_TI_/_   TGACACAACT YATCAAGGAA ATGGCCAACA ACCACAAGGC TCGTGGAATT
bathophilus_(n-2_/_AR_/_32)      TGACACAACT CATCAAGGAA ATGGCCAACA ACCACAAAGC TCGTGGAATT
bathophilus_(n-1_/_AR_/_33)      TGACACAACT CATCAAGGAA ATGGCCAACA ACCACAAAGC TCGTGGAATT
bathophilus_(n-1_/_DP_/_34)      TGACWCAACT YATCAAGGAA ATGGCCAACA ACCACAAAGC TCGTGGAATT
bathophilus_(n-1_/_OP_/_35)      TGACACAACT YATCAAGGAA ATGGCCAACA ACCACAARGC TCGTGGAATT
bathophilus_(n-2_/_SJ_/_37)      TGACACAACT TATCAAGGAA ATGGCCAACA ACCACAAGGC TCGTGGAATT
C._pallidivittatus_(AF110170)    TGAAAACACT TATCAACGAA ATGGCCGCCA ACCACAAAGC TCGTGGAATT
C._pallidivittatus_(AF110169)    TGACAACACT TATCAACCAA ATGGCTGCCA ATCACAAAGC TCGTGGAATT
C._pallidivittatus_(AF110168)    TGACAACACT TATCAACCAA ATGGCTGCCA ATCACAAAGC TCGTGGAATT
C._pallidivittatus_(AF110167)    TGATAACACT TATCAACCAA ATGGCTGCCA ATCACAAAGC TCGTGGAATT
C._dilutus_(AF110173)            TGGAAACACT TATCAACGAA ATGGCTGCCA GCCACAAAGC TCGTAAAATT
C._dilutus_(AF110174)            TGGAAACACT TATCAACGAA ATGGCTGCCA GCCACAAAGC TCGTAAAATT
gb7A_-_C._thummi_(AJ003812)      TTCAAACCTT AGTCGGACAA CTCGCWGCTA GCCACAAGGC ACGTGGAATC
gb7A_-_C._nr._atroviridis_(sp.   TCCAAACCCT CGTCGGACAA CTSGCTGCCA CCCACAARGC CCGTGGAATC
gb7A_-_C._melanotus_(AJ003797)   TCAACACATT GGTCGGACAA CTCGCTGCCA GCCACAAGGG CCGTGGAATC
gb9_-_C._tentans_(AJ003809)      TGAACACCCT TATCAACGAA TTGGCAACTA ACCATCACAA CCGTGGAATC
gb9_-_C._zealandicus_(AJ003814   YRGCTACCCT TGTCAACGAA TTGGCCGCAA CCCATCACGC CCGTGGAATC
gb9_-_C._aprilinus_(AJ003787)    TGTCAACCCT CATCAACGAA TTAGCTACAA ACCATCACAA CCGTGGAATC
gb9_-_C._luridus_(AJ003794)      TGGCTACCCT TATCAACGAA TTAGCCACAA GCCATCACAA CCGTGGAATC
gb9_-_C._pallidivittatus_(AJ00   TGAACACCCT TATCAACGAA TTGGCTACAA ACCATCACAA CCGTGGAATC
gb9_-_C._thummi_piger_(AJ00380   TGGCTACCCT CATCAATGAA TTATCAACAA GCCATCACAA CCGTGGAATC
gb9_-_C._cingulatus_(AJ003788)   TGGCTACTTT AATCAACGAA TTGGCCACAA ACCATCACAA CCGTGGAATC
gb9_-_C._oppositus_(AJ003800)    YGGCTACCCT TGTCAACGAA TTGGCCGCTA SCCATCACAA CCGTGGAATC
gb9_-_C._duplex_(AJ003790)       CAGCTACTCT TGTCAACGAA TTGGCCGCAA GCCATCACAA CCGTGGAATC
gb9_-_C._jacksoni_(AJ003793)     TGGCTACCCT TGTCAACGAA TTGGCCGCTA GCCATCACAA CCGTGGAATC


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                         360        370        380        390        400
salinarius_(n-2_/_HA_RA_/_26)    CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCA ATTATTTAAA
salinarius_(n-2_/_RA_RM_/_25)    CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCA ATTATTTAAA
plumosus_(n-4_/_MC_OP_TI_/_18)   CCAAAAGCTC AATTCAATGA ATTCCGTGCC TCACTCGTTG CTTATTTAAA
thummi_(n-3_/_KA_/_14)           ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
thummi_(n-1_/_RA_/_16)           ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
thummi_(n-1_/_PI_/_17)_|_C._an   ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
thummi_(n-1_/_OS_/_15)           ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
thummi_(n-1_/_RA_/_28)           ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
thummi_(n-1_/_AR_/_29)           ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
thummi_(n-1_/_AR_/_30)           ACAAAGGGAC AATTTAATGA GTTCCGAGCT TCCTTAGTTT CTTACCTCCA
C._annularius_(AJ003784)         ACAAAGGGAC AGTTCAATGA GTTCCGTGCC TCATTAGTAT CTTATCTCAA
semireductus/plumosus_(n-1_/_P   ACAAAGGCAC AATTTAATGA ATTCCGTRCC TCATTAGTTG CTTATTTACA
semireductus/plumousus_(n-10_/   ACAAAGGCAC AATTTAATGA ATTCCGTGCC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-2_/_K   ACAAAGGCAC AATTTAATGA ATTCCGTGCC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-1_/_O   ACAAAGGCAC AATTTAATGA ATTCCGTGCC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-7_/_D   CCAAAGGAAC AATTTAACGA GTTCCGTGCC TCACTAGTAT CTTATCTTCA
salinarius_(n-2_/_VA_/_23)_|_C   CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CTTATCTTTC
salinarius_(n-2_/_SJ_/_22)       CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CTTATCTTTC
plumosus_(n-6_/_AR_/_31)         CCAAAGACTC AATTCAACGA ATTCCGTGCC TCACTTGTCT CATATTTGAA
C._entis_(40)                    CCAAAGGAAC AATTTAACGA GTTCCGTGCC TCACTAGTAT CTTATCTTCA
C._entis_(39)                    CCAAAGGAAC AATTTAACGA GTTCCGTGCC TCACTAGTAT CTTATCTTCA
C._acidophilus_(42)              CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTTGTTG ACTACATCAA
C._calligraphus_(43)             CCAAAAGCCC AATTCAATGA ATTCCGTGCC TCACTCGTCT CATACCTCCA
C._sp._u_(41)                    ACAAAGGGAC AATTCAACGA ATTCCGTGCC TCACTCGTTT CTTATCTCAA
C._plumosus_(38)                 ACAAAGGCAC AATTTAATGA GTTCCGTACC TCATTAGTTG CTTATTTACA
```

```
semireductus/plumosus_(n-4_/_A   ACAAAGGCAC AATTTAATGA ATTCCGTGCC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-1_/_F   ACAAAGGCAC AATTTAATGA ATTCCGTRCC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-2_/_P   ACAAAGGCAC AATTTAATGA GTTCCGTACC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-1_/_A   ACAAAGGCAC AATTTAATGA GTTCCGTACC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-5_/_D   ACAAAGGCAC AATTTAATGA GTTCCGTACC TCATTAGTTG CTTATTTACA
semireductus/plumosus_(n-1_/_A   ACAAAGGCAC AATTTAATGA GTTCCGTACC TCATTAGTTG CTTATTTACA
plumosus_(n-1_/_BD_/_19)         CCAAAGGCTC AATTCAACGA ATTCCGTNCC TCACTCGTCT CATACCTCCA
plumosus_(n-9_/_KE_/_20)         CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
bathophilus_(n-3_/_DP_OP_TI_/_   CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CATACCTCAA
bathophilus_(n-2_/_AR_/_32)      CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CATACCTCAA
bathophilus_(n-1_/_AR_/_33)      CCAAAGGCTC AATTCAACGA ATTCCGTGCM TCACTYGTCT CATACCTCAA
bathophilus_(n-1_/_DP_/_34)      CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CATACCTCAA
bathophilus_(n-1_/_OP_/_35)      CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CATACCTCAA
bathophilus_(n-2_/_SJ_/_37)      CCAAAGGCTC AATTCAACGA ATTCCGTGCC TCACTCGTCT CATACCTCAA
C._pallidivittatus_(AF110170)    CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
C._pallidivittatus_(AF110169)    CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
C._pallidivittatus_(AF110168)    CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
C._pallidivittatus_(AF110167)    CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
C._dilutus_(AF110173)            CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
C._dilutus_(AF110174)            CCACAGGCTC AATTCAACGA ATTCCGTGCT TCACTCGTCT CATACCTCCA
gb7A_-_C._thummi_(AJ003812)      TCACAAGCTC AATTCAATGA ATTCCGTGCT GGACTTGTCT CATACGTCTC
gb7A_-_C._nr._atroviridis_(sp.   TCACAAGCTC AATTCAATGA ATTCCGTGCC TCACTYGTCT CATACCTCCA
gb7A_-_C._melanotus_(AJ003797)   CAACAAGCTC AATTTAATGA ATTCAGAACT GCCTTAGTTG CWTATTTACM
gb9_-_C._tentans_(AJ003809)      TCAAAGGCTC AATTCAACGA ATTCCGTGCC TCAATGACCT CATACCTTTC
gb9_-_C._zealandicus_(AJ003814   TCACAAGCTC AATTCAACGA ATTCCGTGGA TCACTCGTCA CATACCTCTC
gb9_-_C._aprilinus_(AJ003787)    ACAAAGGGAC AATTCAACGA ATTCCGTTCA TCACTCGAAT CATACCTTGC
gb9_-_C._luridus_(AJ003794)      ACCAAGGGAC AATTCAACGA ATTCCGTTCA TCACTCGAAT CATACCTCTC
gb9_-_C._pallidivittatus_(AJ00   TCAAAGGCTC AATTCAACGA ATTCCGTGCC TCATTGACCT CATACCTCGC
gb9_-_C._thummi_piger_(AJ00380   ACAAAGGGAC AATTCAATGA ATTCCGTTCA TCACTCGTCT CATACCTCTC
gb9_-_C._cingulatus_(AJ003788)   TCAAAGGCTC AATTCAATGA ATTCCGTGCC TCATTAACAT CTTACCTCCA
gb9_-_C._oppositus_(AJ003800)    TCACAGGCTC AATTCAACGA ATTCCGTGGA TCACTCGTCA CATACCTCTC
gb9_-_C._duplex_(AJ003790)       TCAAAGGATC AGTTCAACGA ATTCCGTGGA TCACTCGTCA CATACCTTTC
gb9_-_C._jacksoni_(AJ003793)     TCACAAGMTC AATTCAACGA ATTCCGTGGA TCACTCGTCA CATACCTCTC


                                 ....|....| ....|....| ....|....| ....|....| ....|....|
                                        410        420        430        440        450
salinarius_(n-2_/_HA_RA_/_26)    AAACCACGCC TCTGGATGGA ACGATGCCAC AGCTGATTCA TGGACTCATG
salinarius_(n-2_/_RA_RM_/_25)    AAACCACGCC TCTGGATGGA ACGATGCCAC AGCTGATTCA TGGACWCATG
plumosus_(n-4_/_MC_OP_TI_/_18)   AGAAAACGT- --GTCATGGA ACGATAATCT CGGCACGGCC TGGACACAAG
thummi_(n-3_/_KA_/_14)           AGCTAATGT- --AGCTTTTG GCGACAATGT TGCTGCTGCC TGGACACAAG
thummi_(n-1_/_RA_/_16)           AGCGAATGT- --TGCTTGGA CAGATAGCCT CGGAGCTGCC TGGACACAAG
thummi_(n-1_/_PI_/_17)_|_C._an   AGCGAATGT- --TGCTTGGT CAGATAGCCT CGGGGCTGCC TGGACACAAG
thummi_(n-1_/_OS_/_15)           AGCGAATGT- --TGCTTGGA CAGATAGCCT CGGAGCTGCC TGGACACAAG
thummi_(n-1_/_RA_/_28)           AGCKAATGT- --WGCTTGKR SMGAYARYST YGSAGCTGCC TGGACACAAG
thummi_(n-1_/_AR_/_29)           AGCKAATGT- --WGCTTGKR SMGAYARYST YGSAGCTGCC TGGACACAAG
thummi_(n-1_/_AR_/_30)           AGCKAATGT- --WGCTTGKR SMGAYARYST YGSAGCTGCC TGGACACAAG
C._annularius_(AJ003784)         AGAAAATGT- --AGCCTGGA GTGATAGCCT CGGAGCTGCC TGGACACAAG
semireductus/plumosus_(n-1_/_P   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumousus_(n-10_/   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-2_/_K   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-1_/_O   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-7_/_D   AGCACATGT- --GACATGGA CCGATAGCCT TGGAGCTGCC TGGACACAAG
salinarius_(n-2_/_VA_/_23)_|_C   AAGCCACGCT CAAAATTGGA ATGATGTCAC AGCTGATGCC TGGACACAAG
salinarius_(n-2_/_SJ_/_22)       AAGCCACGCT CAAAATTGGA ATGATGTCAC AGCTGATGCC TGGACACAAG
plumosus_(n-6_/_AR_/_31)         GGAGCATAC- --AAGCTTTG ATGATGCCAC AGCTTCTGCC TGGACACAAG
C._entis_(40)                    AGCACATGT- --GACATGGA CCGATAGCCT TGGAGCTGCC TGGACACAAG
C._entis_(39)                    AGCACATGT- --GGCATGGA GCGATAGCCT TGGAGCTGCC TGGAAACAAG
C._acidophilus_(42)              AGCTAATGT- --TTCATGGA ACGATAACCT CGGAGATGCC TGGACACAAG
C._calligraphus_(43)             AAACCACGT- --TTCATGGA ATGATAACCT TGGAGCTGCC TGGACACAAG
C._sp._u_(41)                    AAAAAACGT- --TTCATGGA ACGATAACCT CGGAGCTGCC TGGACACAAG
C._plumosus_(38)                 AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-4_/_A   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-1_/_F   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-2_/_P   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-1_/_A   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-5_/_D   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
semireductus/plumosus_(n-1_/_A   AGCGAATAC- --CGAATTTT CATCAACCAC AGCTGCTGCC TGGACACAAG
plumosus_(n-1_/_BD_/_19)         AAGCCACGT- --TTCATGGA ACGATAACCT CGGAGCTGCC TGGACACAAG
plumosus_(n-9_/_KE_/_20)         AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTGCC TGGACACAAG
bathophilus_(n-3_/_DP_OP_TI_/_   GACACATAC- --AAGTTTCG ATGGACCAAC TGAAGCCGCC TGGACACAAG
```

```
bathophilus_(n-2_/_AR_/_32)      GACACATAC- --AAGTTTCG ATGGAGCAAC TGAAGCCGCC TGGACACAAG
bathophilus_(n-1_/_AR_/_33)      GACACATAC- --AAGTTTCG ATGGASCAAC TGAAGCCGCC TGGACACAAG
bathophilus_(n-1_/_DP_/_34)      GACACATAC- --AAGTTTCG ATGGACCAAC TGAAGCCGCC TGGACACAAG
bathophilus_(n-1_/_OP_/_35)      GACACATAC- --AAGTTTCG ATGGACCAAC TGAAGCCGCC TGGACACAAG
bathophilus_(n-2_/_SJ_/_37)      GACACATAC- --AAGTTTCG ATGGACCAAC TGAAGCCGCC TGGACACAAG
C._pallidivittatus_(AF110170)    AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTNNN NNNNNNNNNN
C._pallidivittatus_(AF110169)    AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTNNN NNNNNNNNNN
C._pallidivittatus_(AF110168)    AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTNNN NNNNNNNNNN
C._pallidivittatus_(AF110167)    AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTNNN NNNNNNNNNN
C._dilutus_(AF110173)            AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTNNN NNNNNNNNNN
C._dilutus_(AF110174)            AAGCCACGT- --TACATGGA ACGATAAACT CGGAGCTNNN NNNNNNNNNN
gb7A_-_C._thummi_(AJ003812)      AAGCAATGT- --TGCATGGA ACGCTGCTGC TGAATCAGCA TGGACTGCTN
gb7A_-_C._nr._atroviridis_(sp.   AgCTAATGT- --TGCATGGA GYGACGCTAC AGGTGCTGCC TGGACACAAG
gb7A_-_C._melanotus_(AJ003797)   ATCGAATTT- --AGATTGGT CAGATASCCT CGGAGCTGCC TGGACTGCTN
gb9_-_C._tentans_(AJ003809)      ACACCATAC- --TACATGGA ATGACGCCAC AGCCGCTGCC TGGACTCATG
gb9_-_C._zealandicus_(AJ003814   ATCCCACTC- --ATCATGGA ATGATGCCAC CGCTGCTGCC TGGACCCAAG
gb9_-_C._aprilinus_(AJ003787)    AGCCCACAG- --CACATGGA ATGATGCCAC AGCTGCTGCC TGGACCCATG
gb9_-_C._luridus_(AJ003794)      AAGCCACAG- --CTCATGGA ATGATGCCAC AGCTGCTGCC TGGACCCATG
gb9_-_C._pallidivittatus_(AJ00   CAGCCACGC- --TACATGGA ATGATGCCAC AGCTGCTGCC TGGACCCATG
gb9_-_C._thummi_piger_(AJ00380   AAGCCACGC- --TTCATGGA ATGATGCCAC CGCTGATGCC TGGACCCATG
gb9_-_C._cingulatus_(AJ003788)   AACTCATGC- --TGCTTGGT CAGACGCCAC CGCTGATGCC TGGACCCATG
gb9_-_C._oppositus_(AJ003800)    AAGCCACGC- --CTCATGGA ATGATGCCAC AGCTGCTGCC TGGACCGCCG
gb9_-_C._duplex_(AJ003790)       ACACCACGC- --TACATGGA ATGATGCCAC AGCTGCTGCC TGGACCGCCG
gb9_-_C._jacksoni_(AJ003793)     AAGCCACGC- --TTCATGGA ATGATGCCAC AGCTGCTGCC TGGACCGCCG


                                 ...

salinarius_(n-2_/_HA_RA_/_26)    GTT
salinarius_(n-2_/_RA_RM_/_25)    GTT
plumosus_(n-4_/_MC_OP_TI_/_18)   GTC
thummi_(n-3_/_KA_/_14)           GTC
thummi_(n-1_/_RA_/_16)           GTC
thummi_(n-1_/_PI_/_17)_|_C._an   GTC
thummi_(n-1_/_OS_/_15)           GTC
thummi_(n-1_/_RA_/_28)           GTC
thummi_(n-1_/_AR_/_29)           GTC
thummi_(n-1_/_AR_/_30)           GTC
C._annularius_(AJ003784)         GTC
semireductus/plumosus_(n-1_/_P   GTC
semireductus/plumousus_(n-10_/   GTC
semireductus/plumosus_(n-2_/_K   GTC
semireductus/plumosus_(n-1_/_O   GTC
semireductus/plumosus_(n-7_/_D   GTC
salinarius_(n-2_/_VA_/_23)_|_C   GTC
salinarius_(n-2_/_SJ_/_22)       GTC
plumosus_(n-6_/_AR_/_31)         GTC
C._entis_(40)                    GTC
C._entis_(39)                    GTC
C._acidophilus_(42)              GTC
C._calligraphus_(43)             GAC
C._sp._u_(41)                    GTC
C._plumosus_(38)                 GTC
semireductus/plumosus_(n-4_/_A   GTC
semireductus/plumosus_(n-1_/_F   GTC
semireductus/plumosus_(n-2_/_P   GTC
semireductus/plumosus_(n-1_/_A   GTC
semireductus/plumosus_(n-5_/_D   GTC
semireductus/plumosus_(n-1_/_A   GTC
plumosus_(n-1_/_BD_/_19)         GTC
plumosus_(n-9_/_KE_/_20)         GTC
bathophilus_(n-3_/_DP_OP_TI_/_   GTC
bathophilus_(n-2_/_AR_/_32)      GTC
bathophilus_(n-1_/_AR_/_33)      GTC
bathophilus_(n-1_/_DP_/_34)      GTC
bathophilus_(n-1_/_OP_/_35)      GTC
bathophilus_(n-2_/_SJ_/_37)      GTC
C._pallidivittatus_(AF110170)    NNN
C._pallidivittatus_(AF110169)    NNN
C._pallidivittatus_(AF110168)    NNN
C._pallidivittatus_(AF110167)    NNN
```

```
C._dilutus_(AF110173)            NNN
C._dilutus_(AF110174)            NNN
gb7A_-_C._thummi_(AJ003812)      NNN
gb7A_-_C._nr._atroviridis_(sp.   GTC
gb7A_-_C._melanotus_(AJ003797)   NNN
gb9_-_C._tentans_(AJ003809)      GTC
gb9_-_C._zealandicus_(AJ003814   GTC
gb9_-_C._aprilinus_(AJ003787)    GTC
gb9_-_C._luridus_(AJ003794)      GTC
gb9_-_C._pallidivittatus_(AJ00   GTC
gb9_-_C._thummi_piger_(AJ00380   GAC
gb9_-_C._cingulatus_(AJ003788)   GAC
gb9_-_C._oppositus_(AJ003800)    GTC
gb9_-_C._duplex_(AJ003790)       GTC
gb9_-_C._jacksoni_(AJ003793)     GTC
```

p. 159 Certaines modifications ou ajouts d'information devraient être apportées au tableau S2. Celles-ci sont soulignées en rouge.

Table S2. List of reference *Chironomus* species sequenced.

***Chironomus* species**	**Location**	**Collector (year specimen was colleted)**	**Identified by and how?**	**GenBank Accession #**		**Voucher number**
				cox1	***gb2B***	
C. (*Chaetolabis*) nr. *atroviridis* (sp.2i)	White Lake, Three Mile Bay, ON, Canada	Don R. Oliver	Were originally identified from reared adults by D.R. Oliver in 1966. Identification of the individual larvae was later confirmed by Jon Martin through cytology.	KF278342		Ch.sp2i15m
C. (*Chaetolabis*) nr. *atroviridis* (sp.2i)	White Lake, Three Mile Bay, ON, Canada	Don R. Oliver		KF278360		Chaet.2i16m
C. (*Chaetolabis*) nr. *atroviridis* (sp.2i)	White Lake, Three Mile Bay, ON, Canada	Don R. Oliver			KF278450	DRO.14.6 16M
C. (*Chaetolabis*) *ochreatus*	Little John Jr. Lake, WI, United States	Jon Martin	Was originally identified from reared adults by J.E. Sublette in 1978. The larva was later identified through cytology by Jon Martin.	KF278351		Ch.ochr13F
C. (*Chironomus*) *acidophilus*	Potters Marsh, Anchorage Co., AK, United States	Dave Wartenbee	Was originally identified from reared adults by I.I. Kiknadze (~2006) and then through cytology by Jon Martin.	KF278358		UAK.1.14F or acidUAK1*
C. (*Chironomus*) *anthracinus*	Lake Esrom, Denmark	Henk Vallendduuk	Identified by Claus Lindegaard.	KF278343		ES(DAN)95-BA3
C. (*Chironomus*) *bifurcatus*	Arboretum, Madison, Dane Co., WI, United States	Jon Martin	All larvae were identified by Jon Martin through cytological comparison to material identified by W.F. Wülker.	KF278345		AAW4003*
C. (*Chironomus*) *bifurcatus*	Lake Pleasant, Franklin Co., MA, United States	Sean Werle (2002)		KF278361		bifMa21
C. (*Chironomus*) *bifurcatus*	Arboretum, Madison, Dane Co., WI, United States	Jon Martin (1978)		KF278353		bifMad7
C. (*Chironomus*) *calligraphus*	Gainsville, Alachua Co., FL, United States	Pauline O. Lawrence	Identified cytologically by Jon Martin in 2010.	KF278357	KF278449	ABZ9507* or UFL.2.1 male4.1
C. (*Chironomus*) *dilutus*	Stevens Pond, Madison, Dane Co., WI, United States	Barry T.O. Lee	Identified morphologically and cytologically by Jon Martin.	KF278359		Stevens Pond_Madison WI (Eastern)
C. (*Chironomus*) *entis*	Saginaw Bay, Lake Michigan, MI, United States	Michael H. Winnell (2012)	Identified cytologically by Jon Martin. The identification was later confirmed by *gb2β* DNA sequence.	KF278355	KF278446	C.entisMI22 or UM1.3.1 22
C. (*Chironomus*) *entis*	Brewer Lake, Cass Co., ND, United States	Malcolm G. Butler (1994)			KF278445	UND.2.1 2
C. (*Chironomus*) *harpi*	Bradleys Acid Pit, Jackson Co., IL, United States	Ken D. Yamamoto (1977 as part of material designated as paratypes)	Identification confired cytologically by Jon Martin.	KF278346		AAJ4275*
C. (*Chironomus*) *plumosus*	Saginaw Bay, Lake Michigan, MI, United States	Michael H. Winnell	Identified cytologically by Jon Martin. The identification was later confirmed by *gb2β* DNA sequence.	KF278354	KF278444	C.plumMI21 or UM1.3.1 21

***Chironomus* species**	**Location**	**Collector (year specimen was colleted)**	**Identified by and how?**	**GenBank Accession #**		**Voucher number**
				cox1	***gb2B***	
C. (*Chironomus*) *quinnitukqut*	Truro, Cape Cod, Barnstable Co., MA, United States	Jon Martin (2004)	Identified thourgh cytology by Jon Martin.	KF278347		AAB7030*
C. sp. g	Lake Bat, Algonquin Park, ON, Canada (coll.	Jon Martin (1966)	Adults considered unknown species by J.E. Sublette, and larvae cytologically distinct from other known cytological material.	KF278348		C.spgBatLk
C. sp. h	Lake Bat, Algonquin Park, ON, Canada	Jon Martin (1966)		KF278349		C.sphBatLk
C. sp. u	Calgary, AL, Canada	Jon Martin (1970)	Adults identified by J.E. Sublette as close to *C. hyperboreus*, but cytologically distinct.		KF278447	CAL.2.4 egg mass #3, 3.2f
C. (*Chironomus*) *staegeri*	Lake Pleasant, Franklin Co., MA, United States	S. Werle (2003)	Identified by Jon Martin by cytology.	KF278356		AAW3999*
C. (*Chironomus*) '*tigris*'	Turtle Lake, Becker Co., MN, United States	Malcolm G. Butler (1993)	Adults identified by association to cytologically determined larvae. The identification was probably done by I.I. Kiknadze, but could have been by M.G. Butler himself since the chromosomes of this species are so readily identified.	KF278350		C.tigris_TurtleLk_MN_USA
C. (*Chironomus*) *anthracinus*-gr.	Marion Lake, Garibaldi Prov.Pk., BC, Canada	Andrew L. Hamilton (~1965)	Identified as *C. anthracinus* by A.L. Hamilton in his studies, but he was unaware of the existence of other morphologically identical species.	KF278344		CBC.1.1 14f(1)

*Published in BOLD (BIN #)

ARTICLE 3

Differences in feeding behaviour among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae

Titre courant : Interspecific differences in *Chironomus* feeding behaviour

Isabelle Proulx et Landis Hare

Institut national de la recherche scientifique – Centre Eau Terre Environnement, 490 rue de la Couronne, Université du Québec, 490 rue de la Couronne, Québec (Québec), G1K 9A9, Canada

Publié en 2014 dans *Freshwater Biology* 59(1) : 73-86.

SUMMARY

1. We set out to determine the feeding behaviours of 15 *Chironomus* species collected from 16 boreal-forest lakes by measuring cadmium (Cd) and stable isotopes of carbon (C), nitrogen (N) and sulphur (S) in larvae.
2. Measurements of S stable isotopes and Cd discriminated between *Chironomus* species that feed mainly on oxic particles from those that feed mainly on anoxic particles. Our results suggest that *C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri* feed mainly on oxic particles (in the water column, in sediment or both), whereas *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C*. spp. NAI-NAIII and *C. 'tigris'* feed mainly on anoxic sediments.
3. In our study lakes, larval C isotopic signatures were relatively high, which suggests that they feed little on methanotrophic bacteria. Although larval C and N signatures differed between some sympatric *Chironomus* species, these differences were not consistent among lakes. The absence of a trend among lakes suggests that lake-specific factors determine larval C and N signatures.
4. Differences in feeding habits and Cd concentrations among sympatric (cohabiting) *Chironomus* species suggest that pooling them in ecological, paleolimnological or ecotoxicological studies could obscure trends in nature, thereby limiting their use as monitors of climate change or pollution.

INTRODUCTION

In the muddy bottoms of lakes and rivers, larvae of the insect genus *Chironomus* (Diptera, Chironomidae) often represent a major portion of the invertebrate biomass and as such can be an important component of ecological (Jónasson, 1972), environmental (Mousavi, Primicerio & Amundsen, 2003) and paleolimnological (Brooks, Langdon & Heiri, 2007) studies in this milieu. The genus *Chironomus* comprises several hundred known species (Ashe & Cranston, 1990; Martin, 2013; Ryser, Wuelker & Scholl, 1985) and more than one species is usually present at a given site (Jónasson, 1972; Proulx *et al*., 2013). Because larvae of *Chironomus* species are hard to separate morphologically, they are often pooled together either at the genus level (Nyman, Korhola & Brooks, 2005) or in species groups based on the presence and form of their abdominal tubules (Shobanov, Shilova & Belyanina, 1996) or the shape of their mouth parts (Brooks *et al*., 2007). The disadvantage of doing so is that if species differ in their behaviour and ecology, then grouping them is likely to obscure or confound patterns in nature. For example, sympatric *Chironomus* species are reported to vary by an order of magnitude in their cadmium (Cd) concentrations (Martin, Proulx & Hare, 2008); grouping them could negate their usefulness as biomonitors of Cd exposure. Likewise, grouping *Chironomus* species with other chironomid genera would greatly reduce their effectiveness as water quality indicators (Sæther, 1979).

Ecological differences among *Chironomus* species can be ascribed in part to their feeding habits. Thus some *Chironomus* species filter particles from water overlying sediments (Walshe, 1951), whereas others consume deposited sediment particles (Jónasson, 1972). Larvae feeding on deposited sediment can consume either oxic particles or anoxic sediments from below the oxic zone (Martin *et al*., 2008) (Fig. 1). Oxic particles are located either at the interface between the water column and the surface sediment, or in the walls of burrows through which larvae pump oxygenated overlying water (Gallon, Hare & Tessier, 2008) (Fig. 1). The oxygen demands of most soft muds are high, such that oxic sediment particles are only found within a few millimeters of the overlying oxic water column (Wang, Tessier & Hare, 2001).

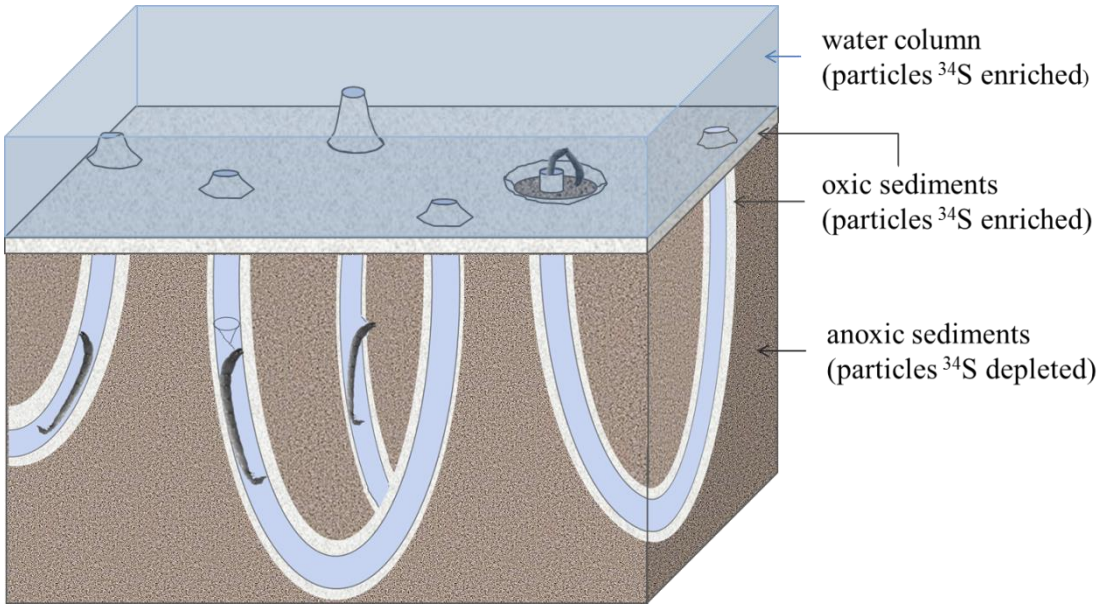


Figure 1. **Hypothetical illustration of *Chironomus* feeding behaviours. From left to right, a *Chironomus* larva feeding on oxic sediments within its tube, filter-feeding, feeding on anoxic sediments within its tube or feeding on anoxic sediments surrounding the mouth of its tube.**

The feeding behaviour of *Chironomus* larvae has been studied by examining gut contents, by observing them in the laboratory (Berg, 1995 and references therein) and by using fatty acid biomarkers (Goedkoop *et al*., 1998). These techniques have been applied to only a small percentage of the known *Chironomus* species, often with inconclusive results (Berg, 1995). Increasingly, stable isotopes of carbon (C), nitrogen (N) and sulphur (S) are used to determine the feeding behaviours of *Chironomus* larvae, as described in the following studies.

Sulphur isotopic signatures ($\delta^{34}S$) are used to determine whether aquatic animals feed on oxic or anoxic particles. Their use is predicated on the fact that $\delta^{34}S$ values tend to be more negative in anoxic sediments than in overlying oxic particles, such that freshwater (Croisetière *et al*., 2009) and marine (Peterson, Howarth & Garritt, 1985; Peterson, 1999) organisms feeding on anoxic or oxic particles show a corresponding difference in their $\delta^{34}S$ signatures. Indeed, Martin *et al*. (2008) showed that larvae of a *Chironomus* species that fed on oxic sediments (*C. staegeri*) had a more positive $\delta^{34}S$ signature than did a congener (*C. 'tigris'*) that fed on anoxic sediments. One possible explanation for these oxic-anoxic differences in $\delta^{34}S$ values is that sulphate-reducing bacteria discriminate against the heavier S isotope (^{34}S), which results in anoxic sediments that have a more negative $\delta^{34}S$ signature than either oxic sediments or the overlying water column (Croisetière *et al*., 2009). The use of $\delta^{34}S$ signatures to compare feeding behaviours among species is simplified by the fact that, on average, $\delta^{34}S$ signatures tend to be

little influenced by trophic level. Thus McCutchan *et al*. (2003) found that the mean (± SE) difference in $\delta^{34}S$ signatures between a variety of consumers and their prey (+0.5 ± 0.56 ‰) was not significantly different from 0. Furthermore, such differences tended to be smaller for non-predators (McCutchan *et al*., 2003), which would favour the use of $\delta^{34}S$ signatures to study the feeding habits of detritivores such as *Chironomus*.

Carbon isotopic signatures ($\delta^{13}C$) have been used to determine whether an aquatic animal's C comes from aquatic or terrestrial sources (Reynolds, 2008; Jones, 1992; Jansson *et al*., 2007), as well as to discriminate between food webs based on either planktonic or benthic algae (Hecky & Hesslein, 1995; Vander Zanden *et al*., 2006). As with S stable isotopes, there is generally little fractionation of C stable isotopes between a consumer and its diet (+0.5 ± 0.13 ‰; mean ± SE; McCutchan *et al*., 2003). $\delta^{13}C$ signatures have also been used to discriminate between *Chironomus* species feeding on methane-oxidising bacteria and those feeding on other carbon sources (for review, see Jones & Grey, 2011). Those that feed on methane-oxidising bacteria tend to be highly depleted in the heavier isotope (^{13}C) relative to other carbon sources because both methanogenesis (Coplen, Hopple & Böhlke, 2002) and methanotrophy (Summons, Jahnke & Roksandic, 1994; Eller *et al*., 2005; Deines, Bodelier & Eller, 2007a) favour assimilation of the lighter (^{12}C) isotope. Lower $\delta^{13}C$ values have been measured for *C. plumosus* relative to *C. anthracinus*, which suggests that the former species feeds to a greater extent on methanotrophic biomass than does the latter (Grey, Kelly & Jones, 2004a; Kelly, Jones & Grey, 2004).

Stable N isotopic signatures ($\delta^{15}N$) are used to indicate trophic level, since consumers are markedly enriched in the heavier isotope (^{15}N) relative to their diet (mean value = 2.3 ± 0.18‰) (McCutchan *et al*., 2003). Although larvae of *Chironomus* species are all primary consumers (detritivores, filter feeders) their $\delta^{15}N$ signatures can vary widely due to differences in the $\delta^{15}N$ signatures of their food sources (Kelly *et al*., 2004; Grey *et al*., 2004a; Grey *et al*., 2004b; Jones & Grey, 2004).

Previous studies using stable isotopes to explain differences in feeding behaviours among *Chironomus* species have tended to focus on a single species pair in a given lake. In contrast, we measured stable isotopes in 15 *Chironomus* species collected from 16 lakes. We also measured Cd concentrations in *Chironomus* larvae, since these are determined in part by larval feeding behaviour (Martin *et al*., 2008). The goal of our study was to answer the following questions: (1) in a given lake, do *Chironomus* species differ in their feeding habits (Fig. 1); if so,

(2) are the relative differences among species the same in all lakes; (3) which variables (C, N and S stable isotopes; Cd) show a consistent pattern among lakes; (4) can these variables be used to group *Chironomus* species of like feeding habits; (5) do the values for *Chironomus* species groups show a logical relationship with those measured in sediments and in other invertebrates that are known to feed on either plankton or deep sediments?

METHODS

Collection of samples

Larvae of *Chironomus* species were collected in late spring to early summer (May-July) in lakes located in the mining areas of Rouyn-Noranda (QC) and Sudbury (ON), as well as in Lake St. Joseph located near Quebec City (QC). Most lakes were sampled once only, at a single depth (exceptions are noted in Table 1). At each collection site, depth and pH were measured (Table 1) as well as vertical profiles of dissolved oxygen and temperature (YSI Model 50B, Yellow Springs, OH, U.S.A.; data not shown). Oxygen concentrations 0.5 m above the sediment surface are reported in Table 1. Water samples, filtered in situ using diffusion samplers (Ponton & Hare, 2009), were collected to measure dissolved organic carbon (DOC; Table 1) that was analysed by combustion and transformation into CO_2 (TOC-VCPH, Shimadzu, Columbia, MD, U.S.A.).

Table 1. **Location, year of collection and trophic status of the study lakes, as well as the depth, oxygen concentration and pH at the specific site where *Chironomus* larvae were collected in each lake.**

Lake	Code	Location	Year	Collecting depth (m)	$[O_2]_{water}$ (mg L^{-1})	pH	Trophic status[a]
QUEBEC CITY (QC)							
Lake St. Joseph	SJ	46°53'N, 71°38'W	2006	9.0	11.9	7.1	oligo-mesotrophic[c]
ROUYN-NORANDA (QC)							
Lake Arnoux	AR	48°15'N, 79°20'W	2010	4.0	9.24	3.8	
Lake d'Alembert	DA	48°23'N, 79°01'W	2006	5.0	6.36		eutrophic (2009-2011)[b]
Lake Duprat	DP	48°20'N, 79°07'W	2010	6.5	8.39	7.6	
Lake Marlon	MN	48°16'N, 79°04'W	2010	1.5	8.67	7.7	meso-eutrophic[b]
Lake Opasatica	OP (3.0m) OP (8.5m)	48°10'N, 79°20'W	2009	3.0 8.5		7.5	mesotrophic (2008)[b]
Lake Osisko	OS	48°15'N, 79°00'W	2010	5.5	8.51	8.5	
Lake Pelletier	PE	48°13'N, 79°03'W	2010	5.0	8.74	8.3	meso-eutrophic[b]
SUDBURY (ON)							
Kasten (Bibby) Lake	KA	46°22'N, 80°58'W	2007	7.5		6.8	oligotrophic (2008)[c]
Hannah Lake	HA	46°27'N, 81°02'W	2007	7.0	10.28	7.4	oligotrophic[c]
Kelly Lake	KE	46°27'N, 81°04'W	2010	1.5	9	7.5	eutrophic (2008)[c]
McFarlane Lake	MC	46°25'N, 80°57'W	2007	10.0		7.8	oligo-mesotrophic[c]
Raft Lake	RA	46°25'N, 80°57'W	2010	10.0	8.06	7.3	oligotrophic[c]
Ramsey Lake	RM	46°28'N, 80°57'W	2007	12.0		7.1	mesotrophic[c]
Silver Lake	SI (07) SI (11)	46°26'N, 81°01'W	2007 2011	4.0		5.9 7.0	oligotrophic[c]
Tilton Lake	TI	46°21'N, 81°04'W	2011	4.0		7.1	oligotrophic[c]

[a] Trophic status determined for collection years unless mention otherwise in parentheses
[b] Trophic status inferred from total phosphorus, chlorophyll a and water transparency (Ministère du Développement durable de l'Environnement et des Parcs, 2012)
[c] Trophic status inferred from total phosphorous measurements (City of Greater Sudbury, 2012)

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 to eliminate fine sediment. Five to 20 grab samples were collected at each site depending on larval densities. Larvae were held in lakewater at field temperatures for transport to the laboratory where they were separated into groups according to the morphology of their abdominal tubules and the colouration of their frontoclypeus. Fourth (final) instars were chosen for study as determined from the head-capsule widths of pre-pupal larvae (Proulx *et al*. 2013).

To identify larvae to species, 10 larvae from each group were sacrificed 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 analysis. Morphological determinations were based on the presence and shape of the ventral and lateral tubules, the colouration of the frontoclypeus and the gula, the teeth of the mentum, mandible and pecten epipharyngis as well as the shape of anterior margin of the ventromental plates. For cytological analyses, giant polytene chromosomes were removed from the salivary glands and stained to determine the number of chromosomes, their structural arrangement and their banding patterns. DNA analysis was conducted through DNA barcoding of the *cox1* and *gb2β* genes. For more details on the identification techniques used and the species collected see Proulx *et al*. (2013).

Chironomus larvae to be used for Cd and stable isotope analyses were held at 4 ^{o}C for a mean of 4 (± 1, SE) days in containers filled with water from their collection site to allow them to empty their gut contents. Excess faecal matter was removed daily to prevent coprophagy. Live larvae with empty guts were stored at -20 ^{o}C until analysis.

For comparative purposes, S stable isotopes were also measured in sediment and in other invertebrates collected with the *Chironomus* larvae. Sediment was collected using an Ekman grab, from which the overlying water was allowed to drain passively so as to not disturb the sediment surface. Using a plastic spatula, we removed three samples of surface oxic sediment (uppermost several mm; identifiable by its light, orange-brown, colour in Lake St. Joseph and the Sudbury lakes or by its greenish colour 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. Invertebrates collected with *Chironomus* included the phantom midge *Chaoborus*, the chironomids *Cryptochironomus*, *Glyptotendipes* and *Procladius*, as well as tubificid oligochaetes (Merritt, Cummins & Berg, 2008; Kathman & Brinkhurst, 1998; Fittkau & Roback, 1983; Pinder & Reiss, 1983), all of which were gut-depurated prior to being

frozen at -20 ^{o}C. Bulk plankton was collected in Ramsey Lake by hauling a 164 µm mesh-aperture net horizontally in the water column. All invertebrate, plankton and sediment samples were frozen at -20 ^{o}C until analysis.

Cadmium analyses

To prevent inadvertent contamination, all labware was soaked in 15% (v/v) nitric acid (TraceMetal grade, Fisher Scientific, Toronto, ON, Canada) for at least 24 hours, rinsed 7 times with ultrapure water (milli-Q system water; >18 MΩ cm^{-1}) and dried under a laminar flow hood. A mean of 4 (±2; SD) *Chironomus* larvae were placed on a weighed piece of Teflon sheeting, freeze-dried (FTS Systems), weighed using a microbalance (Sartorius M2P PRO 11) and placed in an acid-washed High Density Polyethylene (HDPE) bottle. Samples were 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, Sigma-Aldrich, Oakville, ON, Canada; 40 µL per mg dry weight); digest volume was completed to 1 mL per mg dry weight using ultra-pure water. 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, U.S.A.) and lobster hepatopancreas (reference material TORT-2, National Research Council, Ottawa, ON, Canada).

Cadmium concentrations were measured by inductively coupled plasma - mass spectrometry (Thermo Electron Corp., model X7, Waltham, MA, U.S.A.) using external calibration standards and rhodium as an internal standard. For quality control, an analytical blank, a certified standard (900Q30, PT89-7, PT 89-9, PT89-10 and PT91-10; Inter-laboratory study, Environment Canada) and a calibration standard were analysed every 10-15 samples. Data were corrected for signal drift as required. Cadmium concentrations in analytical blanks and digestive blanks were below the detection limit and analytical certified standards were within 10% of the certified value. All measured values were above the detection limit. Cadmium concentrations of certified biological reference materials were within the 95% confidence limit of the certified mean.

Stable isotope measurements

Invertebrates and sediments were freeze-dried and ground to a powder. For measurements of S isotopes in biological material, 2 mg of pooled individuals (means ± SD; 20 ± 11 *Chaoborus*, 4 ± 3 *Chironomus*, 9 ± 10 other Chironomidae, or 11 ± 1 Tubificidae) were placed in tin cups (4 x 3.2 mm) along with 4 mg of vanadium pentoxide. For S isotopes in sediments, 10-20 mg of sediment was placed in a tin cup (8 x 5 mm) along with 10 mg of vanadium pentoxide. For measurements of C and N isotopes in biological material, 1 mg of pooled (3 ± 2) individuals was placed alone in each tin cup (4 x 3.2 mm). We did not have enough specimens to measure C and N stable isotopes in *Chironomus* species from Lake Pelletier and Silver Lake as well as in *C. staegeri* from Lake Duprat and Lake Opasatica.

Stable S, C and N signatures are reported as $\delta^{34}S$, $\delta^{13}C$ or $\delta^{15}N$, respectively, where these variables equal $[(R_{sample}/R_{standard})-1]*10^3$, for which R is the ratio of either $^{34}S/^{32}S$, $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively, and the standards used are S from the Canyon Diablo troilite (CDT), C from the Vienna Peedee Belemnite (VPDB) or atmospheric N, respectively. Stable isotopes were measured by Iso-Analytical Ltd. (Cheshire, UK) on an elemental analysis - isotope ratio mass spectrometer (EA-IRMS; Europa Scientific, Cambridge, U.K.). Analytical details are given in Croisetière *et al*. (2009). Differences between the certified values for standards and measured values were less than 0.4‰ for $\delta^{34}S$, 0.2‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$. Differences between duplicate samples (20% of the total number of samples) were 0.4‰ for $\delta^{34}S$, 0.2‰ for $\delta^{13}C$ and 0.1‰ for $\delta^{15}N$.

Statistical analyses

We used statistical tests to compare means of stable isotope signatures or Cd concentrations in biological and sediment samples from a given lake. We did not compare values among lakes because the lakes differed widely in Cd and stable isotope values due to both natural differences (drainage basin characteristics, geographical region, etc.) and anthropogenic alterations (contaminant levels, lake liming, sewage inputs, etc.). We compared pairs of means ($p \leq 0.05$) using either a t-test, when data satisfied the criteria for a parametric test, or the non-parametric Mann-Whitney Rank Sum test when this was not the case. Likewise, when comparing more than two means ($p \leq 0.05$), we used a one-way analysis of variance (ANOVA) followed by the Holm-Sidak method, when data satisfied the criteria for a parametric test, or the

Kruskal Wallis non-parametric test followed by Dunn's test if this was not the case. Single or duplicate samples are shown on graphs, but were not included in statistical tests. For each lake, relationships among $\delta^{13}C$ and $\delta^{15}N$ values measured in pooled *Chironomus* samples were examined in biplots and tested using the Pearson product-moment correlation after verifying data normality (Shapiro-Wilk test). Statistical tests were performed using SigmaPlot 11.0 (Systat Software, San Jose, CA, U.S.A.).

RESULTS

At the time of collection, the water columns at all sampling sites were well mixed and oxygenated (Table 1). Most of the lakes that we studied can be classified as oligotrophic to mesotrophic and circum-neutral (Table 1). From 2 to 5 *Chironomus* species were collected in a given lake (Fig. 2) and a total of 15 *Chironomus* species were found in the 16 study lakes (Table 2). Twelve of these species have been named, whereas 3 remain unidentified (Table 2; Proulx *et al*., 2013).

S stable-isotope signatures in *Chironomus* species (Fig. 2A)

Values of $\delta^{34}S$ varied by up to 14‰ among *Chironomus* species at one sampling location (Lake Opasatica; OP, Fig. 2A). Some *Chironomus* species had consistently higher $\delta^{34}S$ signatures than did others. Thus values for *C. staegeri* (red circles) from seven lakes were significantly higher than those of *C. bifurcatus* (black triangles; SJ, DA, DP, OP (8.5m), MC, TI), *C*. sp. NAIII (grey stars; DA, MC) and *C. 'tigris'* (white circles; OP (8.5m), MC, SI (07), TI) from these same lakes (Fig. 2A). The $\delta^{34}S$ signatures of *C. staegeri* (red circles) were also more positive, although not significantly higher, than those of *C*. sp. NAII in Silver Lake (white star; SI (07)) and *C. cucini* from Lake St. Joseph (black star; SJ). In contrast, $\delta^{34}S$ values in *C. staegeri* (red circles) did not differ significantly from those measured in both *C. entis* from Lake D'Alembert (yellow circle; DA) and *C. plumosus* from Lakes D'Alembert and Duprat (orange circles; DA, DP).

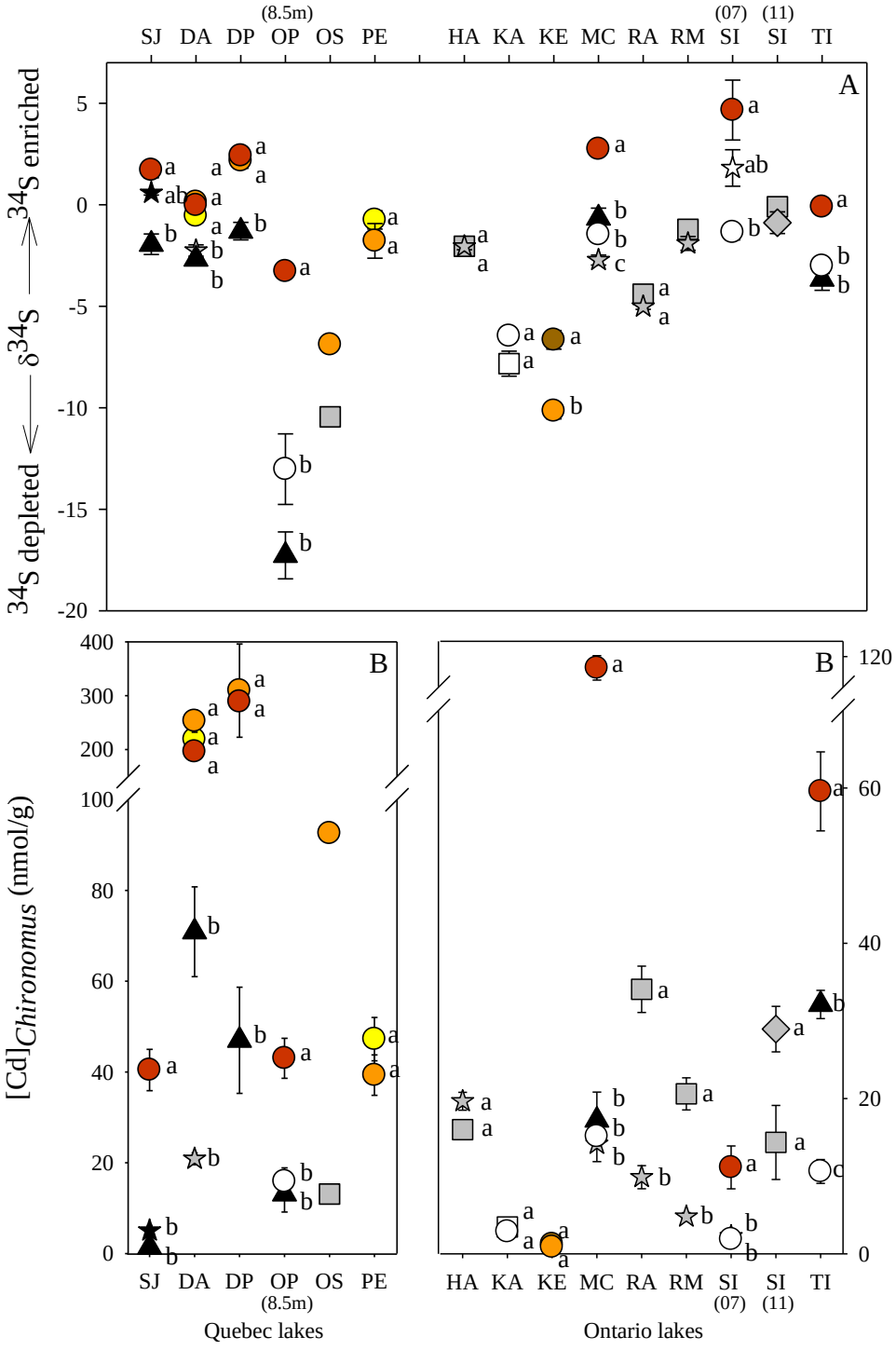


Figure 2. **Mean (±SE; n = 1 or 3-8) (A) δ^{34}S signatures (‰) and (B) Cd concentrations (nmol g^{-1}) in *Chironomus* species (see Table 2 for species names) collected from lakes in eastern Canada (see Table 1 for lake names). *Chironomus* species in Lake Opasatica (OP) were collected at a depth of 8.5 m and *Chironomus* species in Silver Lake (SI) were collected in 2007 and 2011. Note that the vertical scales for Cd concentrations differ between the Ontario and Quebec lakes. For a given lake, values that do not differ significantly (p > 0.05) are followed by the same letter (except for values where n = 1).**

In the five lakes in which *C. plumosus* (orange circles) was collected (Fig. 2A), its $\delta^{34}S$ values were more positive than those of *C. anthracinus* (grey square; OS), *C*. sp. NAIII (grey star; DA) and *C. bifurcatus* (black triangle; DA, DP). However, the $\delta^{34}S$ signatures of *C. plumosus* were not significantly different from those of *C. entis* (yellow circle; DA, PE) or *C. staegeri* (red circle; DA, DP). In Kelly Lake (KE), the mean $\delta^{34}S$ signature of *C. plumosus* (orange circle) was significantly lower than that of *C. dilutus* (brown circle). In Silver Lake (SI (11)), the $\delta^{34}S$ signature of *C. decorus*-group sp. 2 (grey diamond) was not significantly different from that of *C. anthracinus* (grey square), which had a lower $\delta^{34}S$ value than *C. plumosus* (orange circle) in Lake Osisko (OS). In Kasten Lake (KA), the $\delta^{34}S$ value of *C*. sp. NAI (white square) was not significantly different than that of *C. 'tigris'* (white circle), which, in other lakes, always had lower $\delta^{34}S$ values than did *C. staegeri* (red circle).

In light of these trends in $\delta^{34}S$ signatures, we separated our *Chironomus* species into two groups (Table 2): those with more positive S isotopic signatures (coloured circles; *C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri*) and those with more negative S isotopic signatures (grey-scale symbols; *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C*. spp. NAI-NAIII and *C. 'tigris'*).

Table 2. **Feeding zone of *Chironomus* species inferred from $\delta^{34}S$ values and Cd concentrations (see text). ND = not determined. Lake names are given in Table 1.**

***Chironomus* species**	**Symbols in Figs. 1-4**	**Larval type (Shobanov *et al.*, 1996)**	**Relative $\delta^{34}S$ values in *Chironomus* species (lakes where collected)**	**Relative [Cd] in *Chironomus* species (lakes where collected)**	**Inferred feeding zone**
C. entis (Shobanov, 1989)	●	semi-reductus	high (DA,PE)	high (DA, MN, PE)	water column or oxic sediments
C. plumosus (Linnaeus, 1758)	●	semi-reductus to plumosus	high (DA, DP, KE, MN, OS, PE)	high (DA, DP, KE, MN, OS, PE)	water column or oxic sediments
C. dilutus (Shobanov, Kiknadze & Butler, 1999)	●	plumosus	high (KE)	high (KE)	water column or oxic sediments
C. staegeri (Lundbeck, 1898)	●	plumosus	high (DA, DP, MC, OP(8.5m), SI(07), SJ, TI)	high (DA, DP, MC, OP(8.5m), SI(07), SJ, TI)	water column or oxic sediments
C. harpi (Wülker, Sublette & Martin, 1991)	●	plumosus	ND	low (AR)	anoxic sediments
C. 'tigris' (nomen nudum in Martin *et al.* (2008) for *C.* sp. Am1 of Kiknadze *et al.* (1993))	○	plumosus	low (KA, MC, OP(8.5m), SI(07), TI)	low (KA, MC, OP(8.5m), SI(07), TI)	anoxic sediments
***C. decorus*-group sp. 2** (Butler *et al.*, 1995)	◇	bathophilus-fluviatilis (lateral tubules sometimes present)	low (SI(11))	low (OP(3m), SI(11))	anoxic sediments
C. bifurcatus (Wuelker *et al.*, 2009)	▲	bathophilus	low (DA, DP, MC, OP(8.5m), SJ, TI)	low (AR, DA, DP, MC, OP(8.5m), SJ, TI)	anoxic sediments
C. anthracinus (Zetterstedt, 1860)	■	thummi	low (HA, OS, RA, RM, SI(11))	low (HA, OS, RA, RM, SI(11))	anoxic sediments
***C.* nr. *atroviridis* (sp. 2i)** (of Martin, 2013)	■	thummi	ND	low (MN)	anoxic sediments
C. ochreatus (Townes, 1945)	■	thummi	ND	low (OP(3m))	anoxic sediments
***C.* sp. NAI** (of Proulx *et al.*, 2013)	□	thummi	low (KA)	low (KA)	anoxic sediments
C. cucini (Webb, 1969)	★	salinarius	low (SJ)	low (SJ)	anoxic sediments
***C.* sp. NAII** (of Proulx *et al.*, 2013)	☆	salinarius	low (SI(07))	low (SI(07))	anoxic sediments
***C.* sp. NAIII** (of Proulx *et al.*, 2013)	☆	salinarius	low (DA, HA, MC, RA, RM)	low (DA, HA, MC, RA, RM)	anoxic sediments

S stable-isotope signatures in sediments (Fig. 3)

The fact that sympatric *Chironomus* species can be separated into two groups based on their relative $\delta^{34}S$ signatures, suggests that the $\delta^{34}S$ signatures of the oxic and anoxic particles on which these groups are likely to feed should also differ (Fig. 1). To test this idea, we compared $\delta^{34}S$ signatures in the uppermost half-centimeter of oxic sediments with those of anoxic sediments collected well below the sediment-water interface. To simplify the comparison of sediment and insect values, we grouped *Chironomus* species having a more positive $\delta^{34}S$ signature (blue circles; *C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri*) and those having more negative values (grey circles; *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C.* spp. NAI-NAIII and *C. 'tigris'*)

Sediment $\delta^{34}S$ signatures (triangles, Fig. 3) were lowest (< -15‰) in the Sudbury-area lakes Kelly (KE) and Ramsey (RA) as well as in the Rouyn-Noranda-area lakes Osisko (OS) and Pelletier (PE). In other Sudbury-area lakes, $\delta^{34}S$ values ranged from -13 to -5‰, whereas in the Quebec lakes Duprat (DP) and St. Joseph (SJ) they ranged from -5‰ to 0‰. As expected, oxic sediments (blue triangles) were always ^{34}S-enriched relative to anoxic sediments (grey triangles). However, these differences in mean signatures were not always significant, which is probably explained by the shallow depth of the oxic layer in soft lake sediments (usually a few mm; Fig. 1) and the attendant difficulty in obtaining pure samples of surface oxic sediment. With the exception of only two lakes (St. Joseph and Duprat), all *Chironomus* species were ^{34}S-enriched compared to the oxic or anoxic sediments on which they are hypothesised to feed, which suggests that *Chironomus* larvae do not feed on bulk sediment, but select particles on which they feed.

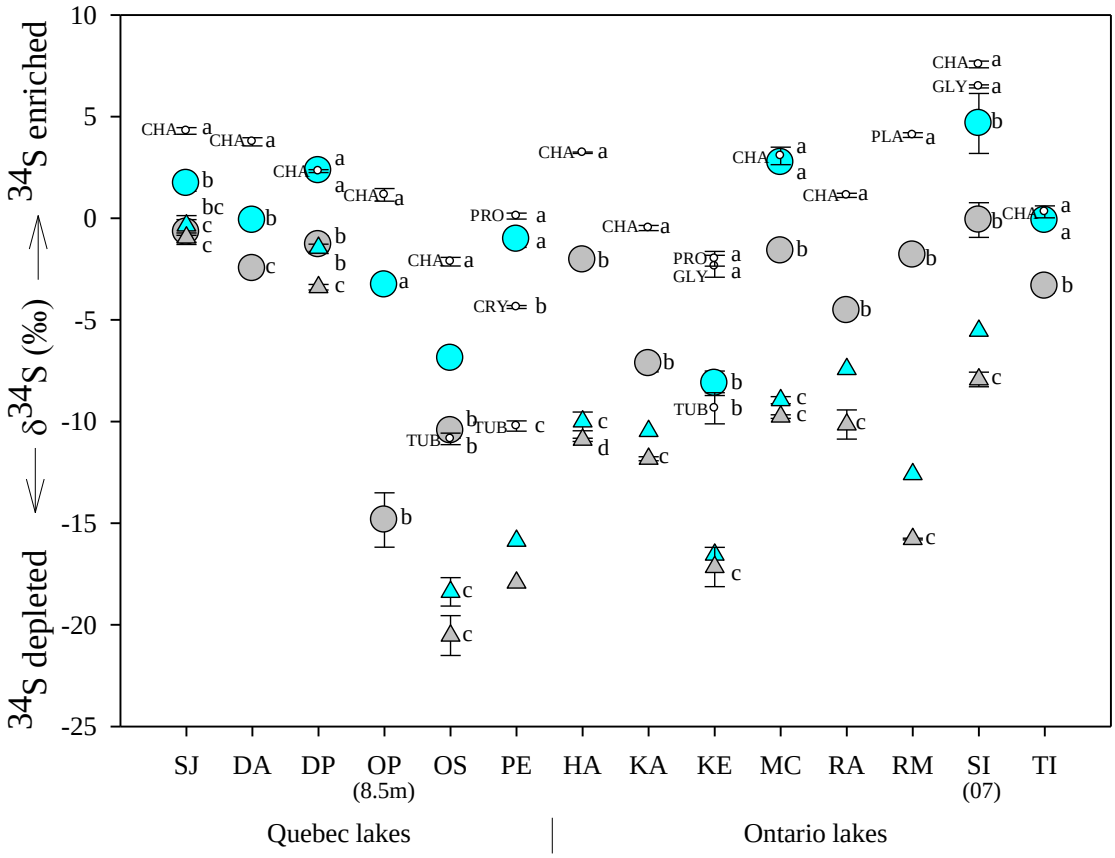


Figure 3. **Mean (±SE, n = 3-12) $\delta^{34}S$ signatures (‰) in *Chironomus* species feeding in either the oxic zone (●) or the anoxic zone (●) compared to mean values for planktonic (CHA = *Chaoborus* and bulk plankton = PLA) (±SE, n = 3-5) and benthic (CRY = *Cryptochironomus*; GLY = *Glyptotendipes*; PRO = *Procladius* and TUB = Tubificidae) (±SE, n = 3) invertebrates as well as those in bulk oxic (▲) and anoxic (▲) sediments (±SE, n = 1 or 3) collected from lakes in Eastern Canada (see Table 1 for lake names). *Chironomus* species in Lake Opasatica (OP) were collected at a depth of 8.5 m, whereas organisms and sediments in Silver Lake (SI) were collected in 2007. In a given lake, values that do not differ significantly (p > 0.05) are followed by the same letter (except for some sediment values where n = 1).**

S stable-isotope signatures in other invertebrates (Fig. 3)

We also measured $\delta^{34}S$ signatures in other types of invertebrates for comparison with those of the two groups of *Chironomus* species (Fig. 3). To obtain a "pure" water-column signal, we collected either larvae of the plankton-feeding insect *Chaoborus* (CHA; Hare & Carter, 1987) or, in one lake (Ramsey, RA), bulk plankton (PLA). As expected, $\delta^{34}S$ signatures of these planktonic organisms were always more positive than those of *Chironomus* species that are hypothesised to feed on anoxic sediments (grey circles, Fig. 3).

Comparing these planktonic signatures (CHA, PLA) to those of *Chironomus* species that feed mainly in the oxic compartment (blue circles) should allow us to discriminate between those that feed solely on oxic particles (including species that filter-feed) and those that include some anoxic particles in their diet and thus must be feeding on deposited sediment (Fig. 1). Our results (Fig. 3) suggest that the feeding habits of a given species vary depending on the lake in which it lives. Thus *C. entis* and *C. plumosus* collected from Lake Duprat (DP) and *C. staegeri* collected from Lakes Duprat, McFarlane (MC), Silver (SI(07)) and Tilton (TI) had $\delta^{34}S$ signatures that did not differ significantly from those of *Chaoborus* (Fig. 3), which suggests that in these lakes these species are feeding only on particles in the oxic zone. In contrast, in Lakes St. Joseph (SJ), D'Alembert (DA), Opasatica (OP) and Osisko (OS), these *Chironomus* species had significantly lower $\delta^{34}S$ signatures than those of *Chaoborus* (Fig. 3), which suggests that in these lakes they are deposit-feeders that consume oxic and anoxic particles.

To obtain a "pure" signal for the anoxic zone, we measured $\delta^{34}S$ signatures in tubificid oligochaetes (TUB, Fig. 3). Tubificids are infaunal burrowers that are reported to feed primarily on anoxic sediments at depths of from 2 to 8 cm (McCall & Tevesz, 1982 and references therein). In Lakes Osisko (OS) and Pelletier (PE), the $\delta^{34}S$ signatures of *Chironomus* species feeding in the water-column or on oxic sediments (blue circles) were significantly higher than those of tubificids (Fig. 3), which confirms that these *Chironomus* species feed little on anoxic sediments. In contrast, in Lake Osisko (OS), there was no significant difference between the mean $\delta^{34}S$ signature of *C. anthracinus* (grey circle) and that of tubificids (Fig. 3), which suggests that this *Chironomus* species feeds on anoxic sediments. Exceptionally, in Kelly Lake (KE), the mean $\delta^{34}S$ signature of species feeding in the oxic zone (blue circle) was not significantly different from that measured in tubificids (Fig. 3). This apparent anomaly can be explained by the fact that this lake has for many years been the recipient of sewage (City of Greater Sudbury, 2013), as well as metals from mining and smelting activities, that could have left the subsurface

sediments uninhabitable even by tubificids. These worms would then have been forced to feed near the surface on the same type of particles that are consumed by *Chironomus* in this lake.

Lastly, we compared the $\delta^{34}S$ signatures of three other chironomid genera to those of *Chironomus* larvae in order to estimate the feeding behaviours of these genera. In the two lakes in which we collected *Procladius* (PRO) larvae (Fig. 3), their $\delta^{34}S$ signatures were equal to (PE) or greater (KE) than those of *Chironomus* species feeding mainly on oxic particles (blue circles), which suggests that larvae of this genus do not consume anoxic particles. Indeed, they are reported to be sprawlers rather than burrowers and algae are an important part of their omnivorous diet (Ferrington, Berg & Coffman, 2008; Berg, 1995). Likewise, *Glyptotendipes* (GLY) larvae appear to feed mainly on oxic particles (Fig. 3) because in Kelly Lake (KE) their $\delta^{34}S$ signature was not significantly different from that of *Procladius* (PRO) and in Lake Silver (SI) its $\delta^{34}S$ signature was not significantly different from that of *Chironomus* species feeding on oxic particles (blue circle). Lastly, *Cryptochironomus* (CRY) larvae from Lake Pelletier (PE) had $\delta^{34}S$ signatures that were significantly lower than those of both *Procladius* larvae (PRO) and of *Chironomus* larvae feeding mainly in the oxic zone (blue circle), but higher than those of tubificids (TUB, Fig. 3), which suggests that *Cryptochironomus* larvae feed on particles from the two zones. Because *Cryptochironomus* larvae are reported to feed primarily on invertebrates (Berg, 1995; Ferrington *et al*., 2008), their $\delta^{34}S$ signatures likely reflect the zone in which their prey feed.

Cadmium concentrations in *Chironomus* species (Fig. 2B & Fig. 4)

We measured enormous differences in Cd concentrations among sympatric *Chironomus* species (Fig. 2B). For instance, in Lakes Duprat (DP) and D'Alembert (DA), Cd concentrations in species symbolised by coloured (yellow, orange, or red) circles were up to 7 and 10 times higher, respectively, than those in species indicated by greyscale (black or grey) symbols (Fig. 2B). In most lakes (SJ, DA, DP, OP, OS, PE, MC, SI (07) and TI), Cd concentrations did not differ significantly among species indicated by coloured (yellow, orange, or red) circles, but were always significantly higher than those measured in species indicated by greyscale (white, grey, or black) symbols (Fig. 2B). Trends for uncommon species are as follows. The mean Cd concentration of *C. decorus*-group sp. 2 larvae (grey diamond) from Silver Lake (SI (11)) was not significantly different from that of sympatric *C. anthracinus* (grey square). Likewise, the mean Cd concentration of *C*. sp. NAI (white square) was identical to that of *C. 'tigris'* (white

circle) in Kasten Lake (KA). Lastly, the Cd concentration of *C. dilutus* (brown circle) was not significantly different from that of *C. plumosus* (orange circle) in Kelly Lake (KE).

These Cd data suggest that the *Chironomus* species that we studied can be separated into two groups (Table 2); those with relatively high Cd concentrations (coloured circles) and those with relatively low Cd concentrations (greyscale symbols). Within each group, sympatric species tend to have similar Cd concentrations. Species with relatively high Cd concentrations include *C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri*, whereas those with relatively low Cd concentrations include *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C*. spp. NAI-NAIII and *C. 'tigris'*.

These species groups are the same as those based on $\delta^{34}S$ signatures (Table 2), which indicates that within a given lake (Fig. 2) *Chironomus* species enriched in ^{34}S also tend to have higher Cd concentrations (species represented by brown, yellow, orange and red circles) relative to their ^{34}S-depleted, low-Cd, congeners (species represented by white, grey and black symbols).

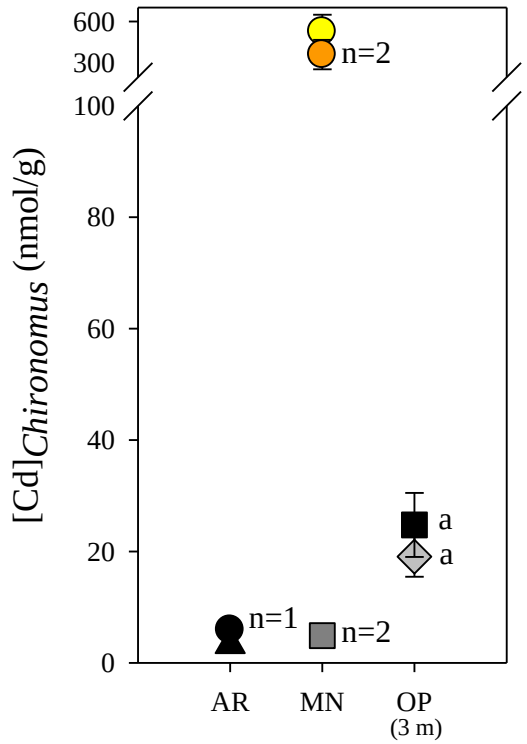


Figure 4. **Mean (±SE; n =1-5) Cd concentrations (nmol g^{-1}) in *Chironomus* species (see Table 2 for species names) collected from three Quebec lakes. *Chironomus* species in Lake Opasatica (OP) were collected at a depth of 3 m. In a given lake, values that do not differ significantly (p > 0.05) are followed by the same letter (except for values where n = 1-2).**

In cases where we had insufficient larval biomass to measure S stable isotopes, we used larval Cd concentrations as a de facto proxy (Fig. 4). Thus, in Lake Arnoux (AR), we collected *C. harpi* (black circle) and *C. bifurcatus* (black triangle), the latter of which feeds on anoxic sediments (Table 2). Since Cd concentrations in both species were similar (Fig. 4), we conclude that their feeding habits are similar and that *C. harpi* also feeds on anoxic sediments. In Lake Marlon (MN, Fig. 4), we collected *C*. nr. *atroviridis* (sp. 2i) (grey square) along with two species that feed on oxic sediments (coloured circles). Since, the latter species had Cd concentrations that were more than an order of magnitude higher than the former, we conclude that *C*. nr. *atroviridis* (sp. 2i) feeds on anoxic sediments. Lastly, we collected *C. ochreatus* (black square) in Lake Opasatica (OP, depth of 3 m) along with *C. decorus*-group sp. 2 (grey diamond). Since Cd concentrations in the two species were not significantly different (Fig. 4), and because the former feeds on anoxic sediment (Table 2), we infer that *C. ochreatus* also feeds on anoxic sediments.

C and N stable-isotope signatures in *Chironomus* species (Fig. 6)

Lake mean $\delta^{13}C$ signatures in a given *Chironomus* species ranged from -41 to -30‰ for the whole of our study lakes (Fig. 6A). In a given lake, we measured species differences of up to 4‰ (DP, OP, MC). The highest $\delta^{13}C$ values (-32 to -30‰) were measured in *Chironomus* species from Lakes Osisko (OS), Kelly (KE) and Ramsey (RM), whereas the lowest values were measured in Lake Duprat (-40 to -36‰; DP) and Lake Opasatica (-40 to -37‰; OP). Within a given lake, there were significant differences in $\delta^{13}C$ signatures among *Chironomus* species. However, from one lake to another, no given species had consistently higher $\delta^{13}C$ values than did others.

Values for mean $\delta^{15}N$ signatures in a given *Chironomus* species from a given lake ranged from 3 to 9‰ for the suite of lakes that we studied (Fig. 6B). Highest $\delta^{15}N$ values (7-9‰) were found in Lakes Osisko (OS), McFarlane (MC) and Ramsey (RM), whereas the lowest $\delta^{15}N$ value (3‰) was measured in *C. 'tigris'* from Tilton Lake (TI). Within a given lake, we measured species differences of up to 2‰ (DP, HA, KE). Although there were significant differences in $\delta^{15}N$ values among sympatric *Chironomus* species in a given lake, among lakes no species had consistently higher $\delta^{15}N$ values than did others.

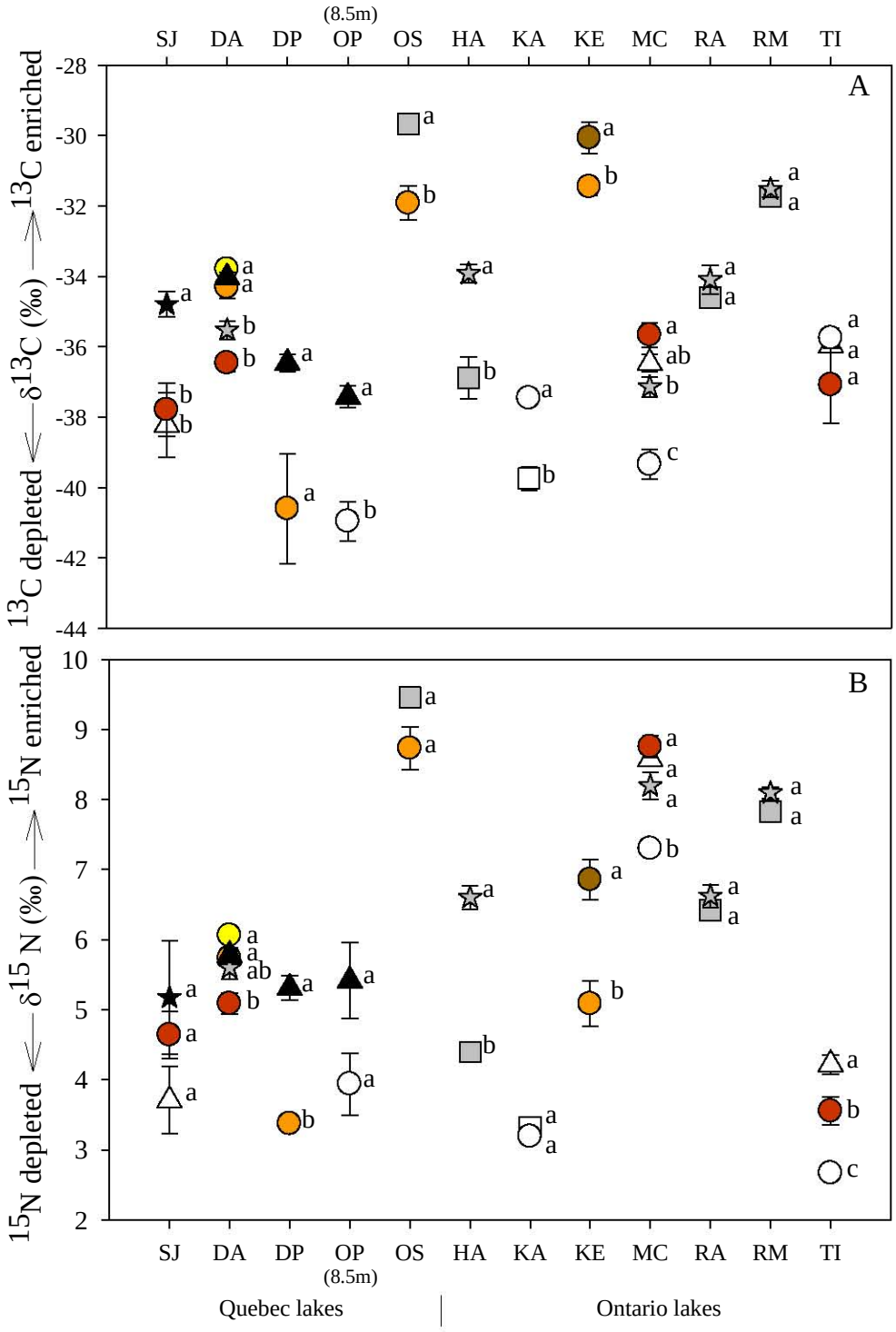


Figure 5. **Mean (±SE; n = 1 or 3-5) (A) δ^{13}C and (B) δ^{15}N signatures (‰) in *Chironomus* species (see Table 2 for species names) collected from lakes in eastern Canada (see Table 1 for lake names). *Chironomus* species in Lake Opasatica (OP) were collected at a depth of 8.5 m. For a given lake, values that do not differ significantly (p > 0.05) are followed by the same letter (except for values where n = 1).**

DISCUSSION

Using S stable isotopes and Cd concentrations to understand *Chironomus* feeding

Croisetière *et al*. (2009) showed that in four lakes from our study area (Rouyn-Noranda, Quebec) the $\delta^{34}S$ signature of sulphide in anoxic sediments is more negative than that of sulphate in the overlying oxic water-column. Furthermore, they showed that animals feeding directly or indirectly on anoxic sediments tended to have a more negative $\delta^{34}S$ signature than did those feeding on particles in the oxic zone. In our study, $\delta^{34}S$ signatures varied by up to 14‰ among sympatric *Chironomus* species, which is greater than the maximum range (8‰) reported by Croisetière *et al*. (2009) for several phyla of planktonic and benthic invertebrates. This wide range in values testifies to the variety of feeding habits among *Chironomus* species.

The differences that we measured in $\delta^{34}S$ values among the various *Chironomus* species suggest that four species (*C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri*) feed mainly on particles in the oxic zone, whereas the remainder feed mainly on anoxic sediments (*C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C*. spp. NAI-NAIII and *C. 'tigris'*). Our results also show that *Chironomus* species having more positive $\delta^{34}S$ values tend to have higher Cd concentrations, which suggests that Cd concentrations and/or Cd bioavailability is higher in particles from the oxic zone than in anoxic sediments. Measurements of Cd concentrations alone suggest that *C. harpi*, *C*. nr. *atroviridis* (sp. 2i) and *C. ochreatus* also feed on anoxic sediments.

Our findings for two *Chironomus* species are corroborated by those of Martin *et al*. (2008) who found that larvae of *C. staegeri* had more positive $\delta^{34}S$ values and higher Cd concentrations than did larvae of *C. 'tigris'*. By measuring the colour of gut contents, these researchers confirmed that larvae of *C. 'tigris'* feed on anoxic sediments, whereas those of *C. staegeri* feed on oxic sediments. Likewise, our results showing that larvae of *C. plumosus* feed to a greater extent on oxic particles than do *C. anthracinus* larvae are consistent with those of Grey & Deines (2005) who reported that in Europe larvae of *C. plumosus* also have more positive $\delta^{34}S$ signatures than do those of *C. anthracinus*. This difference between the species is consistent with what is known about their feeding habits. Thus, *C. plumosus* larvae are reported to feed primarily on particles filtered from the overlying water column, although they can switch to deposit feeding under periods of low plankton availability or in the presence of leaf litter

(Jónasson, 1972; Hodkinson & Williams, 1980). In contrast, *C. anthracinus* is reported to feed on surface sediment around the opening of its tube thereby exposing circular patches of black anoxic sediment (Jónasson, 1972; http://www.youtube.com/watch?v=RQwau_uSyy4). Based on these observations, Jónasson (1972) concluded that *C. anthracinus* larvae feed on surface oxic sediments. However, our $\delta^{34}S$ values suggest that the majority of the particles consumed by *C. anthracinus* are from the anoxic zone. Given the fact that the surface oxic zone in soft lake muds tends to be only a few millimeters thick (Gallon *et al*., 2008; Wang *et al*., 2001), *C. anthracinus* could feed on some surface oxic sediment and yet still be ingesting the majority of its particles from the anoxic zone. As a result, its $\delta^{34}S$ signature would be more negative than that of *C. plumosus*. An alternative explanation is that *C. anthracinus* larvae remove surface particles surrounding the mouth of their tube prior to feeding on anoxic sediments.

We note that in some of our study lakes there were significant differences in $\delta^{34}S$ signatures and in Cd concentrations between sympatric *Chironomus* species feeding on anoxic sediments. These differences could be explained by the fact that *Chironomus* species feeding on anoxic sediments feed at different depths. The quality and age of anoxic sediment varies with depth such that both Cd concentrations (Borgmann *et al*., 2001) and $\delta^{34}S$ values (Nriagu & Soon, 1985; Mayer *et al*., 2007) are also likely to vary.

The fact that most *Chironomus* species were ^{34}S-enriched compared to the oxic or anoxic sediments on which they are hypothesised to feed, suggests that *Chironomus* larvae do not feed on bulk sediment, but select particles on which they feed. This is consistent with the observation that the gut contents of *Chironomus* larvae tend to have higher proportions of algae and lower proportions of detritus than do the surrounding sediments (Kajak & Warda, 1968). Similarly, Johnson, Bostrom & Van De Bund (1989) found a higher proportion of bacteria in the gut of *C. plumosus* than in bulk sediments. Comparing the S signatures of species feeding in the oxic zone to those of plankton-feeding *Chaoborus* larvae indicated that a given species can feed either on suspended particles or surface oxic particle depending on the lake in which it occurs. This observation is consistent with the plasticity in feeding habits reported for larvae of *C. plumosus* larvae, which can switch from filter-feeding to surface-feeding depending on the availability of phytoplankton (Jónasson, 1972; Hodkinson & Williams, 1980).

Using C and N stable isotopes to understand *Chironomus* feeding

The range of $\delta^{13}C$ values in *Chironomus* larvae from our study lakes (-43 to -28‰) is similar to that reported for larvae from lakes in which methane-oxidising bacteria are thought to make a negligible contribution (<20%) to larval biomass (-40 to -29‰; Deines, Wooller & Grey, 2009; Eller *et al*., 2005; Kelly *et al*., 2004). In lakes where methane-derived C is important, the $\delta^{13}C$ signatures of *Chironomus* larvae tend to be much more negative (-56 to -46‰; Deines *et al*., 2009; Eller *et al*., 2005; Kelly *et al*., 2004). There are several plausible reasons why methane-oxidising bacteria are of little importance to *Chironomus* larvae in our study lakes. First, we collected *Chironomus* larvae in the spring when populations of methane-oxidising bacteria are likely to be smaller than those in the late summer to autumn (Jones & Grey, 2011; Yasuno *et al*., 2012) when methane production is thought to reach a peak (Casper *et al*., 2000; Thebrath *et al*., 1993). Second, *Chironomus* larvae having highly negative $\delta^{13}C$ signatures are usually found in eutrophic lakes (Jones & Grey, 2011; Yasuno *et al*., 2012), whereas most of our study lakes are oligotrophic to mesotrophic. Lastly, mining and smelting activities in our study areas have released large quantities of sulphur (Nriagu & Soon, 1985; Mayer *et al*., 2007), which could interfere with methane production in sediments due to competition for substrate between methane-oxidising and sulphate-reducing bacteria (Kelly *et al*., 2004; Deines *et al*., 2007b).

Trends in the $\delta^{13}C$ signatures of *C. plumosus* and *C. anthracinus* from Lake Osisko are consistent with those of previous studies (Deines *et al*., 2009; Grey *et al*., 2004a; Kelly *et al*., 2004; Grey & Deines, 2005) in that $\delta^{13}C$ signatures of the former tend to be more negative than those of the latter species. These authors attributed this difference to the fact that the former species tends to be more dependent on methane-oxidising bacteria than the latter. If the same explanation holds for these species in Lake Osisko, then ^{13}C-depleted methane-oxidising bacteria should be concentrated in oxic sediments since our results suggest that *C. plumosus* larvae feed more heavily on oxic particles than do *C. anthracinus* larvae. To test this idea, we compared the $\delta^{13}C$ signatures of *Chironomus* species feeding on oxic particles with those feeding on anoxic particles, as defined by their $\delta^{34}S$ signatures. In some lakes, there were significant differences in $\delta^{13}C$ values between species living at the same site, but species with more negative $\delta^{13}C$ signatures were not necessarily those feeding on oxic sediments. In fact, among lakes, no *Chironomus* species consistently had more positive or negative $\delta^{13}C$ values relative to the others. Our data suggest either that methane-oxidising bacteria are not restricted

to oxic sediments or that these bacteria make a negligible contribution to *Chironomus* C in our lakes (as supported by the relatively high $\delta^{13}C$ signatures of our larvae; see above).

The range in our *Chironomus* $\delta^{15}N$ values (3 to 9 ‰) falls within that reported for the genus (-7.8 to 15‰: Grey *et al*., 2004b; Jones & Grey, 2004; Kelly *et al*., 2004; Grey *et al*., 2004a). Within a given lake, some *Chironomus* species differed markedly in their $\delta^{15}N$ values. This result is at first sight counter-intuitive because variations in the $\delta^{15}N$ signatures of aquatic animals are thought to be explained by differences in trophic level, whereas *Chironomus* larvae are all primary consumers. This result suggests that interspecific differences in $\delta^{15}N$ signatures among sympatric *Chironomus* species are explained by differences in the $\delta^{15}N$ signatures of their food sources. The absence of consistent trends in relative $\delta^{15}N$ values between species in our various study lakes suggests that lake-specific factors determine larval $\delta^{15}N$ signatures.

Previous studies have reported positive correlations between $\delta^{13}C$ and $\delta^{15}N$ signatures in *Chironomus* larvae due to the fact that ^{13}C-depleted larvae feed mainly on ^{15}N-depleted microbial communities (Grey *et al*., 2004a; Grey *et al*., 2004b; Jones & Grey, 2004; Kelly *et al*., 2004). These authors showed, for example, that *C. plumosus* larvae tend to be depleted in ^{13}C and ^{15}N relative to those of *C. anthracinus*. Likewise, in our study, *C. plumosus* larvae from Lake Osisko were significantly depleted in these isotopes compared to those of *C. anthracinus*. In contrast, the $\delta^{13}C$ and $\delta^{15}N$ signatures of our other study species were correlated in only about half of our study lakes, which suggests that these two variables are not consistently correlated in the particles on which *Chironomus* larvae feed.

CONCLUDING REMARKS

Overall, the results of our study suggest that *C. dilutus*, *C. entis*, *C. plumosus* and *C. staegeri* feed mainly on oxic particles (in the water column, in surface sediment and/or in the walls of their tubes), whereas, *C. anthracinus*, *C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i), *C. ochreatus*, *C*. spp. NAI-NAIII and *C. 'tigris'* feed on anoxic sediment particles. The feeding behaviour of many of these species (*C. bifurcatus*, *C. cucini*, *C. decorus*-group sp. 2, *C. dilutus*, *C. harpi*, *C*. nr. *atroviridis* (sp. 2i) and *C. ochreatus*) was previously unknown. Our results show the utility of measurements of S stable isotopes and contaminant concentrations (Cd in our study) in larvae as tools for discriminating among *Chironomus* species according to their feeding habits. Measurements of S stable isotopes were also useful for

inferring the feeding behavior of other chironomid genera. Overall, $\delta^{13}C$ and $\delta^{15}N$ signatures proved less useful for explaining *Chironomus* feeding habits.

Our results underline the importance of correctly identifying *Chironomus* species when using their larvae in ecological and behavioural studies or as environmental sentinels for contaminants or climate monitors. In spite of this result, in many studies, *Chironomus* larvae are either not identified to species, identified only to species group, or the species identification mentioned is not adequately justified, which makes it difficult to put the results of such studies in context. For example, it is clear from Table 2 that simply identifying larvae as “plumosus type” based on certain aspects of their morphology could lead to ambiguous and even contradictory results among studies. Given the current low cost and ready availability of genetic techniques, reliable *Chironomus* species identifications are now within the reach of most (Proulx *et al*., 2013), which would put the results of future studies on a more solid footing.

ACKNOWLEDGEMENTS

Funding was provided by the Metals In the Human Environment - Strategic Network and the Natural Sciences and Engineering Research Council of Canada. We thank Victor Thomasson and Hugo Lavoie for their assistance in collecting samples. In addition Dominic Ponton and Maikel Rosabal-Rodriguez aided in sample collection and provided some water quality measurements for our study lakes. We thank two anonymous reviewers for their helpful comments

.

REFERENCES

Ashe P. & Cranston P.S. (1990) Family Chironomidae. In: *Catalogue of Palearctic Diptera. Vol. 2: Psychodidae - Chironomidae* (Eds A. Soos & L. Papp), pp. 113-355. Elsevier, Amsterdam.

Berg M.B. (1995) Larval food and feeding behaviour. In: *The Chironomidae: The biology and ecology of non-biting midges* (Eds P. Armitage, P.S. Cranston & L.C.V. Pinder), pp. 136-168. Chapman & Hall, London.

Borgmann U., Norwood W.P., Reynoldson T.B. & Rosa F. (2001) Identifying cause in sediment assessments: bioavailability and the Sediment Quality Triad. *Canadian Journal of Fisheries and Aquatic Sciences,* **58**, 950-960.

Brooks S.J., Langdon P.G. & Heiri O. (2007) *The identification and use of Palearctic Chironomidae larvae in palaeoecology,* QRA Technical Guide No. 10, Quaternary Research Association, London.

Casper P., Maberly S.C., Hall G.H. & Finlay B.J. (2000) Fluxes of methane and carbon dioxide from a small productive lake to the atmosphere. *Biogeochemistry,* **49**, 1-19.

City of Greater Sudbury (2013) Individual Lake Information. http://www.greatersudbury.ca/living/lakes-facts/local-lake-descriptions/. Accessed 17 September 2013.

Coplen T.B., Hopple J.A. & Böhlke J.K. (2002) Compilation of Minimum and Maximum Isotope Ratios of Selected Elements in Naturally Occuring Terrestrial Materials and Reagents., U.S. Geological Survey Water-Resources Investigations Report 01-4222, Reston, Virginia.

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.

Deines P., Bodelier P.L.E. & Eller G. (2007a) Methane-derived carbon flows through methane-oxidizing bacteria to higher trophic levels in aquatic systems. *Environmental Microbiology,* **9**, 1126-1134.

Deines P., Grey J., Richnow H.H. & Eller G. (2007b) Linking larval chironomids to methane: Seasonal variation of the microbial methane cycle and chironomid $\delta^{13}C$. *Aquatic Microbial Ecology,* **46**, 273-282.

Deines P., Wooller M.J. & Grey J. (2009) Unravelling complexities in benthic food webs using a dual stable isotope (hydrogen and carbon) approach. *Freshwater Biology,* **54**, 2243-2251.

Eller G., Deines P., Grey J., Richnow H.H. & Krüger M. (2005) Methane cycling in lake sediments and its influence on chironomid larval $\delta^{13}C$. *FEMS Microbiology Ecology,* **54**, 339-350.

Ferrington L.C., Berg M.B. & Coffman W.P. (2008) Chironomidae. In: *An Introduction to the Aquatic Insects of North America, 4th edition* (Eds R.W. Merritt, K.W. Cummins & M.B. Berg), pp. 847-989. Kendall/Hunt Publishing Compagny, Iowa.

Fittkau E.J. & Roback S.S. (1983) The larvae of Tanypodinae (Diptera: Chironomidae) of the Holarctic region - Keys and diagnoses. *Entomologica Scandinavica Supplements,* **19**, 33-110.

Gallon C., Hare L. & Tessier A. (2008) Surviving in anoxic surroundings: How burrowing aquatic insects create an oxic microhabitat. *Journal of the North American Benthological Society,* **27**, 570-580.

Goedkoop W., Sonesten L., Markensten H. & Ahlgren G. (1998) Fatty acid biomarkers show dietary differences between dominant chironomid taxa in Lake Erken. *Freshwater Biology,* **40**, 135-143.

Grey J. & Deines P. (2005) Differential assimilation of methanotrophic and chemoautotrophic bacteria by lake chironomid larvae. *Aquatic Microbial Ecology,* **40**, 61-66.

Grey J., Kelly A. & Jones R.I. (2004a) High intraspecific variability in carbon and nitrogen stable isotope ratios of lake chironomid larvae. *Limnology and Oceanography,* **49**, 239-244.

Grey J., Kelly A., Ward S., Sommerwerk N. & Jones R.I. (2004b) Seasonal changes in the stable isotope values of lake-dwelling chironomid larvae in relation to feeding and life cycle variability. *Freshwater Biology,* **49**, 681-689.

Hare L. & Carter J.C.H. (1987) Chironomidae (Diptera, Insecta) from the environs of a natural West African lake. *Entomologica Scandinavica Supplements,* **29**, 65-74.

Hecky R.E. & Hesslein R.H. (1995) Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *Journal of the North American Benthological Society,* 14, 631-653.

Hodkinson I.D. & Williams K.A. (1980) Tube formation and distribution of *Chironomus plumosus* L. (Diptera: Chironomidae) in a eutrophic woodland pond. In: *Chironomidae: Ecology, Systematics, Cytology and Physiology* (Ed. D.A. Murray), pp. 331-337. Pergamon Press, Oxford.

Jansson M., Persson L., De Roos A.M., Jones R.I. & Tranvik L.J. (2007) Terrestrial carbon and intraspecific size-variation shape lake ecosystems. *Trends in Ecology and Evolution,* **22**, 316-322.
Johnson R.K., Bostrom B. & Van De Bund W. (1989) Interactions between *Chironomus plumosus* (L.) and the microbial community in surficial sediments of a shallow, eutrophic lake. *Limnology & Oceanography,* 34, 992-1003.
Jónasson P.M. (1972) Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. *Oikos Supplement,* 14, 1-148.
Jones R.I. (1992) The influence of humic substances on lacustrine planktonic food chains. *Hydrobiologia,* **229**, 73-91.
Jones R.I. & Grey J. (2004) Stable isotope analysis of chironomid larvae from some Finnish forest lakes indicates dietary contribution from biogenic methane. *Boreal Environment Research,* **9**, 17-23.
Jones R.I. & Grey J. (2011) Biogenic methane in freshwater food webs. *Freshwater Biology,* **56**, 213-229.
Kajak Z. & Warda J. (1968) Feeding on benthic nonpredatory Chironomidae in lakes. *Annales Zoologici Fennici,* **5**, 49-56.
Kathman R.D. & Brinkhurst R.O. (1998) *Guide to the Freshwater Oligochaetes of North America,* Aquatic Resources Center, College Grove, Tennessee.
Kelly A., Jones R.I. & Grey J. (2004) Stable isotope analysis provides fresh insights into dietary separation between *Chironomus anthracinus* and *C. plumosus*. *Journal of the North American Benthological Society,* **23**, 287-296.
Martin J. (2013) North American species of the genus *Chironomus*. http://genetics.unimelb.edu.au/Martin/NACytfiles/NAChiron.html. Accessed 17 septembre 2013.
Martin S., Proulx I. & Hare L. (2008) Explaining metal concentrations in sympatric *Chironomus* species. *Limnology and Oceanography,* **53**, 411-419.
Mayer B., Alpay S., Gould W.D., Lortie L. & Rosa F. (2007) The onset of anthropogenic activity recorded in lake sediments in the vicinity of the Horne smelter in Quebec, Canada: Sulfur isotope evidence. *Applied Geochemistry,* **22**, 397-414.
McCall P.L. & Tevesz M.J.S. (1982) The Effects of Benthos on Physical Properties of Freshwater Sediments. In: *Animal-Sediment Relations. The Biogenic Alteration of Sediments* (Eds P.L. McCall & M.J.S. Tevesz), pp. 105-176. Plenum Press, New York.
McCutchan J.H., Lewis W.M., Kendall C. & McGrath C.C. (2003) Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos,* **102**, 378-390.
Merritt R.W., Cummins K.W. & Berg M.B. (2008) *An Introduction to the Aquatic Insects of North America,* Kendall/Hunt Publishing, Iowa.
Mousavi S.K., Primicerio R. & Amundsen P.A. (2003) Diversity and structure of Chironomidae (Diptera) communities along a gradient of heavy metal contamination in a subarctic watercourse. *Science of the Total Environment,* **307**, 93-110.
Nriagu J.O. & Soon Y.K. (1985) Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. *Geochimica et Cosmochimica Acta,* **49**, 823-834.
Nyman M., Korhola A. & Brooks S.J. (2005) The distribution and diversity of Chironomidae (Insecta: Diptera) in western Finnish Lapland, with special emphasis on shallow lakes. *Global Ecology and Biogeography,* 14, 137-153.
Peterson B.J. (1999) Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. *Acta Oecologica,* **20**, 479-487.
Peterson B.J., Howarth R.W. & Garritt R.H. (1985) Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. *Science,* **227**, 1361-1363.
Pinder L.C.V. & Reiss F. (1983) 10. The larvae of Chironominae (Diptera: Chironomidae) the Holarctic region - Keys and diagnoses. *Entomologica Scandinavica Supplements,* **19**, 293-435.
Ponton D.E. & Hare L. (2009) Assessment of nickel contamination in lakes using the phantom midge *Chaoborus* as a biomonitor. *Environmental Science and Technology,* **43**, 6529-6534.
Proulx I., Martin J., Carew M. & Hare L. (2013) Using various lines of evidence to identify *Chironomus* species in eastern Canadian lakes. *Zootaxa,* **3741**, 401-458.
Reynolds C.S. (2008) A changing paradigm of pelagic food webs. *International Review of Hydrobiology,* **93**, 517-531.
Ryser H.M., Wuelker W. & Scholl A. (1985) Revision der Gattung *Chironomus* Meigen (Diptera) X: *LoboChironomus* n. subgen. (*C. montuosus* n. sp., *C. storai* Goetgh., *C. mendax* Stora). *Revue Suisse de Zoologie,* **92**, 385-404.

Sæther O.A. (1979) Chironomid communities as water quality indicators. *Holarctic Ecology,* **2**, 65-74.
Shobanov N.A., Shilova A.I. & Belyanina S.I. (1996) Extent and content of the genus *Chironomus* Meig. (Diptera, Chironomidae): review of world fauna (In Russian). In: *Ecology, Evolution and Systematics of Chironomids* (Eds N.A. Shobanov & T.D. Zinchenko), pp. 44-96, Inst. Biol. Inland Waters & Inst. Ecol. Volga Basin, Russian Academy of Sciences, Tolyatti (Boruk, Russia).
Summons R.E., Jahnke L.L. & Roksandic Z. (1994) Carbon isotopic fractionation in lipids from methanotrophic bacteria: Relevance for interpretation of the geochemical record of biomarkers. *Geochimica et Cosmochimica Acta,* **58**, 2853-2863.
Thebrath B., Rothfuss F., Whiticar M.J. & Conrad R. (1993) Methane production in littoral sediment of Lake Constance. *FEMS Microbiology Ecology,* **102**, 279-289.
Vander Zanden M.J., Chandra S., Park S.K., Vadeboncoeur Y. & Goldman C.R. (2006) Efficiencies of benthic and pelagic trophic pathways in a subalpine lake. *Canadian Journal of Fisheries and Aquatic Sciences,* **63**, 2608-2620.
Walshe B.M. (1951) The feeding habits of certain chironomid larvae (subfamily Tendipendinae). *Proceedings of the Zoological Society of London,* **121**, 63-79.
Wang F., Tessier A. & Hare L. (2001) Oxygen measurements in the burrows of freshwater insects. *Freshwater Biology,* **46**, 317-327.
Yasuno N., Shikano S., Muraoka A., Shimada T., Ito T. & Kikuchi E. (2012) Seasonal increase of methane in sediment decreases δ^{13}C of larval chironomids in a eutrophic shallow lake. *Limnology,* 13, 107-116.

ADDENDA - ARTICLE 3

p. 177 Le titre dans la 2^{e} colonne devrait plutôt être “Symbols in Figs. 2 and 4”, car ces symboles ne sont pas utilisés dans les figures 1 et 3.

p. 177 À la place de « **of** Kiknadze *et al.* (1993) », « **of** Martin, 2013 » et « **of** Proulx *et al.*, 2013 », il aurait fallu écrire « **sensu** Kiknadze *et al.* (1993) », « **sensu** Martin, 2013 » et « **sensu** Proulx *et al.*, 2013 », respectivement.

ARTICLE 4

Using *Chironomus* larvae to assess the bioavailability of trace elements in sediments

Isabelle Proulx et Landis Hare

Institut national de la recherche scientifique – Centre Eau Terre Environnement, 490 rue de la Couronne, Université du Québec, 490 rue de la Couronne, Québec (Québec), G1K 9A9, Canada

À soumettre

ABSTRACT

Larvae of the insect genus *Chironomus* (Diptera, Chironomidae) have great potential for estimating the bioavailability of trace elements in lake sediments since they are common in fine sediments and tolerate high concentrations of these contaminants. A potential drawback to their use as biomonitors is that they are difficult to identify to species, and species can differ in their trace element concentrations. Thus, we measured 9 trace elements in 15 *Chironomus* species collected from 22 lakes to determine if pooling species would compromise their use as trace element biomonitors. On the one hand, 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. On the other hand, we found that Cd, Se and Zn concentrations differed between sympatric *Chironomus* species, which can be explained by differences in 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. Given these differences among species, using unidentified *Chironomus* larvae to estimate site-to-site differences in Cd, Se or Zn bioavailability could give misleading results. Lastly, we compared trace element concentrations in *Chironomus* larvae to those in their gut contents, sediments and lakewater. We conclude that *Chironomus* larvae are useful biomonitors for sedimentary trace elements and we suggest practical means of using them to this end.

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, which can lead to toxic effects. Consequently, assessing the bioavailability of sedimentary trace elements is important in ecological risk assessments designed to protect benthic communities. It is generally recognized that the total concentration of contaminants in bulk sediments are difficult to relate to those in benthic animals because sediment values do not consider the availability of contaminants to the biota. To this end, approaches that consider the concentrations of various sediment components that influence trace element bioavailability have been used to improve predictions of trace element bioaccumulation or toxicity. However such approaches cannot consider all of the important processes that affect sediment metal bioavailability and thus their predictions tend to be approximate at best (Luoma & 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 & Rainbow, 2008) or sentinels (Beeby, 2001).

In lakes and slow-flowing rivers, a good candidate for monitoring sedimentary trace elements are non-biting midge larvae of the genus *Chironomus*. Its larvae are in close contact with the substrate since they are tube builders and most species feed on sediment. Furthermore, the genus is distributed worldwide, and larvae are tolerant to and accumulate trace elements (Armitage, Cranston & Pinder, 1995). Although cultured *Chironomus* larvae have been widely used to assess trace element availability and sediment toxicity in the laboratory (ex. Marinković *et al.*, 2012; Mogren *et al.*, 2012; Galluba, Oetken & Oehlmann, 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 together in environmental and paleolimnological studies (ex. Nyman, Korhola & Brooks, 2005; Larocque, Velle & Rolland, 2010), which could confound trends if there are important behavioral and physiological differences among species. Hence, studies on annelids (Bryan & Gibbs, 1987), arthropods (Moore & Rainbow, 1987; Rainbow *et al.*, 1993; Croteau, Hare & Tessier, 2001; Cain, Croteau & Luoma, 2011) 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 Cd concentrations between sympatric species (Martin, Proulx & Hare, 2008; Proulx & Hare, 2008).

We investigated the practicality of using *Chironomus* larvae as biomonitors to evaluate the bioavailability of sedimentary trace elements: (1) by determining if arsenic (As), barium (Ba), cadmium (Cd), cobalt (Co), copper (Cu), manganese (Mn), nickel (Ni), selenium (Se), and zinc (Zn) concentrations differed between species living at the same site, (2) by determining why some sympatric *Chironomus* species differ in their trace element concentrations and (3) by comparing the concentrations of trace elements in larvae, their feces/gut contents, lakewater and sediments.

MATERIAL AND METHODS

Collection of samples

We collected *Chironomus* larvae, lakewater and sediments in late spring to early summer in different years (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) (Table 1). In Rouyn-Noranda, local Cu ores were processed by the Horne smelter from 1927 until the closure of the Horne mine in 1976. Since then, the Horne smelter has processed ores (with varying metals composition) from suppliers around the world and also recycles metal-rich materials. In Sudbury, Ni and Cu mining and smelting operations have taken place since 1902. In both regions, lakes have been contaminated with a variety of trace elements, and in some cases acidified, through atmospheric deposition from smelters and localized runoff from mine tailings. Despite a reduction in smelter emissions (since 1987 for Rouyn-Noranda and the 1970s and 1990s for Sudbury), many lakes near to and downwind from these point sources remain highly contaminated (Nriagu *et al.*, 1998; Croteau, Hare & Tessier, 2002). Cadmium and Cu have been determined to be the metals that are most likely to cause toxicity to organisms in Rouyn-Noranda lakes (Borgmann *et al.*, 2004), whereas Ni is thought to be the most likely to cause toxicity in Sudbury lakes (Borgmann *et al.*, 2001).

The lakes selected for our study encompass a wide range in pH and trace element concentrations in water and sediments, since some are located close to and downwind of smelters whereas others are located upwind or distant from them (Table 1; Figs. 1 and 2). Some of the selected lakes have also received acid mine drainage (Dufault, Osisko and Kelly) and/or untreated hospital or municipal sewage (Osisko and Kelly, respectively). At the time of collection

(2010), Lake Arnoux was still receiving acidic drainage from an abandoned metals mine that has since been rehabilitated.

Table 1. Location, year and depth of collection, as well as water chemistry of the collecting sites.

	Code	Year	Depth (m)	Location	pH	Species collected	Distance and direction from main smelter
ROUYN-NORANDA (QC)							
Lake Arnoux[1]	AR (1.5m)	2010	1.5	48°15'N, 79°20'W	3.83	*C. bifurcatus*, *C. harpi*	24 km W (268°)
	AR (4.5m)		4.5		4.36	*C. anthracinus*	
Lake Bousquet	BO	2006	14	48°13'N, 78°39'W	6.9[a]	*C. cucini*	29 km SE (100°)
Lake D'Alembert	DA	2006	5	48°23'N, 79°01'W		*C. bifurcatus*, *C. entis*, *C. plumosus*, C. sp. NAIII, *C. staegeri*	14 km NE (14°)
Lake Dufault[2]	DF	2006	4	48°17'N, 79°00'W	7.7	*C. decorus*-group sp. 2	3 km NE (16°)
Lake Duprat	DP	2010	6.5	48°20'N, 79°07'W	7.6	*C. bifurcatus*, *C. plumosus*, *C. staegeri*	12 km NW (317°)
Lake Fortune	FO	2006	5-6	48°11'N, 79°19'W	7.6	*C. plumosus*	23 km SW (251°)
Lake Marlon	MN	2010	1-2	48°16'N, 79°04'W	7.7	*C. entis*, *C.* nr. *atroviridis* (sp. 2i), *C. plumosus*	4 km NW (287°)
	OP (07)	2007	9	48°10'N, 79°20'W	8.2	*C. bifurcatus*, *C. plumosus*, *C. staegeri*, *C. 'tigris'*	
Lake Opasatica	OP (09 - 3m)	2009	3	48°10'N, 79°20'W		*C. decorus*-group sp. 2, *C. ochreatus*	26 km SW (246°)
	OP (09 - 8.5m)	2009	8.5	48°10'N, 79°20'W		*C. bifurcatus*, *C. staegeri*, *C. 'tigris'*	
Lake Osisko[3]	OS	2010	6	48°15'N, 79°00'W	8.5	*C. anthracinus*, *C. plumosus*	2 km SE (135°)
Lake Pelletier	PE	2010	5	48°13'N, 79°03'W	8.3	*C. entis*, *C. plumosus*	5 km SW (212°)
Lake Rouyn[4]	RO	2010	4	48°15'N, 78°57'W	8	*C. plumosus*, *C. staegeri*	6 km SE (105°)
SUDBURY (ON)							
Clearwater Lake	CL	2007	19	46°22'N, 80°03'W	6.2	*C. cucini*	12 km S (178°)
Crooked Lake	CR	2010	5-6	46°25'N, 81°02'W	6.7	*C. staegeri*	7 km SE (166°)
Hannah Lake	HA	2007	7	46°27'N, 81°02'W	7.4	*C. anthracinus*, *C.* sp. NAIII	4 km SE (160°)
Kasten (Bibby) Lake	KA	2007	7.5	46°22'N, 80°58'W	6.8	*C.* sp. NAI, *C. 'tigris'*	14 km SE (152°)
Kelly Lake[5]	KE	2010	5	46°27'N, 81°04'W	7.5	*C. dilutus*, *C. plumosus*	4 km S (172°)
McFarlane Lake	MC	2007	10	46°25'N, 80°57'W	7.8	*C. bifurcatus*, *C.* sp. NAIII, *C. staegeri*, *C. 'tigris'*	10 km SE (133°)
Pine Lake	PI	2010	4-6	46°23'N, 81°01'W	5.7	*C. anthracinus*	12 km SE (168°)
Raft Lake	RA	2010	10	46°25'N, 80°57'W	7.3	*C. anthracinus*, *C.* sp. NAIII	12 km SE (133°)
Ramsey Lake	RM	2007	12	46°28'N, 80°57'W	7.1	*C. anthracinus*, *C.* sp. NAIII	8 km E (94°)
Silver Lake	SI (07)	2007	4	46°26'N, 81°01'W	5.9	*C.* sp. NAII, *C. staegeri*, *C. 'tigris'*	6 km SE (149°)
	SI (11)	2011			7	*C. anthracinus*, *C. decorus*-group sp. 2	
Tilton Lake	TI	2007	4	46°21'N, 81°04'W	6.6	*C. staegeri*, *C. 'tigris'*	14 km S (185°)

[1] Receives acid mine drainage
[2] Adjacent to a mining site
[3] As received untreated sewage from an hospital and discharges from smelting operations
[4] Lake connect to Lake Osisko
[5] Lake also contaminated by untreated sewage from the city of Sudbury (1880s-1972) and discharges from mining, milling and smelting operation (1880s-present) (City of Greater Sudbury, 2013)

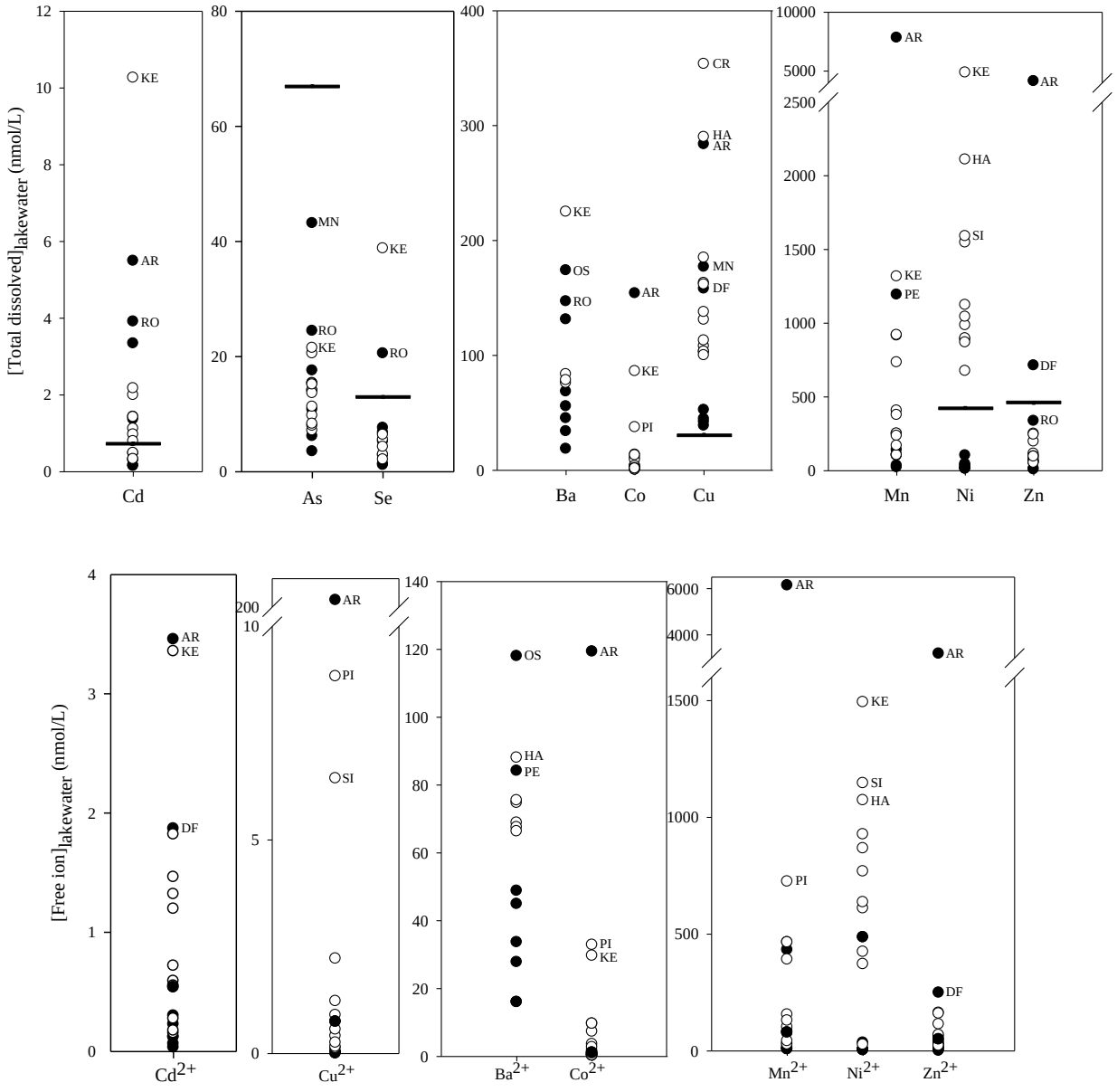


Figure 1. **Mean total dissolved concentrations (n=3, nM) of As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn (upper panels) and mean (n=3, nmol/L) free-ions concentrations (n=3; nmol/L) of Ba, Cd, Co, Cu, Mn, Ni and Zn in water of lakes in the regions of Rouyn-Noranda (●) and Sudbury (○). To simplify the figure, standard deviations (given in Tables S4-S12) were omitted since the coefficient of variation was in general <10%. Lakes where total dissolved and free ion concentrations were highest are labelled (lake abbreviations are given in Table 1). In the upper panel, the horizontal bars represent the threshold values for the protection of aquatic life. For As, Cd, Co, Ni and Zn, threshold values were taken from CCME (2014) whereas for Se the threshold value was taken from Lemly (2002).**

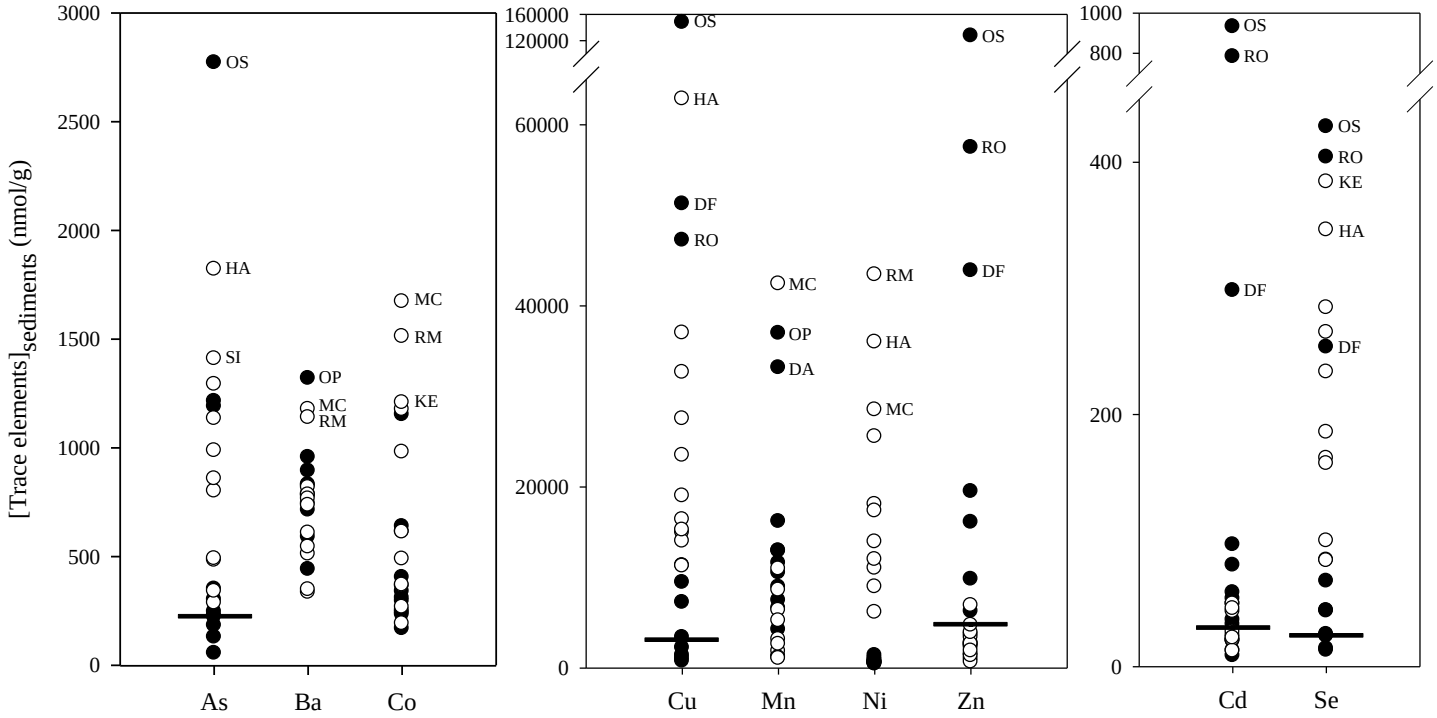


Figure 2. **Mean (n=6) As, Ba, Co, Cu, Mn, Ni, Zn, Cd and Se concentrations (nmol/g) in oxic and anoxic sediments from lakes near Rouyn-Noranda (●) and Sudbury (○). Standard deviations and values for oxic and anoxic sediments were omitted for simplicity (given in Tables S4-S12). The coefficient of variation of sedimentary trace element concentrations for each lake was generally <30%. Lakes where sediment trace element concentrations were highest are labelled (lake abbreviations are given in Table 1). Horizontal bars represent threshold values for the protection of aquatic life (CCME, 2014).**

Chironomus larvae

We collected *Chironomus* larvae 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 to eliminate fine sediment. Five to 20 grab samples were collected at each site depending on larval densities. Fourth (final) instars were chosen for study as determined from the head-capsule widths of pre-pupal larvae (Proulx *et al.*, 2013). Larvae were held in lakewater at field temperatures for transport to the laboratory where they were tentatively separated to species according to the presence/absence and morphology of their abdominal tubules as well as the coloration of their frontoclypeus (Table S1; Proulx *et al.*, 2013).

To confirm that larvae were correctly separated to species and to identifiy them, 10 larvae from each morphological group were sacrificed 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 determinations 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 anterior margin of the ventromental plates. 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. DNA analysis was conducted through DNA barcoding of the *cox1* and *gb2B* genes. The results of these analyses are presented in Proulx *et al.* (2013). Larvae from the lakes presented in this study were correctly separated to species.

The other larvae were kept for trace element measurements. They were held at 4^{o}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 retained for the measurement of trace elements. Additional larvae were dissected to remove their gut contents for trace element analyses. Larvae with empty guts, as well as larval feces and gut contents 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. 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, Hare & Tessier, 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) 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 detail in Ponton & 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 collected 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.

In 2010, three samples were removed from the grab sampler using a 2.5 cm diameter plastic syringe (open at both ends) inserted to a depth of 5 cm for the measurement of acid volatile sulfides (AVS) and simultaneously extracted metals (SEM). These sediment samples were placed into Whirl-Pak bags that were closed carefully to eliminate as much air as possible. They were then inserted into a large sack containing sediments and held at 4^{o}C until analysis.

Analyses

To prevent inadvertent contamination, all labware used for trace element analyses was soaked in 15% (v/v) nitric acid (TraceMetal grade, Fisher Scientific) 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. 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 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 digestive 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 ratio. 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.

Trace element concentrations in digestive 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 85-90% 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 (RSD) between the measurements were generally <10%.

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 from 1 to 14 days (Fig. S1). 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.

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 section 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 & Hare (2013) who used an alternative method (atomic fluorescence spectrometry; Millennium Excalifur System, PS Analytical) to measure Se in our water samples. Measurements of dissolved organo-Se, selenite (Se (IV)) and selenate (Se(VI)) were also taken from Ponton & Hare (2013).

Free 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, Lofts & Sonke, 2011). Measured WHAM input parameters included pH, total dissolved concentrations of metals (Al, Ba, Cd, Co, Cu, Fe, Mn, Ni and Zn), major cations (Al, Ca, Fe, K, Mg, Mn and Na), anions (SO_4^{2-}, NO_3^{2-} and Cl^-), inorganic carbon and fulvic and humic acids (Table S2). 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 DOC-to-DOM (dissolved organic matter) ratio was 2 (Buffle, 1988), that 65% of DOM was active in the complexation of metals (Bryan, Tipping & Hamilton-Taylor, 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 INRS-ETE quality assurance/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, gut contents and fecal matter

Sediments in all the study lakes were composed of very fine clay and silt particles. Oxic and anoxic sediments were homogenized and dried at 60^{o}C for 12 hours whereas fecal matter and gut contents 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 above for *Chironomus* 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. As, Cd, Co and Se concentrations were measured by ICP-MS, whereas Ba, Cu, Mn, Ni and Zn concentrations were measured by ICP-AES. The quality of the analytical process was controlled as described for the trace element analyses in larvae.

Certified reference materials MESS-3 (marine sediments, National Research Council of Canada (NRC), Ottawa, ON), PACS-3 (marine sediments, NRC, Ottawa, ON) and 7110 C 513 (2-31) (St. Lawrence River sediment, COREM and INRS-ETE, Quebec City, QC) were also digested and analyzed as a means to evaluate the efficacy of our sediment digestion method. Certified values for reference materials MESS-3 and PACS-3 were obtained from total digestions, whereas certified values for 7110 C 513 (2-31) are based on two partial digestions (HNO_3/H_2O_2/HCl with intervals of heating and cooling (Ministère de l'Environnement et de la Faune du Québec, 1996) or HNO_3/HCl in a block digester (Ministère de l'Environnement du Québec, 1987). Comparisons between measured and certified values for the three reference materials are given in supplementary Table S3. Briefly, certified values for MESS-3 and PACS-3

are generally somewhat higher (except for Se) than the values we measured in these reference materials whereas measured values fall within the 95% confidence limits of the certified values for reference material 7110 C 513 (2-31). No sediment certified reference materials were available for Ba determination.

To determine trace element concentrations in the particles ingested by *Chironomus* larvae, we collected samples of their gut contents or, where not available, feces. To determine if trace element concentrations in gut contents and feces were equivalent, we compared values for samples taken from *C. staegeri* from Crooked Lake and *C. anthracinus* from Hannah Lake. Barium, Cu, Se and Zn concentrations did not significantly differ ($p>0.05$) between gut contents and feces. Although concentrations did differ for As, Cd, Co, Mn and Ni, these differences were very small (data not shown) compared to the range of concentrations in the feces/gut contents of *Chironomus* larvae among our study lakes.

The organic matter content of sediments was measured by digesting ~0.5 g of dried sediment in 50 mL of 0.1 N H_2SO_4 and the mixture was shaken for 15 minutes to allow the loss of carbonates. The solution was filtered through a preweighed 42.5 mm glass-fibre filter (GF/C 1.2 µm) and the filter, along with the sediment retained, was dried at 55^{o}C then organic carbon was measured in a CHN analyser (Leco model CHNS-932, St. Joseph, MI, USA). Instrumental blanks were under the detection limit and the values of standards (OAS, sediment standard – high organic content, Isomass Scientific Inc.; PACS-2, marine sediment, NRC) were within the certified limits. At each site, organic matter content was only measured in one oxic and one anoxic sediments sample. Measurements (data not shown) demonstrated that organic matter content varies little between sediment samples collected within a site (n=3, coefficient of variation <2%)

Concentrations of AVS and SEM were measured in sediments collected in 2010. For measurements of AVS, sulfides were converted to gaseous H_2S by digesting 0.5 g of wet sediment in 10 mL of 6 N HCl in a N-purged reaction cell containing 50 mL of demineralized water and 200 µL of 1 N NaOH. The H_2S was trapped in two tubes filled with 50 mL of 0.25 N degassed NaOH. After 45 minutes, the solution of H_2S and NaOH was colored with methylene blue and the S concentration was measured by atomic absorption spectrophotometry at 670 nm. The spectrophotometer was calibrated using sulfide standards. Following H_2S release, the acidified mixture was filtered (0.45 µm) and metals in this solution (SEM) were measured by ICP-AES (Varian Vista AX) using external standards and yttrium as an internal standard.

Filtration blanks were close to or below the limit of detection. Control samples were also used to verify measurement reliability (900-Q30: Inter-laboratory study, Environment Canada).

Statistical analysis

All numerical data are presented as means ± standard error (SE). Statistical analyses were conducted using SigmaPlot 11.0.

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 ($p\leq0.05$). Mean values obtained from 2 samples or less were not included in the statistical tests.

Relationships between trace element concentrations in larvae, fecal matter, water and sediment were assessed through linear regressions. First, relationships were examined through bivariate scatterplots. We then checked that the data were normally distributed around the regression line (Kolmogorov-Smirnov test) and that the variance of the dependent variable was constant regardless of the value of the independent variable (Shapiro-Wilk test). When these assumptions were not met ($p<0.05$), data were $\log_{10}$-transformed. If the assumptions were still not met after $\log_{10}$-transforming data, correlations between the variables were assessed through the non-parametric Spearman Rank Order Correlation.

RESULTS/DISCUSSION

Trace element concentrations in *Chironomus* species

At the time of collection, the water columns at all sampling sites were well mixed and the sediment-water interfaces were well oxygenated (data not shown). Overall, 15 *Chironomus* species were collected, of which 12 have been identified (Table 2) whereas the status of three others remains uncertain (*C*. sp. NAI-III; Table 2). The species are described in detail in Proulx *et al.* (2013). At each sampling site, 1 to 5 *Chironomus* species were collected (Table 1). In Figs. 3 and 4, we present the trace element concentrations measured in *Chironomus* species

from sites where more than one species was collected. Concentrations of trace elements in *Chironomus* larvae from lakes where only one species was found are presented in the Tables S4-S12.

Table 2. Larval type and feeding behavior of collected *Chironomus* species.

***Chironomus* species**	**Symbols in Figs. 1 and 2**	**Larval type (see Table 1; Proulx *et al.*, 2013)**	**Inferred feeding zone (Proulx & Hare, 2014)**
C. entis (Shobanov, 1989)	●	semireductus	water column or oxic sediments
C. plumosus (Linnaeus, 1758)	●	semi-reductus to plumosus	water column or oxic sediments
C. dilutus (Shobanov, Kiknadze & Butler, 1999)	●	plumosus	water column or oxic sediments
C. staegeri (Lundbeck, 1898)	●	plumosus	water column or oxic sediments
C. harpi (Wülker, Sublette & Martin, 1991)	●	plumosus	anoxic sediments
C. 'tigris' (nomen nudum in Martin *et al.* (2008) for *C.* sp. Am1 sensu Kiknadze *et al.* (1993))	○	plumosus	anoxic sediments
***C. decorus*-group sp. 2** (Butler *et al.*, 1995)	◇	bathophilus, fluviatilis or melanotus	anoxic sediments
C. bifurcatus (Wuelker *et al.*, 2009)	▲	bathophilus	anoxic sediments
C. anthracinus (Zetterstedt, 1860)	□	thummi	anoxic sediments
C.* nr. *atroviridis (sensu Martin, 2013)	■	thummi	anoxic sediments
C. ochreatus (Townes, 1945)	■	thummi	anoxic sediments
***C.* sp. NAI** (sensu Proulx *et al.*, 2013)	□	thummi	anoxic sediments
C. cucini (Webb, 1969)	★	salinarius	anoxic sediments
***C.* sp. NAII** (sensu Proulx *et al.*, 2013)	☆	salinarius	anoxic sediments
***C.* sp. NAIII** (sensu Proulx *et al.*, 2013)	☆	salinarius	anoxic sediments

Enormous differences in Cd concentrations were measured among sympatric *Chironomus* species (Fig. 3A). In fact, in Lake Marlon, the range in Cd concentrations among species (3 to 520 nmol/g) is almost as high as the range in Cd concentrations that we measured in *Chironomus* species among lakes (1 to 520 nmol/g). Some of these Cd concentrations have been published in Proulx & Hare (2014). Cadmium concentrations did not differ significantly among the species indicated by colored (brown, orange, red, or yellow) circles, but were always significantly higher than those measured in species indicated by greyscale (white, grey, or

black) symbols (Fig. 3A; see Table 2 for species names). As in our study, Martin *et al.* (2008) also reported that *C. staegeri* (yellow circle in Fig. 3A) had higher Cd concentrations than *C. 'tigris'* (white circle in Fig. 3A).

Within a given lake, Zn concentrations often differed significantly among sympatric *Chironomus* species (Fig. 3B). 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 with Cd larval concentrations. Martin *et al.* (2008) also reported higher Zn concentrations in *C. staegeri* (yellow circle in Fig. 3B) than in *C. 'tigris'* (white circle in Fig. 3B).

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 (Fig. 3C). 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 (except for the *Chironomus* species collected in Kelly, McFarlane, Raft, and Silver Lakes) (Fig. 3C).

In general, the concentrations of As, Ba, Co, Cu, Mn and Ni did not significantly differ between sympatric *Chironomus* species (Fig. 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* (yellow circle in Fig. 4D) and *C. 'tigris'* (white circle in Fig. 4D) (Martin *et al.*, 2008)

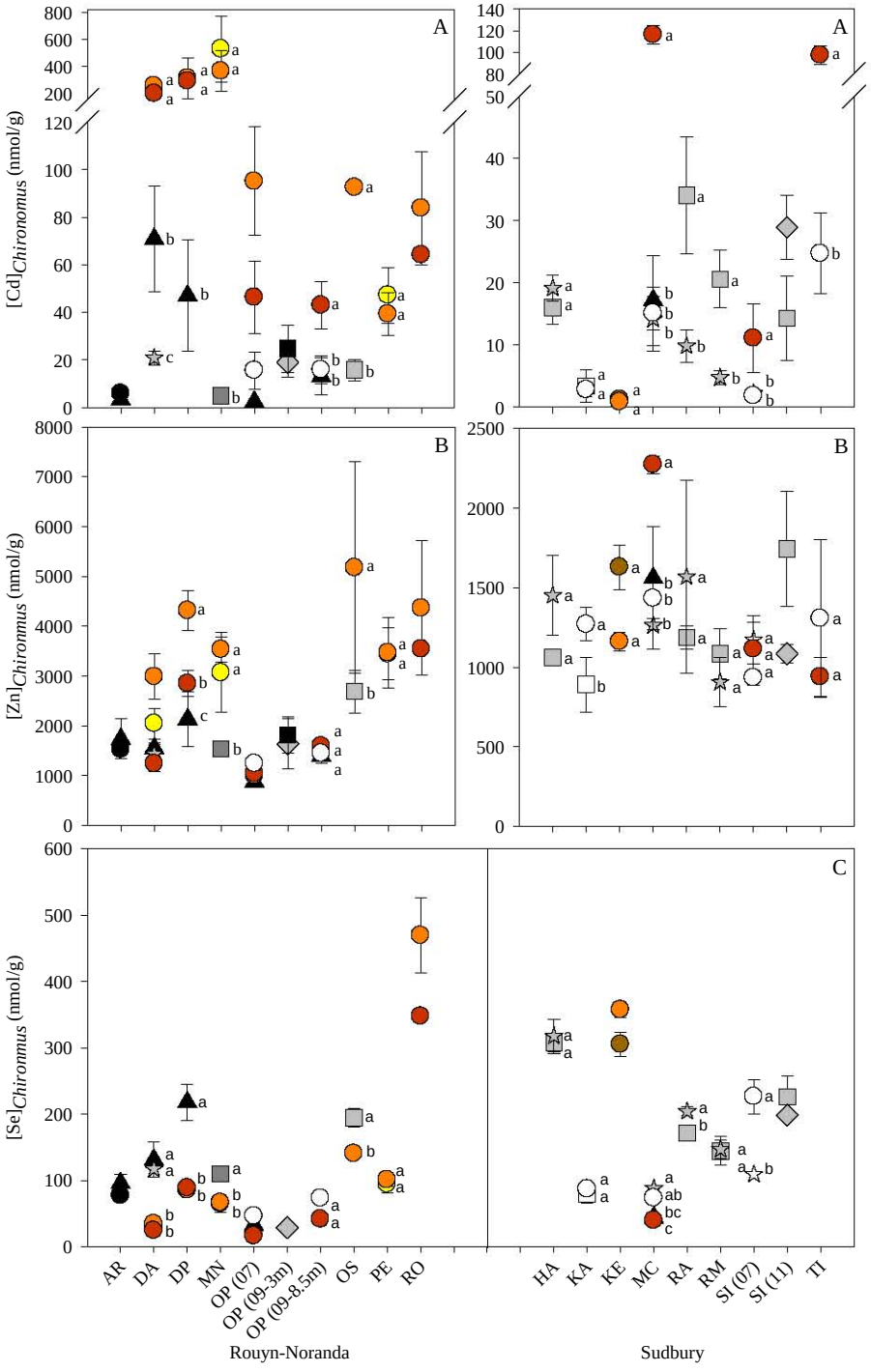


Figure 3. **Mean (± SD; n=3-8) (A) Cd, (B) Zn and (C) Se concentrations (nmol/g dry weight) in *Chironomus* species (see Table 2 for species names) collected from various lakes (see Table 1 for lake abbreviations). Note the differences in the vertical scales for Cd and Zn concentrations. For a given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

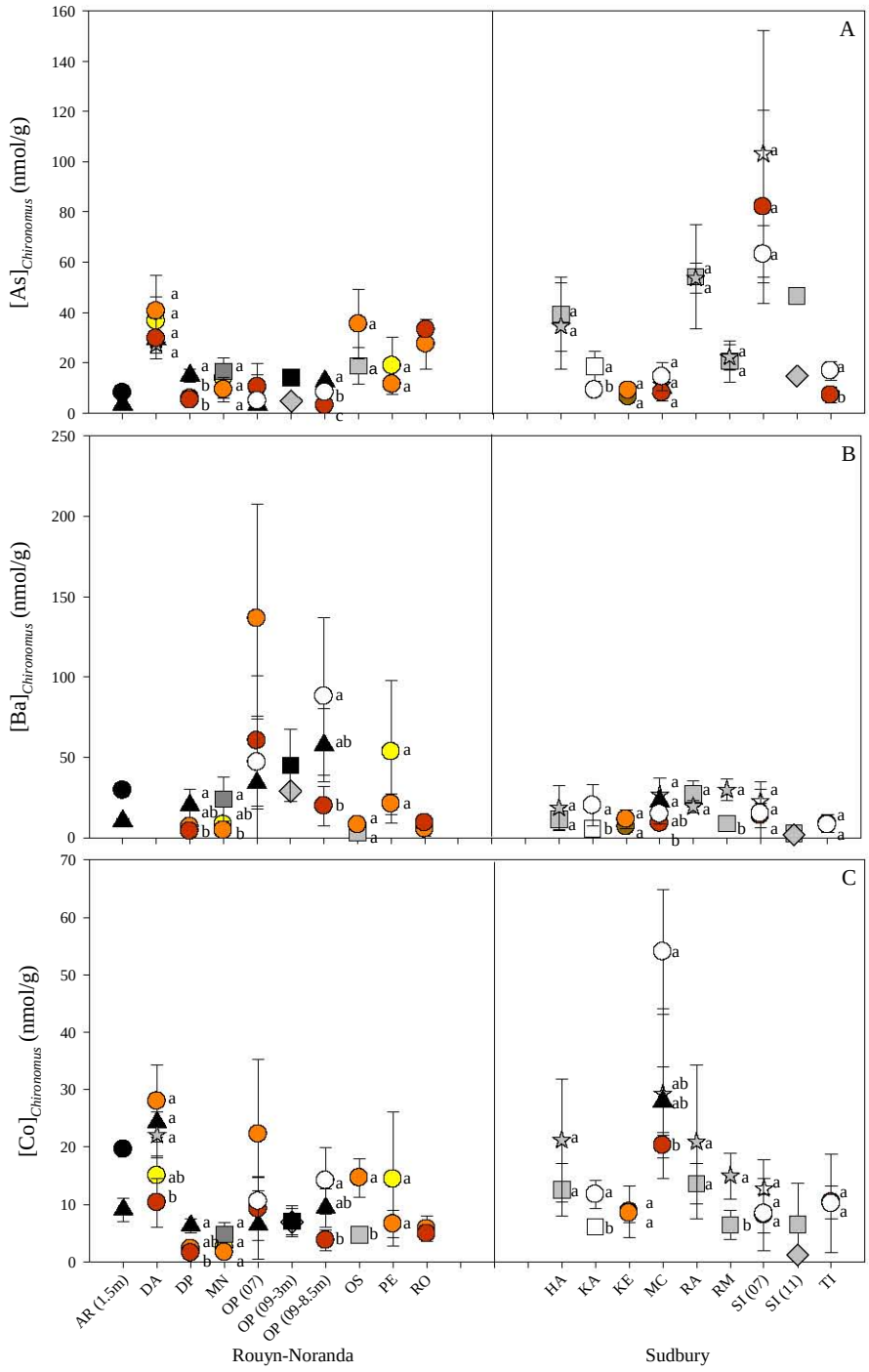
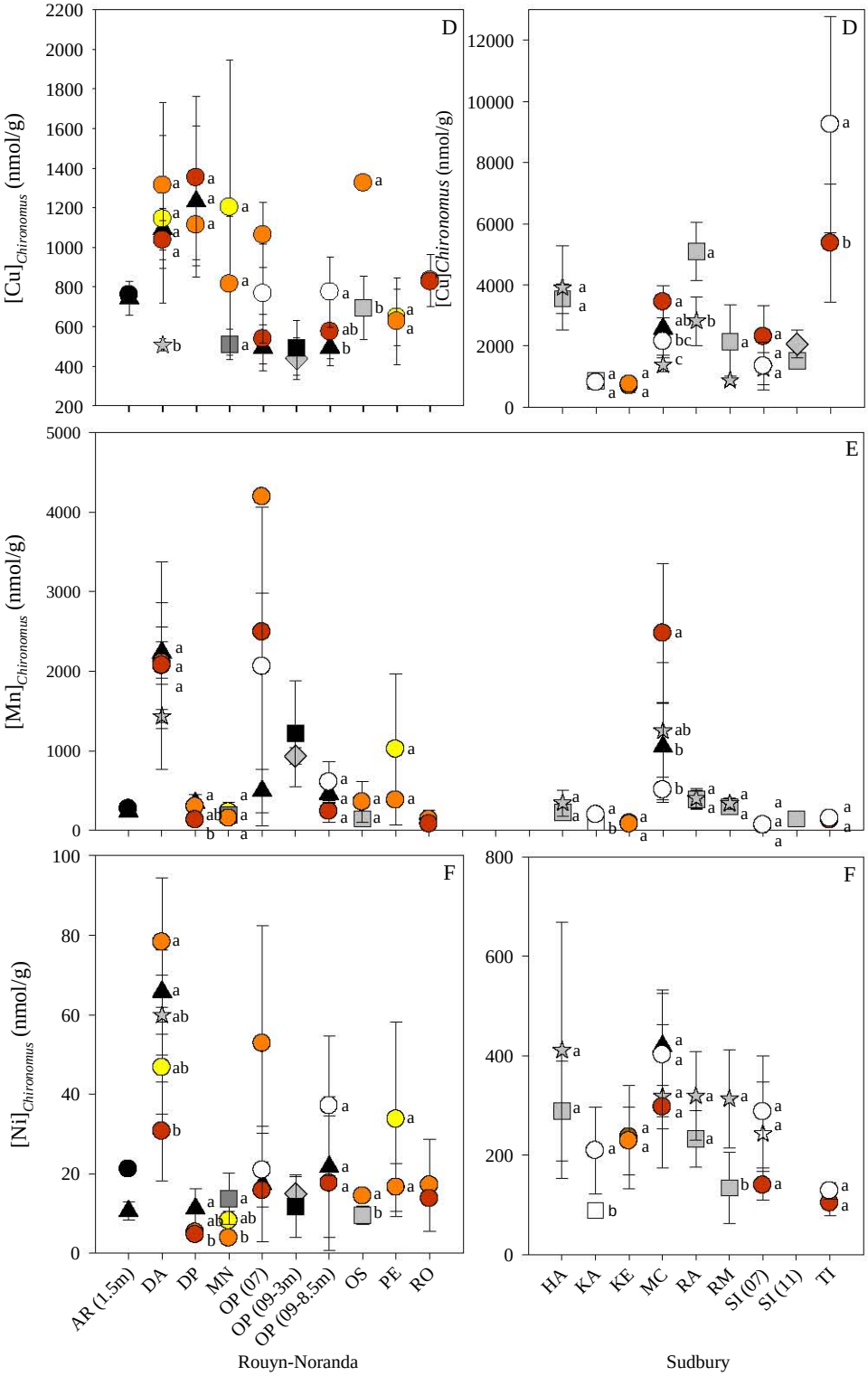


Figure 4. **Mean (± SD; n=3-8) (A) As, (B) Ba, (C) Co, (D) Cu, (E) Mn and (F) Ni concentrations in *Chironomus* species (see Table 2 for species names) collected from our study lakes (see Table 1 for lake abbreviations). Note the differences in the vertical scales for Cu and Ni. For a given lake, values that do not differ significantly (p>0.05) are followed by the same letter (except for values where n=1).**

Fig. 4 (cont.)


D
E
F
[Cu]Chironomus (nmol/g)
[Mn]Chironomus (nmol/g)
[Ni]Chironomus (nmol/g)
AR (1.5m)
DA
DP
MN
OP (07)
OP (09-3m)
OP (09-8.5m)
OS
PE
RO
HA
KA
KE
MC
RA
RM
SI (07)
SI (11)
TI
Rouyn-Noranda
Sudbury

Explaining differences in trace element concentrations among sympatric *Chironomus* species

The surprisingly large differences that we measured in the concentrations of some trace elements among sympatric *Chironomus* species can be explained by several factors. We have structured our discussion of these factors around the terms of the biodynamic model given in equation 1, in which changes over time in the 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,

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

(uptake from water) (uptake from food) (physiological loss) ("loss" via growth dilution)

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), Eq. (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 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 ($[TE_{water}]$).

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.

Although there are no published comparisons of uptake rates of dissolved trace elements between *Chironomus* species, Buchwalter & Luoma (2005) found that rates of dissolved metal uptake were similar among species of the same insect genus, but differed among insects from

different orders (Ephemeroptera, Plecoptera and Trichoptera). Likewise, Cain *et al.* (2011) reported that rates of metal uptake from water were similar among species of a given mayfly genus but differed among mayfly genera. Overall, 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 it oxygenated (Jónasson, 1972; Charbonneau & Hare, 1998; Stief & De Beer, 2002; Huryn, Wallace & Anderson, 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, Tessier & Hare, 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 & Wang, 2001), studies on insects in the same order as *Chironomus* (the dipteran *Chaoborus*) have shown that they do not differ among species (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 & 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.

Trace element influx from food

The uptake rate of a trace element from food is determined by the product of the concentration of the trace element in food ($[TE_{food}]$), the ingestion rate of food (IR) and the assimilation efficiency (AE) of the trace element by gut epithelial cells. In the following discussion, we do not consider 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, Tessier & Campbell, 1991b; 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 species of the same genus. 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 predatory phantom midge *Chaoborus* (Croteau *et al.*, 2001).

The type of particle that *Chironomus* larvae ingest will determine in part the trace element concentrations ($[TE_{food}]$) to which they are exposed. 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). 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 2; Proulx & Hare, 2014). In fact, species feeding on oxic particles (represented by the colored circles in Figs. 3 and 4) had higher Cd and usually Zn concentrations but lower Se concentrations than species feeding on anoxic particles (represented by the greyscale symbols in Figs. 3 and 4). Differences in feeding behavior have been corroborated by the visual examination of gut contents in two sympatric species by Martin *et al.* (2008). One of these species (*C. staegeri*) had gut contents that were rusty orange in

color, as were the oxic sediments collected with larvae, whereas the other species (*C. 'tigris')* had gut contents that were grey in color, like those of associated anoxic sediments. *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. In addition to [TE_{food}], the quality/composition of the food particles will influence the efficiency with which larvae are able to assimilate (AE) 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 & Rainbow, 2008). We evaluate these possibilities in the following subsections.

Do the Chironomus species we studied feed on phytoplankton or sediment?

To evaluate the likelihood that the *Chironomus* species feeding on oxic particles assimilate trace elements mainly from suspended phytoplankton, we compared larval concentrations of all the trace elements measured in this study to those in lakewater (Fig. S2, Table S13). We also compared the concentrations of trace elements in larvae feeding on anoxic particles to those in lakewater to further confirm that these larvae do not feed on phytoplankton (Fig. S2, Table S13). For these comparisons, we grouped species according to their feeding behavior (Table 2). Data from Lake Arnoux (1.5 m), Lake Opasatica in 2009 and Silver Lake in 2011 were not included in these comparisons (or those with sediment discussed below) because we did not measure trace elements in water and sediments, either at the site (Lake Arnoux) or in those years. 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 because concentrations of trace elements in phytoplankton should reflect those in water. In theory, trace elements concentrations in phytoplankton should reflect those in water. The precedent for such comparisons is that strong correlations have been reported between Cd, Se and Ni concentrations in lakewater and those in the predatory insect *Chaoborus* (Munger & Hare, 1997; Croteau *et al.*, 1998; Ponton & Hare, 2009; Ponton & Hare, 2013) even though this insect takes up these trace elements mainly from its zooplankton food (Orvoine *et al.,* 2006; Ponton & Hare, 2013).

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 (Fig. S2, Table S13). 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 ion rather than their total dissolved concentration (Campbell, 1995), we also calculated free ion concentrations in lakewater (Fig. 1) for comparison with those in *Chironomus* larvae. The percentages of Ba (34-89%), Cd (21-84%), Co (21-88%), Mn (10-79%), Ni (14-86%), and Zn (10-80%) present as free ions varied widely among lakes due to differences in lakewater chemistry. In the case of Cu, free-ion concentrations were very low (<10%) in all lakes except for Lake Arnoux where it represented 75% of the total dissolved Cu. In fact, in this highly acidic lake (pH ~4), 70-80% of the Ba, Cd, Co, Cu, Mn, Ni and Zn were present as free ions and the concentrations of Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} were several orders of magnitude higher than those in the other study lakes. 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 (Fig. S3, Table S13).

In contrast, Ni concentrations in *Chironomus* larvae were correlated ($p<0.05$) with those in lakewater (total and free ion; Figs. S1G and S2F, Table S13), as were Ni concentrations between lakewater and sediment ($p<0.001$). However, Sudbury lakes have received high loadings of Ni compared to those in the Rouyn-Noranda area such that mean Ni concentrations in sediment, water and larvae from the former region were respectively 27, 44 and 9 times higher than in those of the latter region. In contrast, within a given region there were no significant ($p>0.05$) relationships between the concentrations of Ni in *Chironomus* larvae and in lakewater.

In the case of anions such as Se, their bioavailability is influenced by their oxidation state (Luoma & Rainbow, 2008). Even so, comparing Se concentrations in *Chironomus* larvae to those of various Se species (organo-Se, selenite, selenate, organo-Se + selenite and organo-Se + selenate), for a subset of our study lakes (Ponton & Hare, 2013), did not markedly improve relationships between Se concentrations in *Chironomus* and those in lakewater (Fig. S4). Although the Se concentrations of *Chironomus* species feeding on oxic sediments were correlated with total dissolved Se concentrations ($p=0.04$; Fig. S2H, Table S13), this is likely a consequence of the fact that Se concentrations in water were significantly correlated with those in oxic sediments ($p=0.02$).

Since we collected *Chironomus* larvae from lakes having a wide range in pH (3.8-8.3) and Ca concentrations (Ca=0.03-4.60 mmol/L; Ca^{2+}=0.02-2.03 mmol/L), 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 lakewater. 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 (Ponton & Hare, 2009; Hare, Tessier & Croteau, 2008; Craig, Hare & Tessier, 1999) into planktonic food chains (Orvoine, Hare & Tessier, 2006). Likewise, high Ca^{2+} concentrations are reported to decrease Cd accumulation by larvae of *Chironomus 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 lakewater and in *Chironomus* larvae (data not shown).

Lastly, if *Chironomus* larvae are feeding on phytoplankton, then trace element concentrations in their gut contents/feces should be correlated with those in lakewater. This was not the case for most trace elements ($p>0.05$ except for Ni and Zn; Table S14), which further supports that idea that the *Chironomus* species we studied do not feed on phytoplankton in the water column.

Overall, the lack of relationships between trace element concentrations in lakewater and in larvae (or their feces/gut contents) confirms that *Chironomus* species feeding on anoxic particles do not ingest particles 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, Hare & Tessier, 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.

Assessing whether the concentrations and/or bioavailability of Cd, Se and Zn differ between oxic and anoxic food particles

On the one hand, the differences that we measured between Cd, Se and Zn concentrations in some sympatric *Chironomus* species could be explained by differences in the bioavailability of these trace elements in oxic and anoxic food particles, which would influence the efficiency with which larvae assimilate trace elements from these particles (AE). On the other hand, the concentration of trace elements, that is $[TE]_{food}$, could differ between the two sediment types. If the latter is the case, then the concentrations of Cd and Zn should have been higher in oxic sediments than in anoxic sediments, which was not the case. 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 the oxic sediments (Tables S6 and S12). Likewise, in most lakes, the concentrations of Se did not differ significantly ($p>0.05$) between oxic and anoxic sediments (Table S11) in spite of the fact that Se concentrations were higher in larvae feeding on anoxic sediments.

The lack of a trend between trace element concentrations in sediments and in larvae could be obscured if larvae select the particles on which they feed such that the trace element concentrations in bulk sediments do not represent those in the particles that larvae actually ingest (that is, $[TE]_{food}$). To test this possibility, we compared As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn concentrations in larval feces/gut contents to those in sediments (Fig. 5, Table S14). If larvae do not select particles, then the concentrations of all elements in these two media should be correlated. Such correlations were observed for several elements which further support the idea that the *Chironomus* species we studied feed mainly on deposited particles. However, in the case of *Chironomus* species feeding on oxic particles, the concentrations of Cd in feces/gut contents were not correlated with those in oxic sediments (Fig. 5C, Table S14). Likewise, in species feeding on anoxic particles the concentrations of As, Cd and Cu in feces/gut contents were not correlated with those anoxic sediments (Figs. 5A, 5C and 5E, Table S14). These results suggest that larvae select in part the particles on which they feed.

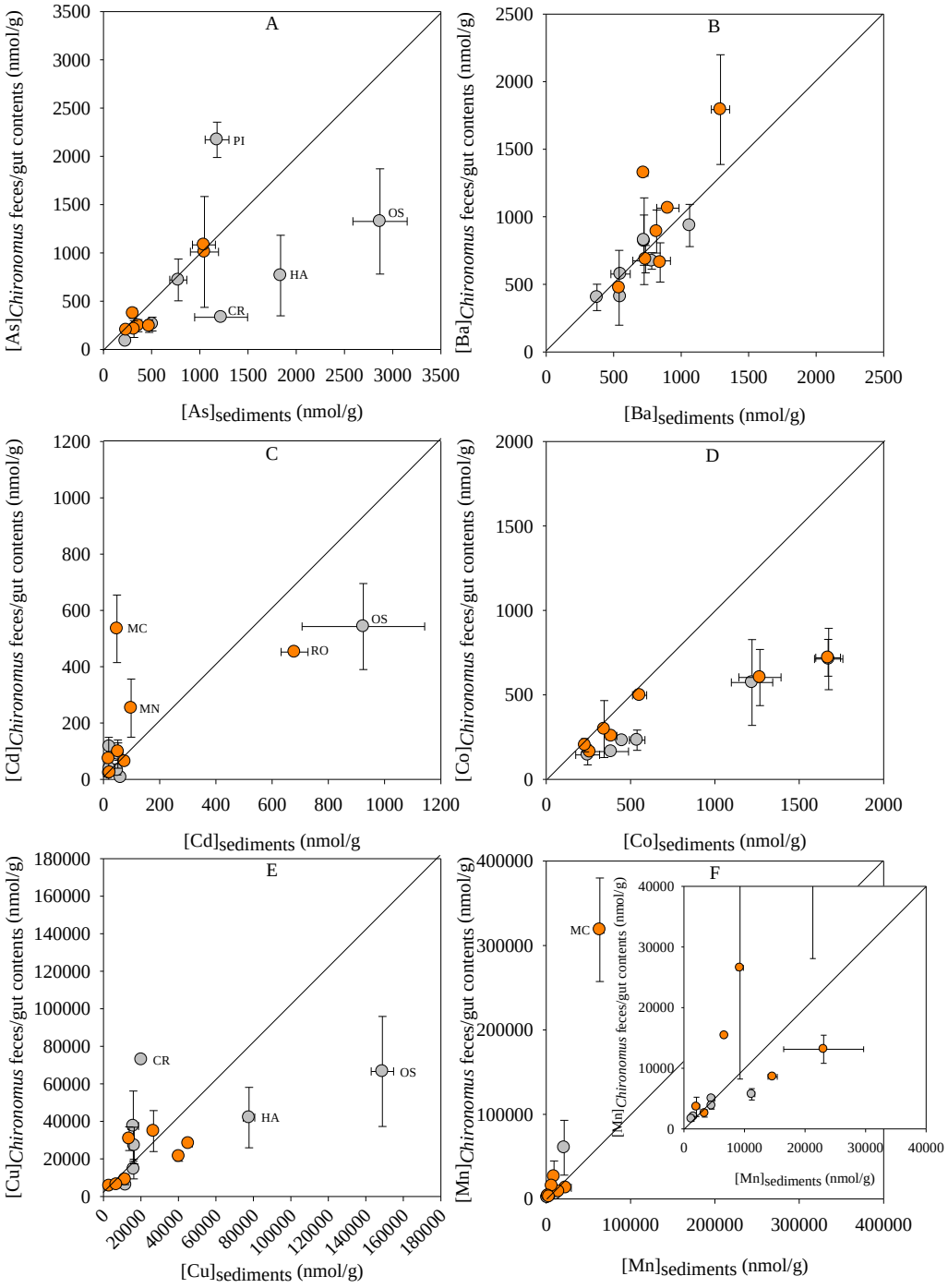
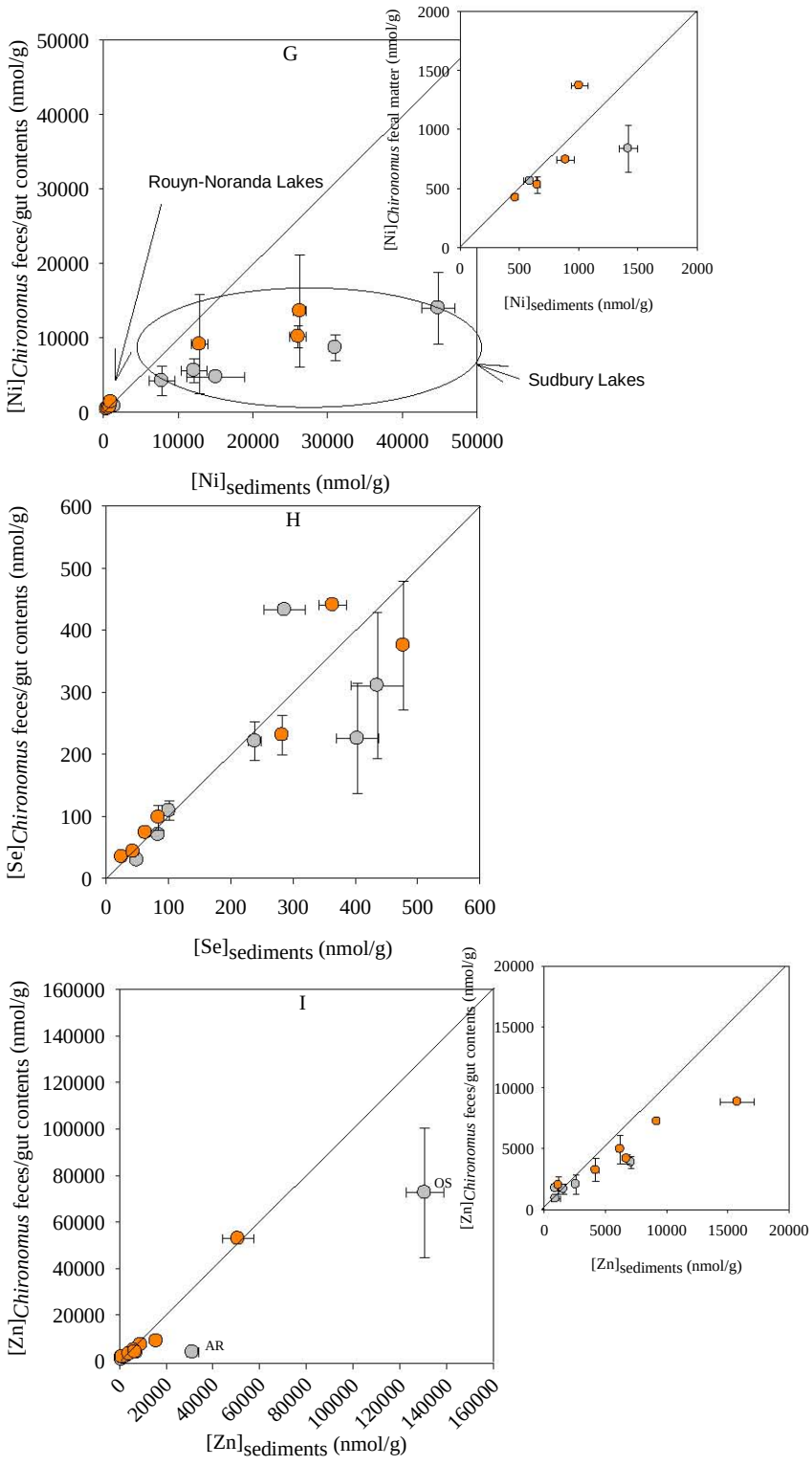


Figure 5. **Bivariate scatterplots of mean (± SD; n=2-19) (A) As, (B) Ba, (C) Cd, (D) Co, (E) Cu, (F) Mn, (G) Ni, (H) Se and (I) Zn concentrations in feces/gut contents of *Chironomus* larvae feeding on oxic particles (n=1-7) to those of oxic sediments (n=3) upon which larvae are thought to feed (●) and in feces/gut contents of *Chironomus* larvae feeding on anoxic particles (n=1-6) to those of anoxic sediments (n=3) on which larvae are thought to feed (●). The 1:1 line is shown. Lake names are given in Table 1.**

Fig. 5 (cont.)


G
Rouyn-Noranda Lakes
Sudbury Lakes
[Ni]Chironomus feces/gut contents (nmol/g)
[Ni]sediments (nmol/g)
[Ni]Chironomus fecal matter (nmol/g)
H
[Se]Chironomus feces/gut contents (nmol/g)
[Se]sediments (nmol/g)
I
OS
AR
[Zn]Chironomus feces/gut contents (nmol/g)
[Zn]sediments (nmol/g)

To evaluate the suitability of using measurements in bulk sediments to estimate [Cd, Se or Zn]$_{food}$, we compared the ratios of Cd, Se and Zn concentrations in bulk sediments to those in larval feces/gut contents. The ratios of Se concentrations in feces/gut contents of *Chironomus* species feeding on oxic or anoxic sediments to Se concentrations in sediments were close to unity (Fig. 5H), as were those for Zn (Fig. 5I) with a few exceptions (Zn concentrations in feces/gut contents were lower for species feeding on anoxic particles in Lakes Arnoux and Osisko). Thus for Zn and Se, oxic or anoxic sediments collected in a larva's surroundings are a reasonable surrogate for the trace element concentrations in the particles that it actually consumes. In contrast, Cd concentrations in feces/gut contents of *Chironomus* species feeding on oxic or anoxic sediments were not correlated with those in bulk sediments indicating that Cd concentrations in bulk sediments do not represent those in the particles ingested by larvae (Fig. 5C). Thus, to further test whether Cd concentrations differed between the oxic and anoxic particles ingested by larvae, we compared Cd concentrations in the feces/gut contents of sympatric *Chironomus* species feeding on these particle types in two of our study lakes (McFarlane and Crooked). Concentrations of Cd were not necessarily higher in the feces/gut contents of larvae feeding on oxic particles than in those feeding on anoxic particles (Tables S6). Thus, Cd, Se and Zn concentrations in sediments or in feces/gut contents indicate that differences in larval trace element concentrations are most likely due to differences in the bioavailability of these trace elements in food rather than to differences in their concentrations.

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 Zn differ between oxic and anoxic sediments in our study lakes. In addition, our results indicate that all of our study species ingest deposited particles and that larvae do not feed on bulk sediments, but select particles, such that trace element concentrations in bulk sediments do not always represent those in larval food.

Using *Chironomus* larvae to evaluate the bioavailability of trace elements in sediments

If *Chironomus* larvae are to be used to assess the bioavailability of a given trace element in sediments, they should not be able maintain constant their concentrations of that trace element. In addition, larvae should take up the majority of the trace element from sediments rather than from the overlying water that they pump through their tube for respiratory purposes. To evaluate the likelihood that larvae take up their trace elements from water, we compared concentrations of trace elements in larvae to those in lakewater. Since the bioavailable concentration of metals can be more easily estimated in water (i.e. free metal ions) than in sediments, we consider that food is the main source of a given metal when the concentration of that metal in larvae does not correlate with that in water even if it does not correlate with that in sediments either.

The assessment of *Chironomus* larvae as biomonitors should ideally be based on comparative measurements of trace element concentrations in larvae from a large number of lakes and for many species. Previous data on trace element concentrations in free-living *Chironomus* larvae are limited to those for a few metals (Cd, Cu and Zn) in a small number of lakes (Martin *et al.*, 2008; Hare, Tessier & Warren, 2001; Hare *et al.*, 1991b). To overcome in part this limitation, we present below, the ranges of trace element concentrations in the *Chironomus* species that we studied (detailed values are given in Tables S4-S12) as well as an element-by-element summary of the ranges in concentrations that we measured in water and in sediments. Note that the concentrations of As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn (Figs. 1 and 2; details in Tables S4-S12) in water and sediments were within the range of concentrations previously reported for lakes in our study area (Borgmann *et al.*, 2004; Couture *et al.*, 2008; Pyle, Rajotte & Couture, 2005; Giguère *et al.*, 2006; Giguère *et al.*, 2004; Shuhaimi-Othman *et al.*, 2006; Borgmann & Norwood, 2002).

Table 3. **Range in mean As, Cd, Co, Cu, Mn, Ni and Zn concentrations (nmol/g) in *Chironomus* species collected in Rouyn-Noranda and Sudbury lakes and lethal body concentrations for *Hyalella azteca* determined from chronic toxicity tests (LBC25).**

	As	Cd	Co	Cu	Mn	Ni	Zn
Rouyn-Noranda lakes	3-66	2-526	2-42	439-3847	58-4191	4-66	870-5386
Sudbury lakes	6-103	1-116	1-54	697-9238	62-2476	45-549	805-2272
LBC25	125[a]	270[b]	103[a]	2380[b]	57900[a]	194[b]	4420[b]

[a]Norwood, Borgmann & Dixon (2007)
[b]Borgmann *et al.* (2004)

Although some *Chironomus* species (i.e., *C. riparius*, *C. tentans* and *C. dilutus*) are widely used in toxicity tests with trace elements, data on the concentrations in larvae that result in toxicity are lacking. To this end, we also compare trace element concentrations in *Chironomus* larvae to the lethal body concentrations reported for the crustacean *Hyalella azteca* (As, Cd, Co, Cu, Mn, Ni and Zn; Table 5). Since *H. azteca* is considered to be more sensitive to trace elements than are *Chironomus* larvae (Besser *et al.*, 2013), trace element concentrations in *Chironomus* species that are lower than the lethal body concentrations for *H. azteca* is considered safe.

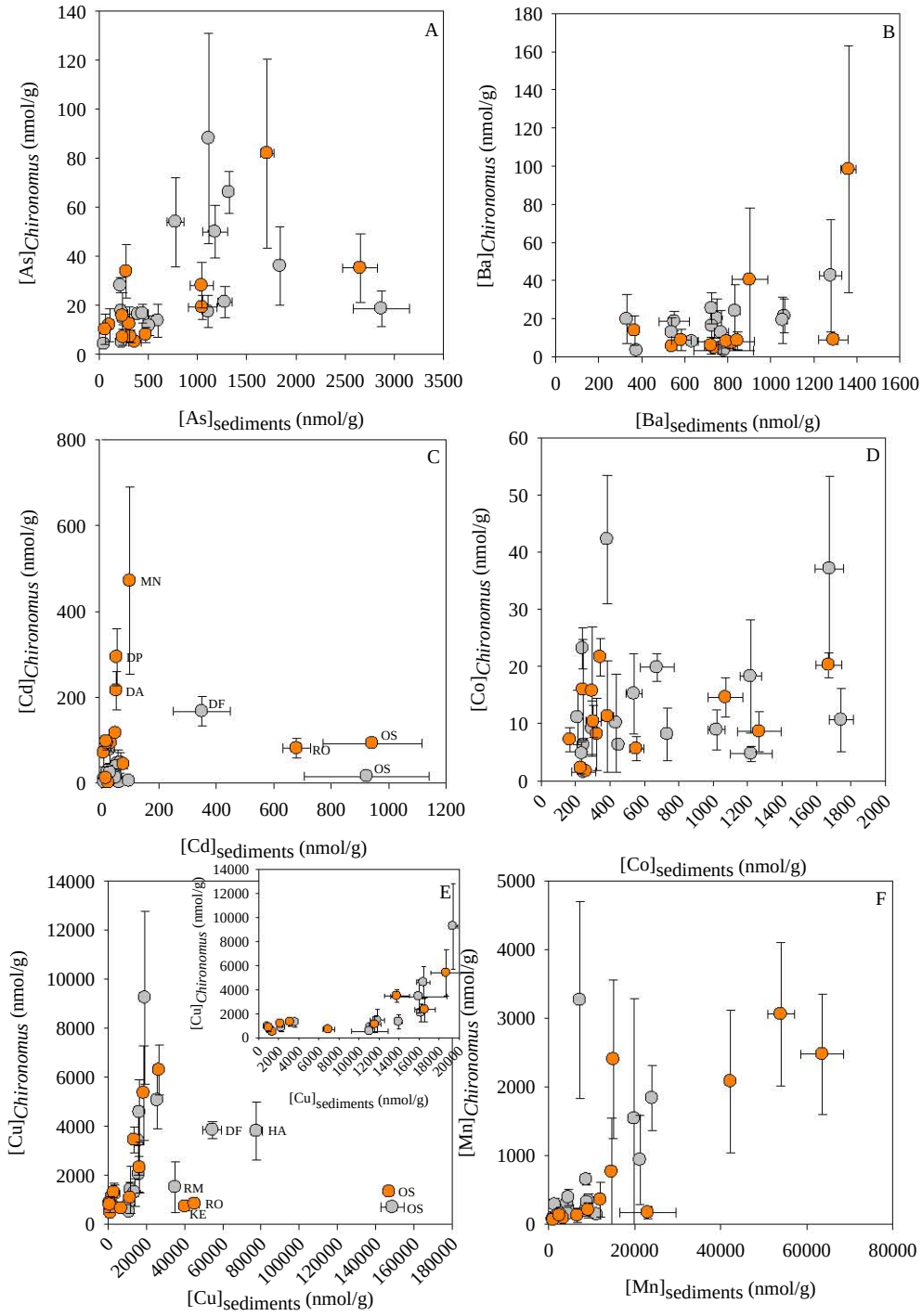
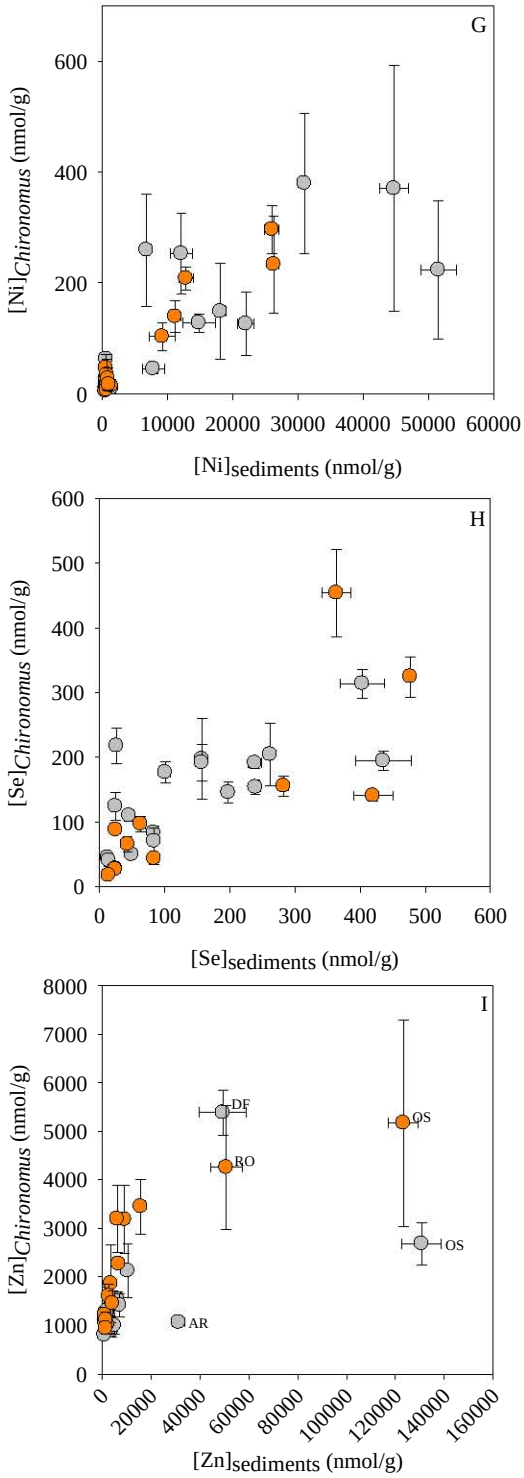


Figure 6. **Bivariate scatterplots of mean (± SD) (A) As, (B) Ba, (C) Cd, (D) Co, (E) Cu, (F) Mn, (G) Ni, (H) Se and (I) Zn concentrations in *Chironomus* larvae feeding on oxic particles (n=2-19) to those of oxic sediments (n=3) upon which larvae are thought to feed (●) and in *Chironomus* larvae feeding on anoxic particles (n=2-17) to those of anoxic sediments (n=3) on which larvae are thought to feed (●). The names of labelled lakes are given in Table 1.**

Fig. 6 (cont.)


G
[Ni]Chironomus (nmol/g)
[Ni]sediments (nmol/g)
H
[Se]Chironomus (nmol/g)
[Se]sediments (nmol/g)
I
[Zn]Chironomus (nmol/g)
[Zn]sediments (nmol/g)
DF
RO
OS
OS
AR

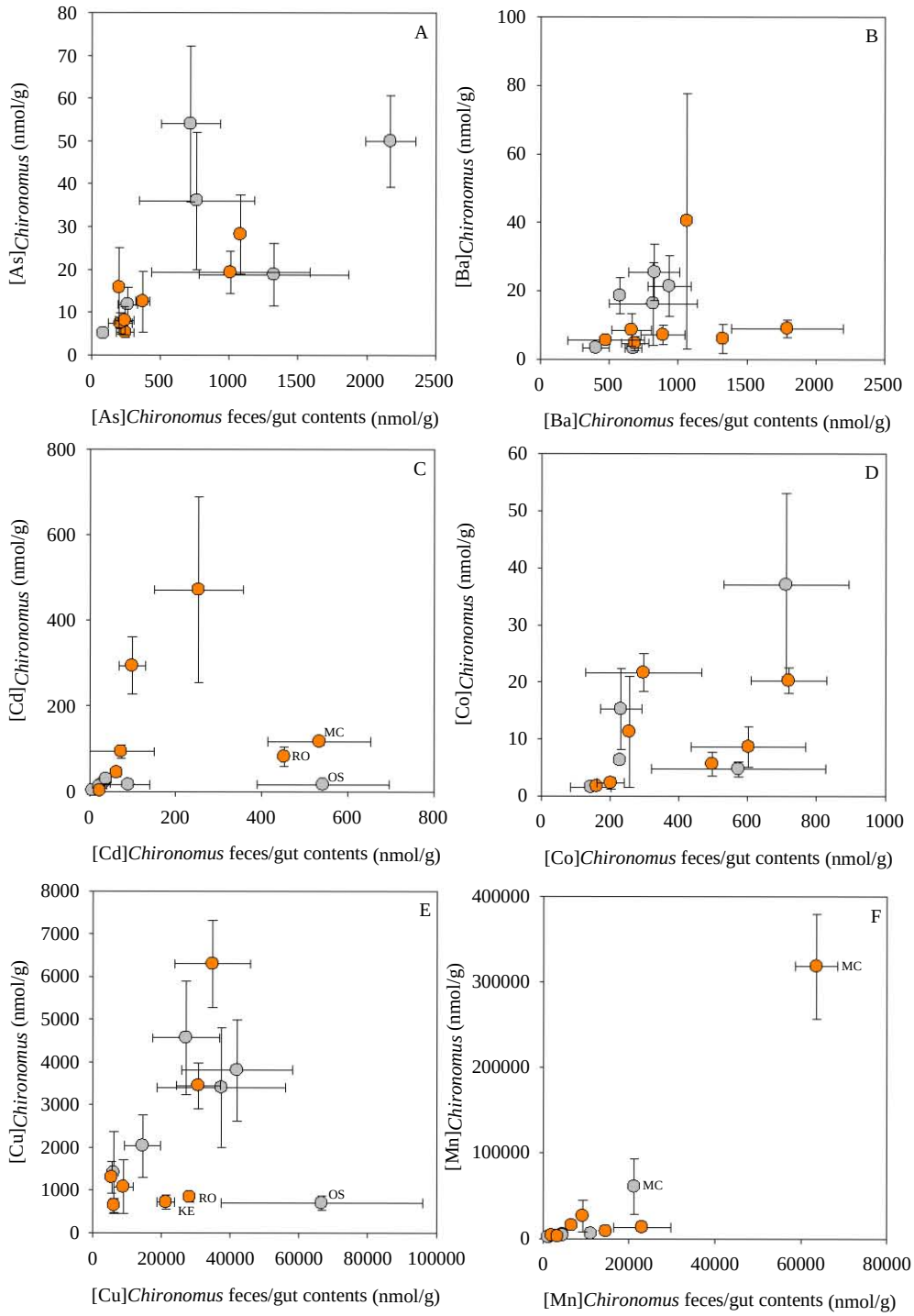
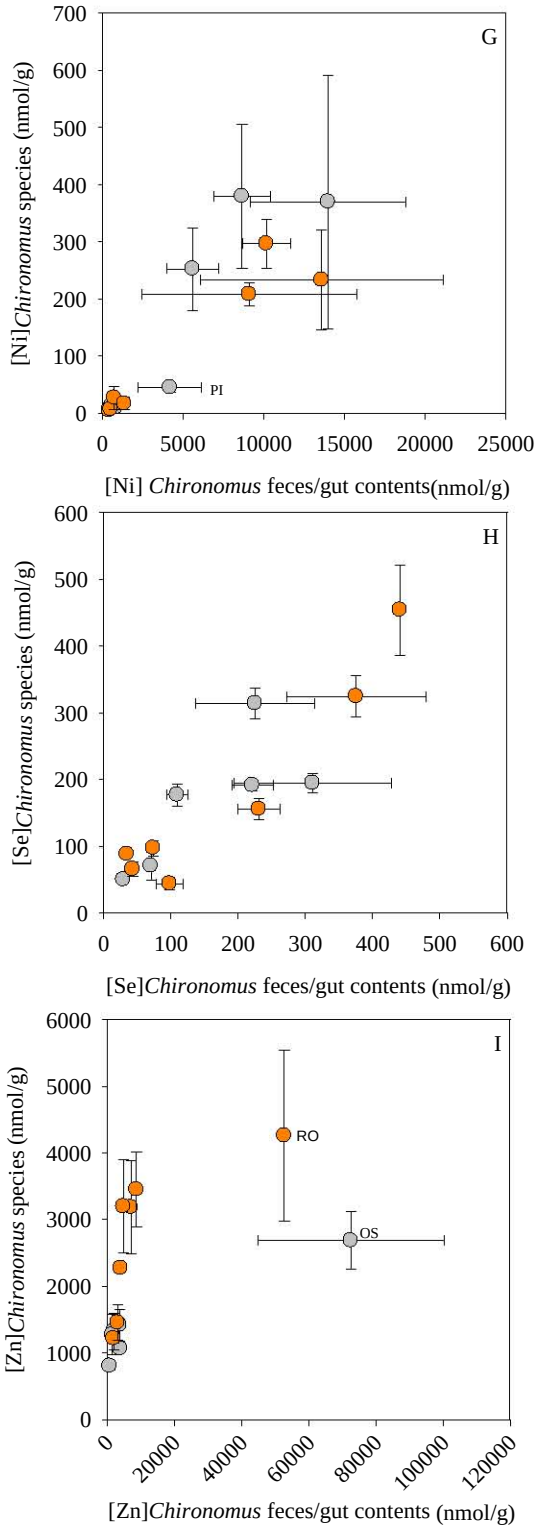


Figure 7. **Bivariate scatterplots of mean (± SD) (A) As, (B) Ba, (C) Cd, (D) Co, (E) Cu, (F) Mn, (G) Ni, (H) Se and (I) Zn concentrations in *Chironomus* larvae feeding on oxic particles (n=2-19) to those in their feces/gut contents (n=1-7) (●) and in *Chironomus* larvae feeding on anoxic particles (n=2-17) to those in their feces/gut contents (n=1-6) (●). Lake names are given in Table 1.**

Fig. 7 (cont.)


G
[Ni]*Chironomus* species (nmol/g)
[Ni] *Chironomus* feces/gut contents(nmol/g)
PI
H
[Se]*Chironomus* species (nmol/g)
[Se]*Chironomus* feces/gut contents (nmol/g)
I
[Zn]*Chironomus* species (nmol/g)
[Zn]*Chironomus* feces/gut contents (nmol/g)
RO
OS

Arsenic (As)

Among lakes, As concentrations varied by 10 fold (4-43 nM; Fig 1) in water and 60 fold (50-2,870 nmol/g; Fig. 2) in sediments (Table S4). Likewise, As concentrations varied by 30 fold in *Chironomus* species (3-103 nmol/g; Fig. 4A, Table S4), which indicates that larvae do not maintain constant their internal concentrations of this essential trace element (Luoma & Rainbow, 2008) and are thus are potential biomonitors for it. In the Rouyn-Noranda and Sudbury lakes, As concentrations in water were below the Canadian probable effect level (PEL) for the protection of aquatic life. However, As concentrations in sediments were above the sediment PEL in all the Sudbury lakes and in all lakes located within a 15-km radius from the Rouyn-Noranda smelter. Despite the concentrations of As being relatively high in some lake sediments, As concentrations in *Chironomus* larvae were lower than the lethal body concentrations for *H. azteca* (Table 3), which suggests that the As in larvae is not toxic to them.

Arsenic concentrations in larvae were not significantly correlated with those in water ($p>0.05$; Fig. S2A, Table S4), but were correlated with those in sediments ($p<0.05$; Fig. 6A, Table S13), indicating that sediments are the most likely source of As to larvae. When significant, As concentrations in sediments explained 30 to 55% of those in larvae (Table S13), which suggests that *Chironomus* larvae have potential for assessing As bioavailability in sediments.

One possible caveat in using burrowing animals as biomonitors for As is that this element can be precipitated with iron oxides on the surface of insects, rather than having been internalized (Hare *et al.*, 1991a; Lacharité, 2011), which could produce misleading correlations between As concentrations in sediments and animals. To verify that the As we measured in *Chironomus* larvae had been truly bioaccumulated, we measured As concentrations in the body, gut, and hemolymph of several *Chironomus* species collected from several lakes (data not shown). On average, 70% of the As was located in the gut and hemolymph of depurated larvae (the balance was in the muscles, exoskeleton, etc.), which suggests that the majority had indeed been assimilated and thus our As measurements in larvae represent bioavailable As.

The 1:1 ratio between As concentrations in oxic sediments and in the feces/gut contents of *Chironomus* larvae feeding on those sediments indicates that As concentrations in bulk oxic sediments represent well the As concentrations that larvae are ingesting (Fig. 5A, Table S14). Additionally, correlations between As concentrations in larvae feeding on oxic particles and As concentrations in their feces/gut contents ($p=0.013$) and oxic sediments ($p=0.027$) indicate that our digestion method estimates well the bioavailable fraction of As in oxic particles (Figs. 6A

and 7A, Table S13-S14). However, for species feeding on anoxic particles, the absence of correlations between As concentrations in feces/gut contents and anoxic sediments indicates that bulk anoxic sediments do not represent well what larvae are ingesting (Fig. 5A; Table S14). Furthermore, the lack of a correlation between As concentrations in larvae feeding on anoxic particles and As concentrations in their feces/gut contents indicate that our digestion method does not estimate well the bioavailable fraction of As in anoxic particles (Figs. 6A and 7A, Table S13-S14).

In the presence of oxygen, oxidized As (As (V)) tends to be strongly bound to Fe oxides, whereas under reducing conditions it is mobilized as dissolved As (III) (Luoma & Rainbow, 2008) and thus potentially more bioavailable. The fact that As concentrations in larvae did not differ between sympatric species feeding on oxic and anoxic sediments suggests that As concentrations in pore waters do not control As concentrations in *Chironomus* larvae, which is consistent with the fact that larvae irrigate their tubes with overlying oxic lakewater. Thus, *Chironomus* larvae can be pooled for As measurements. Overall, the lack of difference in As concentrations between sympatric *Chironomus* species indicates that the bioavailable concentration of As does not differ between the oxic and anoxic particles ingested by these larvae and that species can be pooled in contaminant studies on As.

Barium (Ba)

Among lakes, Ba concentrations varied 5 fold in sediments (295-1,361 nmol/g; Fig. 2), 10 fold in water (20-225 nM; Fig. 1) and 140 fold in *Chironomus* larvae (2-282 nmol/g; Fig. 4B) (Table S5). This wide variation in larval Ba concentrations suggests that larvae have the potential to be biomonitors for this metal.

Barium concentrations in larvae were not correlated with those in water (total dissolved or free ion concentrations) ($p>0.05$; Figs. S1B and S2A, Table S13), which suggests that larvae take up their Ba from food and not from water. However, Ba concentrations in larvae were also not significantly ($p>0.05$) related to those in either sediments (Fig. 6B, Table S13) or feces/gut contents (Fig. 7B, Table S14), which suggests that our digestion method does not provide a good estimate of the Ba that would be released for uptake by gut tissues. The ratios of Ba concentrations in the feces/gut contents of *Chironomus* larvae to those in sediments were close to unity, suggesting that bulk oxic and anoxic sediment concentrations represent well what larvae are ingesting (Fig. 5B, Table S14).

The fact that Ba concentrations did not differ between *Chironomus* species feeding on oxic and anoxic sediments suggests that the bioavailable concentration of Ba does not differ between oxic and anoxic sediments and that *Chironomus* species can be pooled for Ba measurements.

Cadmium (Cd)

Among our study lakes, Cd concentrations varied by 70 fold (0.15-10.27 nM; Fig. 1) in water and 190 fold in sediments (5-943 nmol/g; Fig. 2) (Table S6). Sixty percent of the measured values in water and sediments were above the PEL values for the protection of aquatic life (0.74 µM for water and 31 nmol/g for sediments) (CCME, 2014). Indeed, the results of a previous laboratory study using sediments from one of our study lakes (Lake Dufault) suggest that Cd was the metal responsible for toxic effects to *Hyllela azteca* (Borgmann *et al.*, 2004).

In *Chironomus* larvae, Cd concentrations varied by 530 fold (1-526 nmol/g) among our study lakes (Fig. 3A, Table S6). In fact, in Lakes Duprat and Marlon (Rouyn-Noranda), *Chironomus* larvae feeding on oxic sediments exceeded the lethal body guideline for *H. azteca* (Table 3). This wide range in larval Cd concentrations indicates that larvae have potential as biomonitors for this metal.

There were no correlations between Cd concentrations in larvae and total dissolved or free ion Cd concentrations in water ($p>0.05$; Figs. S1C and S2B, Table S13). Even considering the competitive effect of lakewater pH and Ca concentrations did not result in significant correlations ($p>0.05$), which suggests that larvae do not take up the majority of their Cd from water. However, there was also no correlation between Cd concentrations in larvae and sediments ($p>0.05$; Fig. 6C, Table S13), which is likely due in part to the fact that Cd concentrations in bulk sediments do not represent those in the particles ingested by larvae (Fig. 5C, Table S14). However, Cd concentrations in feces/gut contents were also not correlated with those in larvae ($p>0.05$; Fig. 7C, Table S14). We hypothesize that this lack of correlation suggests that our digestion method does not mimic conditions in the larval gut rather than indicating that larvae do not take up their Cd from food.

Within lakes, Cd concentrations in *Chironomus* species feeding on oxic sediments were always higher than those feeding on anoxic sediments (Fig. 3A), which indicates that species should not be pooled for Cd measurements. Since, at a given site, Cd concentrations in bulk oxic sediments were not higher than in anoxic sediments and were not higher in feces/gut contents of species feeding in oxic sediments than in those feeding in anoxic sediments (Table S6), larval

Cd concentrations suggest that Cd is far less bioavailable in anoxic sediments than in oxic sediments. In anoxic sediments, Cd is likely precipitated with sulfides, which could explain the low bioavailability of Cd to larvae feeding on anoxic particles.

Cobalt (Co)

Among lakes, Co concentrations varied by 370 fold (0.42-154.00 nM; Fig. 1) in water and 10 fold (137-1,744 nmol/g; Fig. 2) in sediments (Table S7). They also varied widely in *Chironomus* larvae (27-fold, 2-54 nmol/g; Fig. 4C, Table S7), even though Co is an essential metal for all animals. These wide variations indicate that larvae do not maintain constant their concentrations of this essential element and thus have potential as biomonitors for it. Cobalt concentrations in larvae were below the lethal body concentrations guideline for *H. azteca* (Table 3) suggesting that in our study lakes, Co concentrations in larvae are not lethal.

Larval Co concentrations were not related to those in water (total dissolved or free ion) ($p>0.05$; Figs. S1D and S2C, Table S13), indicating that larvae most likely accumulate Co from food rather than from water. However, larval Co concentrations were not related to those in sediments ($p>0.05$; Fig. 6D, Table S13) or in their feces/gut contents ($p>0.05$; Fig. 7D, Table S13), which suggests that the digestion method used does not estimate well the bioavailable fraction of Co.

Cobalt concentrations in the feces/gut contents of *Chironomus* larvae were correlated with those in the sediment layer (oxic or anoxic) on which larvae are assumed to feed (Fig. 5D, Table S14). However, Co concentrations in *Chironomus* feces/gut contents were lower than those in bulk sediments, which suggest that larvae feed on particles that are poorer in Co than the average sediment particle.

Cobalt concentrations did not differ between species feeding on oxic and anoxic sediments indicating that the bioavailable concentration of this trace element does not differ between these sediment layers. Thus, *Chironomus* species feeding on these layers can be pooled for Co measurements.

Copper (Cu)

Lakes in which we collected *Chironomus* larvae exhibited a small range in dissolved Cu concentrations (9 fold, 40-350 nM; Fig. 1) relative to those in sediments (230 fold, 650-148,830 nmol/g; Fig. 2) (Table S8). All of our study lakes exceeded the PEL value for dissolved Cu (31

nM; CCME, 2014). Likewise, all lakes in Sudbury exceeded the PEL guideline for sediment (31,000 nmol/g; CCME, 2014) as did Rouyn-Noranda area lakes receiving acid mine drainage or located close (≤14 km) to the smelter.

Because Cu is an essential metal (Rainbow 2002), its tissue concentrations are controlled in aquatic animals such as the crustacean *Hyallella azteca* (Borgmann & Norwood, 1999; Borgmann *et al.*, 2001) and the phantom midge *Chaoborus* (Croteau *et al.*, 1998), which negates the use of these widespread invertebrates as biomonitors for this metal. In contrast, Cu concentrations in *Chironomus* larvae varied widely among lakes (21 fold, 440-9240 nmol/g; Fig. 4D, Table S8), and were usually higher in Sudbury lakes, which indicates that larvae do no control their total Cu concentrations and thus are potential biomonitors of this metal. In fact, in some lakes, Cu concentrations were above the lethal body guideline for *H. azteca* (Table 3).

Copper concentrations in *Chironomus* larvae were not correlated with those in water (total dissolved or free ion concentrations) ($p>0.05$; Figs. S1E and S2D, Table S13), indicating that food is the most likely source of Cu for larvae. However, Cu concentrations in *Chironomus* larvae were not correlated with those in bulk sediments (Fig. 6E, Table S13). This is in part due to Cu concentrations in bulk sediments not representing truly the concentration of Cu larvae are ingesting (Fig. 5E, Table S14), but there was also no correlation between Cu concentrations in larvae and in their feces/gut contents (Fig. 7E, Table S14), which suggests that the digestion method used does not estimate the Cu that would be available in the larval gut. Some of our study lakes have received domestic sewage (Kelly, Osisko, Rouyn), whereas others have not, and Cu bioavailability is known to be strongly influenced by organic matter. Indeed, the Cu concentrations of larvae from lakes that have received sewage were lower than expected given the high concentrations of Cu in their sediments. When we excluded lakes that have received sewage, Cu concentrations in *Chironomus* larvae were correlated with those in their feces/gut contents ($p=0.003$; Fig. 7E). Although the percentage organic carbon was not higher in the sediments of lakes that have received sewage (Table S15), we hypothesize that the Cu is more tightly bound to the type of organic matter associated with sewage than with organic matter of planktonic or terrestrial origin.

Copper concentrations did not significantly vary between sympatric *Chironomus* species feeding on oxic or anoxic sediments, despite the fact that Cu concentrations were sometimes higher in oxic sediments than in anoxic sediments (Table S8). This similarity suggests that the

bioavailable concentration of Cu does not vary greatly between these sediment types and that *Chironomus* species can be pooled for Cu analyses.

Manganese (Mn)

Manganese concentrations varied by 60 fold in sediments (1,010-63,620 nmol/g; Fig. 1) and 360 fold in water (22-7,830 nM; Fig. 2) (Table S9). Manganese concentrations varied by 5 fold in larvae (810-4,190 nmol/g) (Fig. 4E, Table S9) indicating that larvae do not control their internal concentrations of this essential metal and are thus are potential biomonitors for it. In our study lakes, the concentrations of Mn in larvae were all below the toxicity threshold for *H. azteca* (Table 3). Since Mn can be deposited on burrowing animals as Mn oxides (Lacharité, 2011), we measured Mn concentrations in the various parts (body, gut, hemolymph) of depurated *Chironomus* larvae from several lakes (data not shown). Although ~60% of the larval Mn was found in the body portion (exoskeleton, muscles, etc.), the large lake-to-lake range in Mn concentrations that we measured in gut tissues (8,683 nmol/g) as opposed to the body (319 nmol/g) indicates that Mn is assimilated by larvae.

Manganese concentrations in larvae were not correlated with those in water (total dissolved or free ion concentrations) ($p>0.05$; Figs. S1F and S2E, Table S13) but were significantly correlated with those in sediments and in their feces/gut contents (Figs 6F and 7F, Tables S13 and S14), indicating that larvae mainly take their Mn from food. The ratio of Mn concentrations in feces/gut contents of *Chironomus* larvae and those in sediments was close to unity (except for larvae collected in McFarlane Lake), which suggests that Mn concentrations in bulk sediment represents well the Mn concentrations that larvae are ingesting (Fig. 5F, Table S14).

In the presence of oxygen, Mn (as Mn(IV)) is mainly precipitated as Mn oxides, whereas in the absence of oxygen, Mn (as Mn(II)) is in dissolved form (Luoma & Rainbow, 2008). In half of our study lakes, the concentrations of Mn were significantly higher in oxic than in anoxic sediments (Table S9), as would be expected given the diagenetic mobility of this element and its tendency to concentrate in surface oxic sediments (Belzile, De Vitre & Tessier, 1989). Likewise, Mn concentrations were higher in the feces/gut contents of larvae feeding on oxic sediments than in those feeding on anoxic sediments (Table S9). However, Mn concentrations in larvae did not differ between larvae feeding in oxic sediments and those feeding in anoxic sediments indicating that overall, the bioavailable concentration of Mn does not differ between oxic and anoxic sediments in our study lakes. Thus, *Chironomus* larvae can be pooled for Mn measurements.

Nickel (Ni)

Nickel concentrations in water (680-4,870 nM) and sediments (4,510-51,560 nmol/g) from Sudbury lakes were considerably higher than those in water (10-100 nM) and sediments (350-740 nmol/g) from lakes near Rouyn-Noranda (Figs. 1 and 2, Table S10). This marked difference between the two regions is consistent with the fact that for over a century Sudbury has been home to nickel mining, milling and smelting activities, and it is this metal that is reported to cause toxicity to benthic invertebrates in impacted Sudbury lakes (Borgmann *et al.*, 2001).

Nickel concentrations in *Chironomus* larvae from Sudbury lakes (45-400 nmol/g) were likewise generally higher than in larvae collected from Rouyn-Noranda area lakes (4-78 nmol/g) (Fig. 4F, Table S10). In some Sudbury lakes, Ni concentrations in larvae exceeded the lethal body concentrations of *H. azteca* (Table 3). The marked difference in *Chironomus* Ni concentrations between the Rouyn-Noranda and Sudbury lakes indicates that larvae do not regulate their internal concentrations of this essential metal and are thus potential biomonitors for it.

Nickel concentrations in larvae were significantly correlated with those in water ($p>0.05$; Figs. S1G and S2F, Table S13), in sediments (Fig. 6G, Table S13), as well as in the feces/gut contents of larvae (Fig. 7G, Table S13). These correlations are driven by the fact that Ni concentrations are higher in all media in the Sudbury lakes. Within each region, no significant correlations ($p>0.05$) were found between Ni concentrations in larvae and in water (total dissolved or free ion concentrations), sediments or larval feces/gut contents. The fact that Ni concentrations in feces/gut contents of *Chironomus* larvae in Sudbury lakes were lower than those in bulk sediments suggests that Ni measurements in bulk sediments overestimate those to which larvae are actually exposed (Fig. 5G).

Nickel concentrations did not usually differ between sympatric *Chironomus* species and where they did, no species had consistently higher Ni concentrations than did others (Fig. 4G). Thus, *Chironomus* species could be pooled when using larvae as Ni biomonitors.

Selenium (Se)

Dissolved Se concentrations ranged from 1.4 to 38.8 nM (Fig 1, Table S11), with those in Kelly Lake (38.8 nM) being above a suggested dissolved Se threshold concentration (25 nM) for the protection of wildlife (Lemly (2002). In sediments, Se concentrations ranged from 13-477 nmol/g (Fig. 2, Table S11). With the exception of three lakes (Osisko, Rouyn and Dufault), Se concentrations were higher in sediments from the Sudbury lakes than from Rouyn-Noranda

lakes. There was a positive correlation (p=0.02) between total dissolved Se concentrations in lake water and Se concentrations in oxic sediments. Such a correlation has been reported previously (Wiramanaden, Forster & Liber, 2010; Muscatello, Belknap & Janz, 2008) and is likely the consequence of Se uptake by plankton and its later deposition onto sediment.

The wide range in larval Se concentrations (17 to 454 nmol/g; Fig. 3C, Table S11) indicates that *Chironomus* larvae do not regulate their internal concentrations of this essential element and thus are potential biomonitors for it. Selenium concentrations in larvae exceeded the suggested threshold in invertebrates (38 nmol/g) for the protection of animals higher in the food chain (Lemly, 2002) in all but three lakes (species feeding on oxic particles in Lakes D'Alembert, Fortune and Opasatica). A wide range in Se concentrations (~100-1,000 nmol/g) has also been reported for unidentified chironomid larvae collected at various distances downstream from a uranium mine (Tse *et al.*, 2012).

Selenium concentrations in water were not correlated with Se concentrations in *Chironomus* species feeding on anoxic particles, but were correlated with Se concentrations in *Chironomus* species feeding on oxic particles (Figs. S1H, Table S13). Since dissolved Se concentrations were correlating with those in oxic sediments (see above), it is likely that the linear relationship between Se concentrations in water and in larvae feeding on oxic particles is indirect. Selenium concentrations in *Chironomus* larvae feeding on either oxic or anoxic particles were significantly correlated with Se concentrations in sediments (Fig. 6H, Table S13) and in larval feces/gut contents (Fig. 7H, Table S14), indicating that *Chironomus* larvae most likely take up their Se from food and could be used as biomonitors for sedimentary Se. These correlations are consistent with the idea that chironomid larvae take up Se mainly from their diet, as suggested by the results of an in situ experiment in which *Chironomus dilutus* larvae exposed to Se in both sediment and overlying water accumulated much higher concentrations of Se than did those exposed to Se in water only (Franz *et al.*, 2013). Consistent with our hypothesis that larvae do not take up the majority of their Se from water is the report that rates of Se uptake from solution tend to be very slow (Luoma & Rainbow, 2008). Selenium concentrations in the feces/gut contents of *Chironomus* larvae were significantly correlated with those in sediments (Fig. 5H, Table S14) and the ratio of the two was close to unity, which suggests that Se concentrations in bulk sediment represent reasonably well those in sediment ingested by larvae.

At a given site, larval Se concentrations were always higher in *Chironomus* species feeding on anoxic sediments than in those feeding on oxic particles (Fig. 3C), which indicates that caution

should be used when pooling larvae for Se analyses. However, Se concentrations in oxic and anoxic sediments did not significantly differ ($p>0.05$) in two-thirds of our study lakes (Table S11), and where there were significant differences neither sediment type had consistently higher Se concentrations. Furthermore, in a given lake, Se concentrations in the feces/gut contents of *Chironomus* species feeding on anoxic particles were not necessarily higher than Se concentrations in the feces/gut contents of those feeding on oxic particles (Table S11). Overall, these measurements indicate that consistent differences in larval Se concentrations between sympatric species are attributable to differences in Se bioavailability between oxic and anoxic sediments rather than to differences in total Se concentrations. This difference in Se bioavailability is likely related to the fact that Se reaching the sediment surface is mainly organo-Se in algal and other plant cells. As this organic material is buried and decomposed by bacteria, the Se it contains is both solubilized and converted to bacterial biomass which renders it more bioavailable to *Chironomus* larvae feeding on anoxic sediments.

Zinc (Zn)

Zinc concentrations varied by 520 fold (8-4,143 nM) in water (Fig. 1) and 310 fold (420-130,720 nmol/g) in sediments (Fig. 2) (Table S12). Zinc concentrations in Lake Arnoux and Lake Dufault were above the PEL water guideline (459 nM; CCME, 2014). Zinc concentrations in sediments of Rouyn-Noranda lakes were higher than in those of Sudbury lakes and were generally above the PEL sediment threshold value (4,816 nmol/g; CCME, 2014).

Zinc concentrations in *Chironomus* larvae varied widely among lakes (7 fold, 810-5,390 nmol/g), and were generally higher in larvae collected from Rouyn-Noranda lakes (Fig. 3B, Table S12), which suggests that larvae could be used as Zn biomonitors. In contrast to *Chironomus*, several aquatic crustaceans (Borgmann & Norwood, 1999; Borgmann *et al.*, 2001), insects (Croteau *et al.*, 1998) and polychaetes (Bryan & Hummerstone, 1973; Amiard *et al.*, 1987) maintain their total concentrations of this essential element constant, thereby negating their use as Zn biomonitors. In some lakes in Rouyn-Noranda, Zn concentrations in larvae were above the toxicity threshold for *H. azteca* (Table 3).

Zinc concentrations in *Chironomus* larvae were not correlated with those in lakewater (total dissolved or free ion concentrations) ($p>0.05$; Figs S1I-S2G, Table S13), but were correlated with those in sediments ($p<0.05$; Fig. 6I, Table S13) and in the feces/gut contents of larvae ($p<0.05$; Fig. 7I, Table S14). This indicates that larvae do not take up Zn mainly from water, but

from food. With the exception of two lakes (Arnoux and Osisko), Zn concentrations in the feces/gut contents of larvae were similar to those in sediments (Fig. 5I, Table S14).

In some, but not all lakes, *Chironomus* species feeding on oxic particles had higher Zn concentrations (~2 times) than did species feeding on anoxic particles (Fig. 3B). In these lakes, Zn concentrations in oxic sediments were not necessarily higher than those in anoxic sediments (Table S12), which suggests that in some lakes Zn is more bioavailable in oxic sediments than in anoxic sediments. The frequent difference in Zn concentrations among sympatric *Chironomus* species suggests that caution be used when interpreting the results of pooled larval samples.

Normalizing sedimentary trace element concentrations to improve predictions of their bioavailability

Overall, positive correlations between the concentrations of As, Mn, Ni, Se and Zn in sediments and in *Chironomus* larvae suggest that *Chironomus* larvae can be used to monitor bioavailable concentrations of these elements in sediments. In contrast, there were no significant correlations between the concentrations of Ba, Cd, Co and Cu in larvae and in sediments. Although this could mean that *Chironomus* larvae are ineffective for assessing the bioavailability of these elements in sediments, it more likely demonstrates our inability to assess trace element bioavailability based on simplistic sediment digestions. In fact, concentrations of trace elements in species feeding in oxic sediments were not better correlated to those in oxic sediments than to those in anoxic sediments (Table S13). This also applies to *Chironomus* species feeding on anoxic sediments (Table S13). Below we consider the efficacy of several normalization methods designed to improve correlations between trace element concentrations in sediments and in benthic invertebrates.

Percentage organic matter

Since some trace elements bind to sedimentary organic matter, normalizing sediment trace element concentrations by the percentage of organic carbon has proven useful for predicting trace element bioaccumulation (Luoma & Rainbow, 2008). However, despite that in our lakes, the percentage of organic carbon ranged from 2.5 to 15.3%. (Table S15), normalizing sediment trace element concentrations by the percentage of organic carbon did not necessarily strengthen the relationships between trace element concentrations in *Chironomus* larvae and those in sediments from various lakes (Table S13). Furthermore, because the percentage of

organic matter was not always significantly higher in oxic sediments than in anoxic sediments, this variable cannot explain the differences in Cd, Zn and Se bioavailability between oxic and anoxic sediments (Table S15). We note, however, that the percentage organic matter considers neither the type of organic matter (e.g., algae, terrestrial vegetation, etc.) nor its state of decomposition, both of which could influence its capacity to bind trace elements.

Fe and Mn oxides in oxic sediments

In oxic sediments, Fe and Mn oxides can bind trace elements such that normalizing their concentrations with respect to these binding phases can improve relationships between sediment and animal trace element concentrations (Pb: Luoma & Bryan, 1978; As: Langston, 1980; Cu and Pb: Tessier *et al.*, 1984; Cu: Tessier, Campbell & Auclair, 1983). Since we did not measure the concentrations of Fe and Mn oxides in sediments, we cannot assess their potential influence on the availability of trace elements among lakes to *Chironomus* species feeding on oxic sediments.

Sulfides in anoxic sediments

In anoxic sediments, trace elements can be precipitated as sulfides and thus be less available for uptake by benthic invertebrates (Luoma & Rainbow, 2008; ICMM, 2007). The SEM-AVS model for predicting the toxicity of sedimentary metals (i.e., Ag, Cd, Cu, Ni, Pb, Zn) is based on this premise. It states that if the concentration of acid-extractable sulfides ([AVS]) exceeds that of the sum of trace metals associated with this phase (∑[SEM]) then there will be no toxicity because porewater metal concentrations will be negligible and it is these dissolved metals that control toxicity (Luoma & Rainbow, 2008; ICMM, 2007). As a corollary, the model predicts that sedimentary trace metals can be bioavailable where ∑[SEM] exceeds [AVS], which could lead to toxicity (depending on the sensitivity of the animals and the metal involved). Despite the fact that the SEM-AVS model has been successful in predicting metal toxicity in the laboratory, and thus has gained credence for use in ecological risk assessments (especially in the USA), its use remains controversial (Campbell, Chapman & Hale, 2006). This is due in part to the fact that it tends to be poor at predicting metal accumulation by sediment-dwelling animals (De Jonge *et al.*, 2009; Hare *et al.*, 2001; De Jonge, Blust & Bervoets, 2010).

We evaluated the utility of the SEM-AVS model for predicting trace metal bioaccumulation by measuring the ∑[SEM] and [AVS] in anoxic sediments and comparing them to the concentrations of Cd, Cu, Ni and Zn in *Chironomus* species feeding on anoxic particles in

several of our study lakes (Fig. 8). Since ∑[SEM] did not exceed [AVS] in three lakes (Osisko, Arnoux and Duprat) *Chironomus* species feeding on anoxic sediments in these lakes should have very low concentrations of these trace metals. The opposite was the case in four of our study lakes (Marlon, Pine, Raft and Hannah). We hypothesize that, in these latter lakes, metals having the lowest solubility product with sulfur will be most likely to precipitate such that the binding order of metals to [AVS] will be Ag, Cu, Pb, Cd, Zn and Ni (ICMM, 2007). Applying this logic suggests that in anoxic sediment from Lake Marlon only part of the Zn and all of the Ni should be bioavailable, whereas in anoxic sediments from Lakes Pine, Raft and Hanna a portion of Cu and all the Pb, Cd, Zn and Ni should be bioavailable.

As predicted, in lakes where Ni is likely not bound to AVS (Pine, Raft, Hannah) *Chironomus* larvae had higher Ni concentrations than those from lakes in which all of the Ni was likely bound to sulfides (Osisko, Arnoux, Duprat). In contrast, Ni concentrations in larvae from Lake Marlon (where all of the SEM_{Ni} should be in solution) were not higher than in larvae from Lakes Osisko, Arnoux and Duprat (where Ni should be bound to AVS). Copper concentrations in *Chironomus* larvae from lakes where Cu is likely not precipitated with AVS were significantly higher than those in Lake Osisko where [AVS] far exceeded those of ∑[SEM]. Neither Cd nor Zn concentrations in larvae followed the expected pattern because Cd concentrations in larvae did not differ between lakes where Cd was likely not precipitated as a sulfide and those where it was, whereas larval Zn concentrations were higher in a lake (Osisko) where the [AVS] far exceeded that of ∑[SEM] compared to others where Zn should also have been in excess (Marlon, Pine, Raft, Hannah).

Overall, our results suggest that the SEM-AVS model is unable to account for the bioavailability of Cd, Cu, Ni, and Zn in *Chironomus* larvae feeding on anoxic sediments. We explain this incongruity by the fact that the [SEM]-[AVS] model assumes that sediment-dwelling invertebrates take up their metals from anoxic pore-waters. However, since *Chironomus* larvae pump surface oxic water through their burrows, they are likely not exposed to anoxic interstitial water. An added constraint is the fact that since *Chironomus* larvae likely take up much of their metal from food, metal concentrations in anoxic sediments would have to be in equilibrium with those in pore waters for the SEM-AVS model to apply.

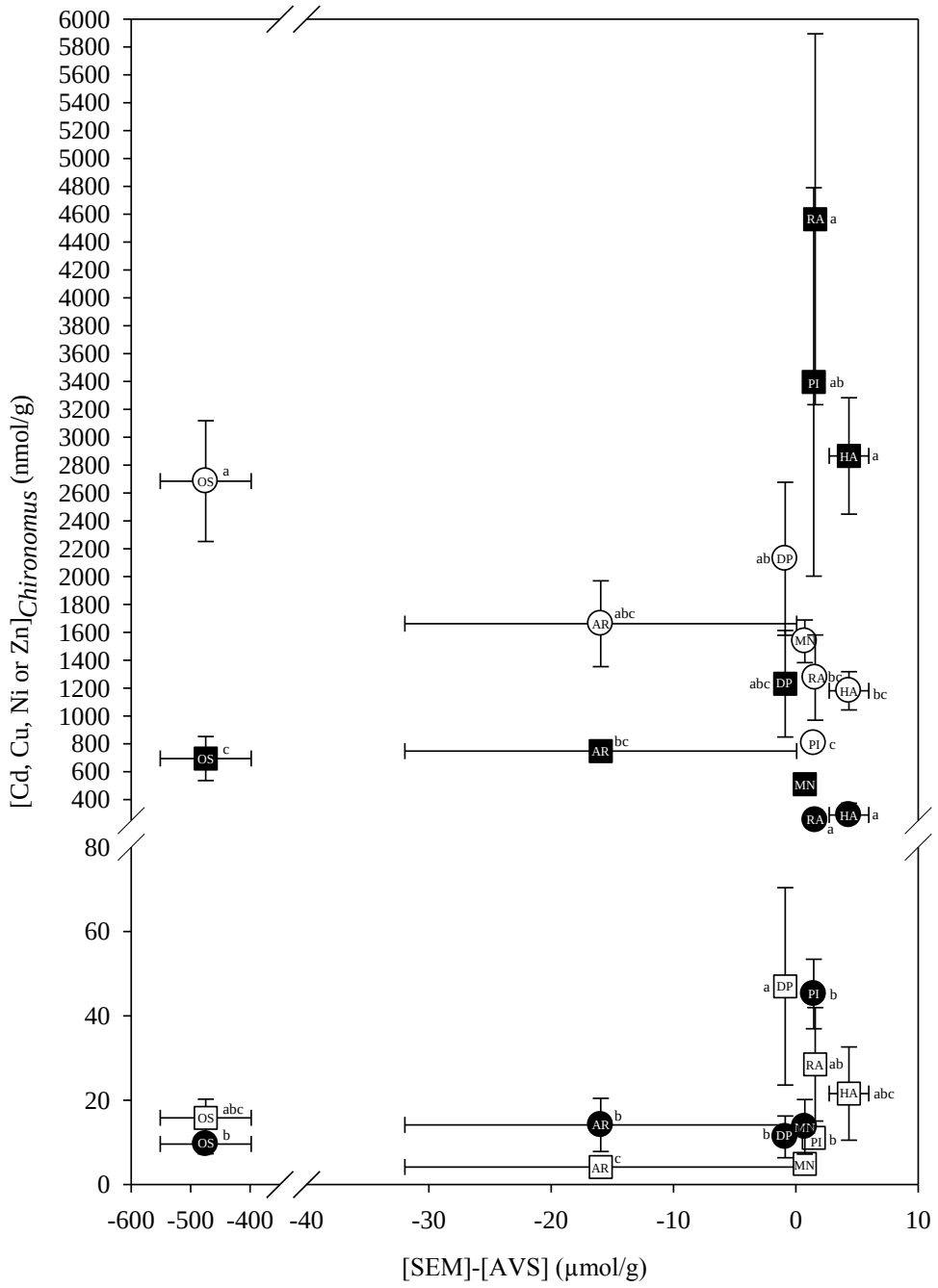


Figure 8. **Mean (± SD; n=2-17) Cd (□), Cu (■), Ni (●) and Zn (○) concentrations (nmol/g) in *Chironomus* species feeding on anoxic particles in relation to mean concentrations of ∑SEM-AVS (± SD; n=3; µmol/g) in sediments of lakes in which they were collected. For clarity's sake, lakes are labelled for each set of corresponding data (see Table 1 for lake abbreviations). For a given trace element, values that do not differ significantly (p>0.05) are followed by the same letter (except for values from Lake Marlon where n=2).**

Using *Chironomus* larvae to compare trace element bioavailability in oxic and anoxic 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 benthic invertebrates have shown 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, was more bioavailable in oxic sediments than in anoxic sediments. These results for Cd and Zn are consistent with those for a polychaete (Wang, Stupakoff & Fisher, 1999) and two species of bivalves (Chong & 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 among lakes without identifying species could confound 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 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 S1; 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 & Hare, 2014). We hope that this tentative key can be expanded in the future to include larvae from different regions and of 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) ……...……. 4
4. Pale frontoclypeus …… **larvae feed on oxic particles**
- Dark frontoclypeus ……..… **larvae can either feed on oxic or anoxic particles**

ACKNOWLEDGEMENTS

Funding was provided by the Metals In the Human Environment – Strategic Network and the Natural Sciences and Engineering Research Council of Canada. We thank Dominic Ponton,

Maikel Rosabal-Rodrigue, Victor Thomasson and Julien Lacharité for their assistance in collecting samples.

REFERENCES

Amiard J.C., Amiard-Triquet C., Berthet B. & Metayer C. (1987) Comparative study of the patterns of bioaccumulation of essential (Cu, Zn) and non-essential (Cd, Pb) trace metals in various estuarine and coastal organisms. *Journal of Experimental Marine Biology and Ecology,* **106**, 73-89.

Armitage P., Cranston P.S. & Pinder L.C.V. (1995) *The Chironomidae - The biology and ecology of non-biting midges,* Chapman & Hall.

Beeby A. (2001) What do sentinels stand for? *Environmental Pollution,* **112**, 285-298.

Belzile N., De Vitre R.R. & Tessier A. (1989) In situ collection of diagenetic iron and manganese oxyhydroxides from natural sediments. *Nature,* **340**, 376-377.

Besser J.M., Brumbaugh W.G., Ingersoll C.G., Ivey C.D., Kunz J.L., Kemble N.E., Schlekat C.E. & Garman E.R. (2013) Chronic toxicity of nickel-spiked freshwater sediments: Variation in toxicity among eight invertebrate taxa and eight sediments. *Environmental Toxicology and Chemistry,* **32**, 2495-2506.

Borgmann U. & Norwood W.P. (1999) Assessing the tixicity of lead in sediments to *Hyalella azteca*: The significance of bioaccumulation and dissolved metal. *Canadian Journal of Fisheries and Aquatic Sciences,* **56**, 1494-1503.

Borgmann U. & Norwood W.P. (2002) Metal bioavailability and toxicity through a sediment core. *Environmental Pollution,* **116**, 159-168.

Borgmann U., Norwood W.P., Reynoldson T.B. & Rosa F. (2001) Identifying cause in sediment assessments: bioavailability and the Sediment Quality Triad. *Canadian Journal of Fisheries and Aquatic Sciences,* **58**, 950-960.

Borgmann U., Nowierski M., Grapentine L.C. & Dixon D.G. (2004) Assessing the cause of impacts on benthic organisms near Rouyn-Noranda, Quebec. *Environmental Pollution,* **129**, 39-48.

Bryan G.W. & Gibbs P.E. (1987) Polychaetes as indicators of heavy-metal availability in marine deposits. In: *Oceanic Processes in Marine Pollution* (Eds J.M. Capuzzo, D.R. Kester & I.W. Duedall), pp. 37-49. Kreiger Publishing Compagny, Malabar, Florida.

Bryan G.W. & Hummerstone L.G. (1973) Adaptation of the polychaete *Nereis diversicolor* to estuarine sediments containing high concentrations of zinc and cadmium. *Journal of the Marine Biological Association of the United Kingdom,* **53**, 839-857.

Bryan S.E., Tipping E. & Hamilton-Taylor J. (2002) Comparison of measured and modelled copper binding by natural organic matter in freshwaters. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology,* **133**, 37-49.

Buchwalter D.B. & Luoma S.N. (2005) Differences in dissolved cadmium and zinc uptake among stream insects: Mechanistic explanations. *Environmental Science and Technology,* **39**, 498-504.

Buffle J. (1988) *Complexation Reactions in Aquatic Systems: An Analytical Approach,* Ellis Horwood Ltd., Chichester, UK.

Butler M.G., Kiknadze I.I., Cooper J.K. & Siirin M.T. (1995) Cytologically identified *Chironomus* species from lakes in North Dakota and Minnesota, USA. In: *Chironomids, from Gene to Ecosystems - Proceedings of the 12th International Symposium on Chironomidae (Canberra, January 23-26, 1994)* (Ed. P.S. Cranston), pp. 31-37. CSIRO, Canberra.

Cain D., Croteau M.N. & Luoma S. (2011) Bioaccumulation dynamics and exposure routes of Cd and Cu among species of aquatic mayflies. *Environmental Toxicology and Chemistry,* **30**, 2532-2541.

Campbell P.G.C. (1995) Interaction between trace metals and aquatic organisms: a critique of the free-ion activity model. In: *Metal speciation and aquatic systems* (Eds A. Tessier & D.R. Turner), pp. 45-102. John Wiley, New York.

Campbell P.G.C., Chapman P.M. & Hale B.A. (2006) Risk Assessment of Metals in the Environment. In: *Chemicals in the Environment: Assessing and Managing Risk* (Eds R.E. Hester & R.M. Harrison), pp. 102-126.

CCME (2014) Canadian Environmental Quality Guidelines. http://ceqg-rcqe.ccme.ca/. Accessed May 27, 2014.

Charbonneau P. & Hare L. (1998) Burrowing behavior and biogenic structures of mud-dwelling insects. *Journal of the North American Benthological Society,* **17**, 239-249.

Chong K. & Wang W.X. (2000) Bioavailability of sediment-bound Cd, Cr and Zn to the green mussel *Perna viridis* and the manila clam *Ruditapes philippinarum*. *Journal of Experimental Marine Biology and Ecology,* **255**, 75-92.

City of Greater Sudbury (2013) Individual Lake Information. http://www.greatersudbury.ca/living/lakes-facts/local-lake-descriptions/. Accessed 17 September 2013.

Couture P., Busby P., Gauthier C., Rajotte J.W. & Pyle G.G. (2008) Seasonal and regional variations of metal contamination and condition indicators in yellow perch (*Perca flavescens*) along two polymetallic gradients. I. Factors influencing tissue metal concentrations. *Human and Ecological Risk Assessment,* **14**, 97-125.

Craig A., Hare L., Charest P.M. & Tessier A. (1998) Effect of exposure regime on the internal distribution of cadmium in *Chironomus staegeri* larvae (insecta, diptera). *Aquatic Toxicology,* **41**, 265-275.

Craig A., Hare L. & Tessier A. (1999) Experimental evidence for cadmium uptake via calcium channels in the aquatic insect *Chironomus staegeri*. *Aquatic Toxicology,* **44**, 255-262.

Croteau M.N., Hare L. & Tessier A. (1998) Refining and testing a trace metal biomonitor (*Chaoborus*) in highly acidic lakes. *Environmental Science and Technology,* **32**, 1348-1353.

Croteau M.N., Hare L. & Tessier A. (2001) Differences in Cd accumulation among species of the lake-dwelling biomonitor *Chaoborus*. *Canadian Journal of Fisheries and Aquatic Sciences,* **58**, 1737-1746.

Croteau M.N., Hare L. & Tessier A. (2002) Increases in food web cadmium following reductions in atmospheric inputs to some lakes. *Environmental Science & Technology,* **36**, 3079-3082.

De Jonge M., Blust R. & Bervoets L. (2010) The relation between Acid Volatile Sulfides (AVS) and metal accumulation in aquatic invertebrates: Implications of feeding behavior and ecology. *Environmental Pollution,* **158**, 1381-1391.

De Jonge M., Dreesen F., De Paepe J., Blust R. & Bervoets L. (2009) Do acid volatile sulfides (AVS) influence the accumulation of sediment-bound metals to benthic invertebrates under natural field conditions? *Environmental Science and Technology,* **43**, 4510-4516.

Franz E.D., Wiramanaden C.I.E., Gallego-Gallegos M., Tse J.J., Phibbs J., Janz D.M., Pickering I.J. & Liber K. (2013) An in situ assessment of selenium bioaccumulation from water-, sediment-, and dietary exposure pathways using caged *Chironomus dilutus* larvae. *Environmental Toxicology and Chemistry,* **32**, 2836-2848.

Gallon C., Hare L. & Tessier A. (2008) Surviving in anoxic surroundings: How burrowing aquatic insects create an oxic microhabitat. *Journal of the North American Benthological Society,* **27**, 570-580.

Galluba S., Oetken M. & Oehlmann J. (2012) Comprehensive sediment toxicity assessment of Hessian surface waters using *Lumbriculus variegatus* and *Chironomus riparius*. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering,* **47**, 507-521.

Giguère A., Campbell P.G.C., Hare L. & Couture P. (2006) Sub-cellular partitioning of cadmium, copper, nickel and zinc in indigenous yellow perch (*Perca flavescens*) sampled along a polymetallic gradient. *Aquatic Toxicology,* **77**, 178-189.

Giguère A., Campbell P.G.C., Hare L., McDonald D.G. & Rasmussen J.B. (2004) Influence of lake chemistry and fish age on cadmium, copper, and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*). *Canadian Journal of Fisheries and Aquatic Sciences,* **61**, 1702-1716.

Hare L., Saouter E., Campbell P.G.C., Tessier A., Ribeyre F. & Boudou A. (1991a) Dynamics of cadmium, lead, and zinc exchange between nymphs of the burrowing mayfly *Hexagenia rigida* (Ephemeroptera) and the environment. *Canadian Journal of Fisheries and Aquatic Sciences,* **48**, 39-47.

Hare L., Tessier A. & Campbell P.G.C. (1991b) Trace element distributions in aquatic insects: variations among genera, elements, and lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* **48**, 1481-1491.

Hare L., Tessier A. & Croteau M.N. (2008) A biomonitor for tracking changes in the availability of lakewater cadmium over space and time. *Human and Ecological Risk Assessment,* **14**, 229-242.

Hare L., Tessier A. & Warren L. (2001) Cadmium accumulation by invertebrates living at the sediment-water interface. *Environmental Toxicology and Chemistry,* **20**, 880-889.

Huryn A.D., Wallace J.B. & Anderson N.H. (2008) Habitat, life history, secondary production, and behavioral adaptations of aquatic insects. In: *An introduction to the aquatic insects of North America* (Eds R.W. Merritt, K.W. Cummins & M.B. Berg), pp. 55-103. Kendall/Hunt Publishing Compagny, Iowa.

ICMM (2007) *MERAG: Metals Enviromental Risk Assessment Guidance,* International Council on Mining and Metals, London, UK.

Jónasson P.M. (1972) Ecology and production of the profundal benthos in relation to phytoplankton in Lake Esrom. *Oikos Supplement,* **14**, 1-148.

Ke C. & Wang W.X. (2001) Bioaccumulation of Cd, Se, and Zn in an estuarine oyster (*Crassostrea rivularis*) and a coastal oyster (*Saccostrea glomerata*). *Aquatic Toxicology,* **56**, 33-51.

Kiknadze I.I., Aimanova K.G., Butler M. & Cooper K. (1993) The pattern of the reduction of the chromosome number in the chironomid evolution (In Russian). *Tsitologiya,* **35**, 96-104.

Lacharité J. (2011) *Éléments traces dans le biomoniteur Hexagenia limbata provenant de lacs de la région de Rouyn-Noranda, Québec*Mémoire de maîtrise en Sciences de l'eau, Université du Québec.

Langston W.J. (1980) Arsenic in U.K. estuarine sediments and its availability to benthic organisms. *Journal of the Marine Biological Association of the United Kingdom,* **60**, 869-881.

Larocque I., Velle G. & Rolland N. (2010) Effect of removing small (<150 µm) chironomids on inferring temperature in cold lakes. *Journal of Paleolimnology,* **44**, 709-719.

Lemly A.D. (2002) A procedure for setting environmentally safe total maximum daily loads (TMDLs) for selenium. *Ecotoxicology and Environmental Safety,* **52**, 123-127.

Leonard E.M., Pierce L.M., Gillis P.L., Wood C.M. & O'Donnell M.J. (2009) Cadmium transport by the gut and Malpighian tubules of *Chironomus riparius*. *Aquatic Toxicology,* **92**, 179-186.

Linnaeus C. (1758) *Systema naturae per regna tria naturae (Vol. 1, 10th ed.),* Laurentii Salvii, Stockholm.

Lobel P.B., Belkhode S.P., Jackson S.E. & Longerich H.P. (1990) Recent taxonomic discoveries concerning the mussel *Mytilus*: Implications for biomonitoring. *Archives of Environmental Contamination and Toxicology,* **19**, 508-512.

Lundbeck W. (1898) Diptera groenlandica. *Videnskabelige Meddelelser fra den Naturhistorisk Forening i Kjøbenhavn,* **1898**, 236-314.

Luoma S.N. & Bryan G.W. (1978) Factors controlling the availability of sediment-bound lead to the estuarine bivalve *Scrobicularia plana*. *Journal of the Marine Biological Association of the United Kingdom,* **58**, 793-802.

Luoma S.N. & Rainbow P.S. (2008) *Metal contamination in aquatic environments: science and lateral management,* Cambrige University Press, New York.

Marinković M., De Bruijn K., Asselman M., Bogaert M., Jonker M.J., Kraak M.H.S. & Admiraal W. (2012) Response of the nonbiting midge *Chironomus riparius* to multigeneration toxicant exposure. *Environmental Science and Technology,* **46**, 12105-12111.

Martin J. (2013) North American species of the genus *Chironomus*. http://genetics.unimelb.edu.au/Martin/NACytfiles/NAChiron.html. Accessed 17 September 2013.

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

Mayer L.M., Chen Z., Findlay R.H., Fang J., Sampson S., Self R.F.L., Jumars P.A., Quetel C. & Donard O.F.X. (1996) Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. *Environmental Science and Technology,* **30**, 2641-2645.

Ministère de l'Environnement du Québec (1987) *Sédiments - Détermination des métaux / Méthode par spectrométrie d'émission au plasma d'argon / MENVIQ. 87.09"205 - Mét. 1.1 / Préliminaire,* MEQ.

Ministère de l'Environnement et de la Faune du Québec (1996) *Sols - Détermination des métaux / Méthode par spectrométrie d'émission au plasma d'argon / N^{o}210 - Mét. 1.0 / Préliminaire usage interne,* MEFQ.

Mogren C.L., von Kiparski G.R., Parker D.R. & Trumble J.T. (2012) Survival, reproduction, and arsenic body burdens in *Chironomus riparius* exposed to arsenate and phosphate. *Science of the Total Environment,* **425**, 60-65.

Moore P.G. & Rainbow P.S. (1987) Copper and zinc in an ecological series of talitroidean Amphipoda (Crustacea). *Oecologia,* **73**, 120-126.

Mueller K.K., Lofts S., Fortin C. & Campbell P.G.C. (2012) Trace metal speciation predictions in natural aquatic systems: Incorporation of dissolved organic matter (DOM) spectroscopic quality. *Environmental Chemistry,* **9**, 356-368.

Munger C. & Hare L. (1997) Relative importance of water and food as cadmium sources to an aquatic insect (Chaoborus punctipennis): Implications for predicting Cd bioaccumulation in nature. *Environmental Science and Technology,* **31**, 891-895.

Muscatello J.R., Belknap A.M. & Janz D.M. (2008) Accumulation of selenium in aquatic systems downstream of a uranium mining operation in northern Saskatchewan, Canada. *Environmental Pollution,* **156**, 387-393.

Norwood W.P., Borgmann U. & Dixon D.G. (2007) Chronic toxicity of arsenic, cobalt, chromium and manganese to *Hyalella azteca* in relation to exposure and bioaccumulation. *Environmental Pollution,* **147**, 262-272.

Nriagu J.O., Wong H.K.T., Lawson G. & Daniel P. (1998) Saturation of ecosystems with toxic metals in Sudbury basin, Ontario, Canada. *Science of the Total Environment,* **223**, 99-117.

Nyman M., Korhola A. & Brooks S.J. (2005) The distribution and diversity of Chironomidae (Insecta: Diptera) in western Finnish Lapland, with special emphasis on shallow lakes. *Global Ecology and Biogeography,* **14**, 137-153.

Orvoine J., Hare L. & Tessier A. (2006) Competition between protons and cadmium ions in the planktonic food chain leading to the phantom midge *Chaoborus*. *Limnology and Oceanography,* **51**, 1013-1020.

Ponton D.E. & Hare L. (2009) Assessment of nickel contamination in lakes using the phantom midge *Chaoborus* as a biomonitor. *Environmental Science and Technology,* **43**, 6529-6534.

Ponton D.E. & Hare L. (2013) Relating selenium concentrations in a planktivore to selenium speciation in lakewater. *Environmental Pollution,* **176**, 254-260.

Proulx I. & Hare L. (2008) Why bother to identify animals used for contaminant monitoring? *Integrated environmental assessment and management,* **4**, 125-126.

Proulx I. & Hare L. (2014) Differences in feeding behaviours among *Chironomus* species revealed by measurements of sulphur stable isotopes and cadmium in larvae. *Freshwater Biology,* **59**, 73-86.

Proulx I., Martin J., Carew M. & Hare L. (2013) Using various lines of evidence to identify *Chironomus* species in eastern Canadian lakes. *Zootaxa,* **3741**, 401-458.

Pyle G.G., Rajotte J.W. & Couture P. (2005) Effects of industrial metals on wild fish populations along a metal contamination gradient. *Ecotoxicology and Environmental Safety,* **61**, 287-312.

Rainbow P.S., Huang Z.G., Yan S.-K. & Smith B. (1993) Barnacles as biomonitors of trace metals in the coastal waters near Xiamen, P. R. China. *Asian Marine Biology,* **10**, 109-121.

Roskosch A., Hette N., Hupfer M. & Lewandowski J. (2012) Alteration of *Chironomus plumosus* ventilation activity and bioirrigation-mediated benthic fluxes by changes in temperature, oxygen concentration, and seasonal variations. *Freshwater Science,* **31**, 269-281.

Shobanov N.A. (1989) The morphological differentiation of *Chironomus* species of *plumosus* group (Diptera, Chironomidae). Larvae. *Acta Biologica Debrecina, Supplementum Oecologica Hungarica,* **2**, 335-344.

Shobanov N.A., Kiknadze I.I. & Butler M.G. (1999) Palearctic and Nearctic *Chironomus* (*Camptochironomus*) *tentans* (Fabricius) are different species (Diptera: Chironomidae). *Insect Systematics and Evolution,* **30**, 311-322.

Shuhaimi-Othman M., Pascoe D., Borgmann U. & Norwood W.P. (2006) Reduced metals concentrations of water, sediment and *Hyalella azteca* from lakes in the vicinity of the sudbury metal smelters, Ontario, Canada. *Environmental Monitoring and Assessment,* **117**, 27-44.

Stief P. & De Beer D. (2002) Bioturbation effects of *Chironomus riparius* on the benthic N-cycle as measured using microsensors and microbiological assays. *Aquatic Microbial Ecology,* **27**, 175-185.

Tessier A., Campbell P.G.C. & Auclair J.C. (1983) Relationships between trace metal partitionning in sediments and their bioaccumulation in freshwater pelecypods. In: *Proceedings of the 4th*

International Conference on Heavy Metals in the Environment, Heidelberg, pp. 1086-1089. International Conference - Heavy Metals in the Environment. CEP Consultants Ltd, Edinburg, UK.

Tessier A., Campbell P.G.C., Auclair J.C. & Bisson M. (1984) Relationships between the partitioning of trace metals in sediments and their accumulation in the tissues of the freshwater mollusc *Elliptio complanata* in a mining area. *Canadian Journal of Fisheries and Aquatic Sciences,* **41**, 1463-1472.

Tipping E., Lofts S. & Sonke J.E. (2011) Humic Ion-Binding Model VII: A revised parameterisation of cation-binding by humic substances. *Environmental Chemistry,* **8**, 225-235.

Townes H.K., Jr. (1945) The Nearctic species of Tendipedini [Diptera, Tendipedidae (= Chironomidae)]. *American Midland Naturalist,* **34**, 1-206.

Tse J.J., Gallego-Gallegos M., Franz E.D., Liber K. & Pickering I.J. (2012) Selenium speciation and localization in chironomids from lakes receiving treated metal mine effluent. *Chemosphere,* **89**, 274-279.

Walshe B.M. (1951) The feeding habits of certain chironomid larvae (subfamily Tendipendinae). *Proceedings of the Zoological Society of London,* **121**, 63-79.

Wang F., Tessier A. & Hare L. (2001) Oxygen measurements in the burrows of freshwater insects. *Freshwater Biology,* **46**, 317-327.

Wang W.X., Stupakoff I. & Fisher N.S. (1999) Bioavailability of dissolved and sediment-bound metals to a marine deposit-feeding polychaete. *Marine Ecology Progress Series,* **178**, 281-293.

Webb D.W. (1969) New species of chironomids from Costello Lake, Ontario (Diptera: Chironomidae). *Journal of the Kansas Entomological Society,* **42**, 91-108.

Wiramanaden C.I.E., Forster E.K. & Liber K. (2010) Selenium distribution in a lake system receiving effluent from a metal mining and milling operation in Northern Saskatchewan, Canada. *Environmental Toxicology and Chemistry,* **29**, 606-616.

Wuelker W., Martin J., Kiknadze I.I., Sublette J.E. & Michiels S. (2009) *Chironomus blaylocki* sp. n. and *C. bifurcatus* sp. n., North American species near the base of the *decorus*-group (Diptera: Chironomidae). *Zootaxa,* **2023**, 28-46.

Wülker W., Sublette J.E. & Martin J. (1991) *Chironomus utahensis* Malloch and *Chironomus harpi* new species and their karyosystematic relationships to other species in the *decorus*-group of *Chironomus*. *Spixiana,* **14**, 71-94.

Zetterstedt J.W. (1860) *Diptera Scandinaviae disposita et descripta. Tomus decimus quartus seu ultimus, continens addenda, corrigena & emendada tomis prioribus, una cum indice alphabetico navarum specierum hujus & praecedentis tomi, atque generico omnium tomorum*. *Lundae,* **14**, 6497-6512.

SUPPORTING INFORMATION CAPTIONS

Table S1. 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 $\geq$ the width of 11th segment

short: ventral tubules < the width of 11th segment

[b] Often hard to distinguish from bathophilus-type

Figure S1. **Mean (n=5-7, ± SD) concentrations of trace elements (As, Ba, Cd, Co, Cu, Mn, Ni, Se and Zn) in *Chironomus* larvae from Tilton Lake (●), Crooked Lake (○) and Lake Duprat (△) after they were left to depurate for 1 to 14 days. For each lake, concentrations of trace elements did not significantly differ (p>0,05) between larvae that were left to depurate for various lenght of time. Concentrations that exceptionnally differed are followed by different letters.**

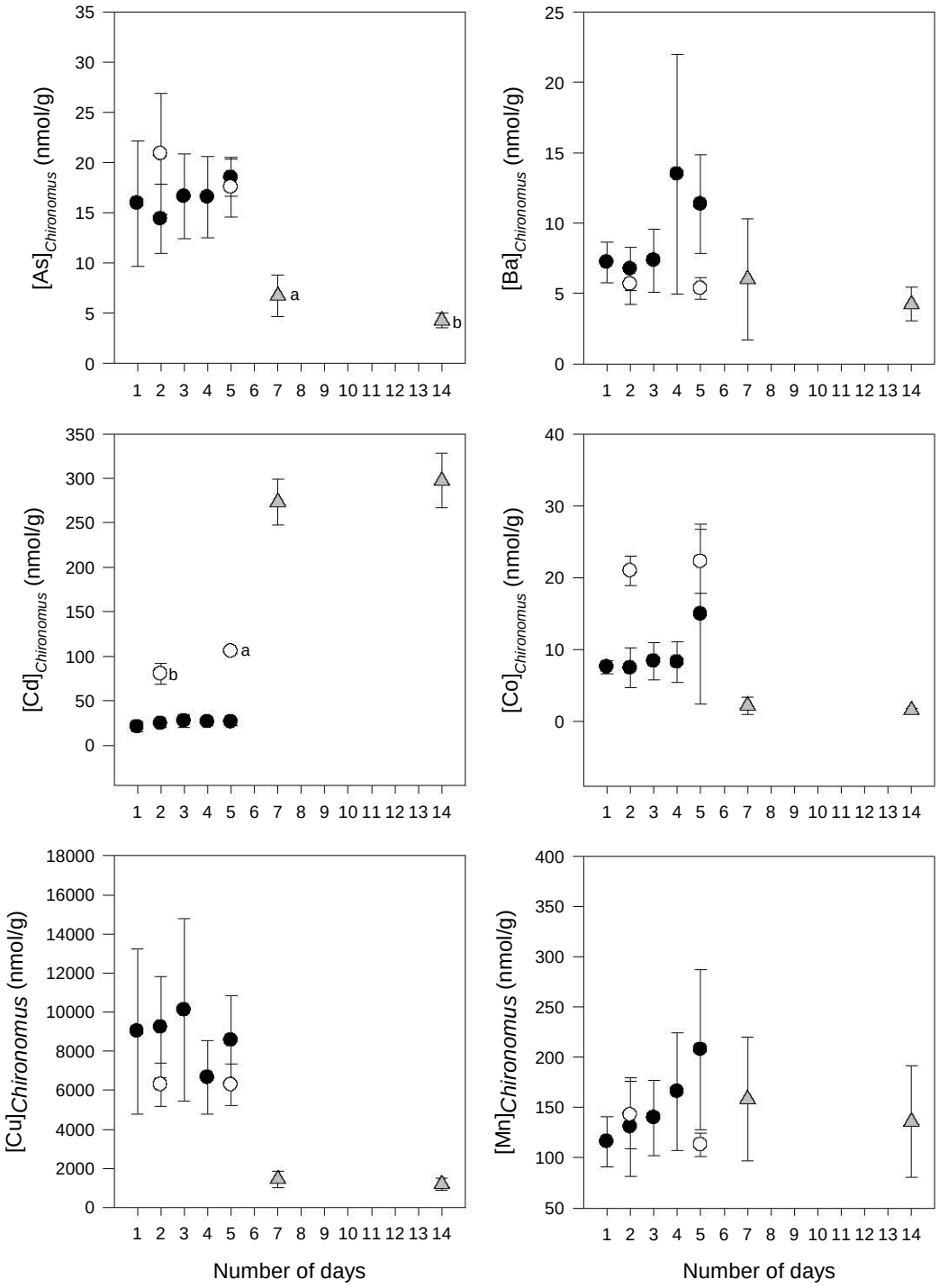


Fig. S1 (cont.)

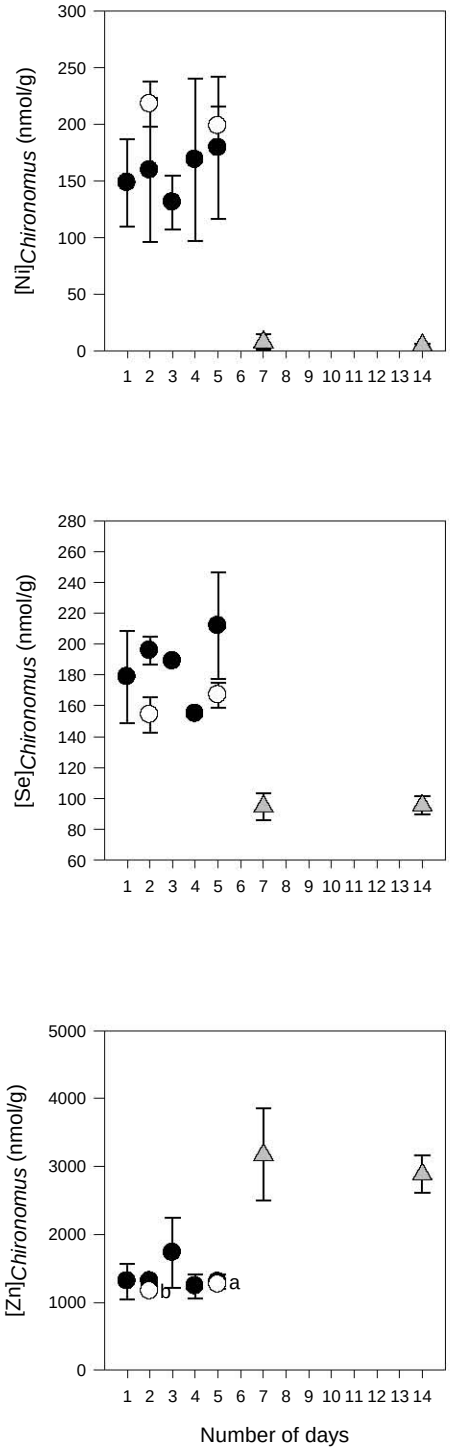

[Ni]Chironomus (nmol/g)
300
250
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14
[Se]Chironomus (nmol/g)
280
260
240
220
200
180
160
140
120
100
80
60
1 2 3 4 5 6 7 8 9 10 11 12 13 14
[Zn]Chironomus (nmol/g)
5000
4000
3000
2000
1000
0
b
a
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Number of days

Table S2. **Mean (n=3, ± SD) pH and concentrations of fulvic acid (FA), sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), barium (Ba), chloride (Cl), sulfate (SO_4), nitrate (NO_3) and carbonate (CO_3) used to calculate the free metal ion concentrations in lakes in Rouyn-Noranda and Sudbury regions.**

Water Body	pH		FA (g/L)		Na (mol/L)		Mg (mol/L)		Al (mol/L)		K (mol/L)	
	Mean*	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ROUYN-NORANDA (QC)												
AR (4.5 m)	4.4	0.0	1.51E-03	8.33E-05	6.18E-05	7.21E-07	6.18E-05	7.21E-07	1.51E-05	5.86E-07	1.71E-05	2.65E-07
DF	7.7	0.0	4.90E-03	1.74E-04	1.69E-04	1.15E-06	1.69E-04	1.15E-06	4.93E-07	4.91E-07	1.59E-05	1.15E-07
DP	7.6	0.0	3.73E-03	2.18E-04	4.80E-05	2.03E-06	4.80E-05	2.03E-06	5.90E-07	1.14E-07	6.15E-06	1.51E-07
MN	7.7	0.0	1.01E-02	1.15E-04	5.53E-05	3.51E-07	5.53E-05	3.51E-07	2.46E-06	1.20E-06	1.58E-05	2.31E-07
OP (07)	8.2	0.1	8.70E-03	3.06E-05	1.33E-04	9.24E-06	1.33E-04	9.24E-06	2.64E-06	1.24E-06	2.46E-05	1.78E-06
OS	8.5	0.0	3.01E-03	9.00E-05	9.72E-04	1.13E-05	9.72E-04	1.13E-05	1.98E-07	1.89E-07	3.99E-05	8.08E-07
PE	8.3	0.2	4.85E-03	1.05E-03	1.31E-03	5.29E-05	1.31E-03	5.29E-05	3.02E-07	1.06E-07	6.95E-05	2.15E-06
RO	8.0	0.0	4.61E-03	2.78E-04	1.10E-03	3.46E-05	1.10E-03	3.46E-05	5.04E-07	2.16E-07	1.24E-04	4.62E-06
SUDBURY (ON)												
CL	6.1	0.2	3.06E-03	2.69E-04	1.90E-04	7.39E-06	4.25E-05	1.92E-06	4.41E-07	5.71E-09	1.56E-05	8.33E-07
CR	5.2	0.3	4.77E-03	5.26E-04	8.01E-05	5.53E-07	4.82E-05	3.70E-07	1.14E-06	3.31E-07	1.81E-05	8.18E-07
HA	7.4	0.1	4.82E-03	2.97E-04	2.10E-03	1.31E-05	1.51E-04	2.89E-07	3.20E-07	3.58E-08	5.39E-05	1.95E-07
KA	6.4	0.3	5.78E-03	9.61E-05	4.61E-05	2.40E-07	4.47E-05	1.98E-07	1.10E-06	8.68E-08	1.12E-05	1.13E-07
KE	7.5	0.0	9.11E-03	6.12E-04	4.01E-03	9.85E-05	4.01E-03	9.85E-05	4.28E-07	1.14E-07	7.31E-04	6.50E-05
MC	7.3	0.0	5.48E-03	6.03E-04	1.87E-03	1.42E-05	2.09E-04	2.08E-07	3.35E-07	3.57E-08	5.29E-05	5.15E-07
PI	5.7	0.1	2.22E-03	5.13E-05	3.08E-05	9.24E-07	3.08E-05	9.24E-07	9.27E-07	3.10E-08	7.29E-06	5.75E-07
RA	7.3	0.1	2.92E-03	1.89E-04	5.72E-05	2.41E-06	5.72E-05	2.41E-06	1.10E-07	1.04E-08	1.31E-05	1.04E-06
RM	7.1	0.1	4.06E-03	9.54E-05	1.96E-03	3.07E-05	1.93E-04	4.73E-07	2.91E-07	2.38E-08	5.09E-05	5.22E-07
SI (07)	5.9	0.1	3.50E-03	2.90E-04	2.06E-03	1.00E-05	1.17E-04	2.08E-07	3.50E-07	4.66E-08	5.09E-05	8.74E-07
TI	6.5	0.2	2.99E-03	3.55E-04	8.86E-05	4.42E-07	4.01E-05	1.39E-07	3.81E-07	3.14E-08	1.18E-05	6.66E-07

*log-average

Table S2 (cont.)

Water Body	Ca (mol/L)		Mn (mol/L)		Fe (mol/L)		Co (mol/L)		Ni (mol/L)		Cu (mol/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ROUYN-NORANDA (QC)												
AR (4.5 m)	2.86E-04	1.15E-06	7.83E-06	3.61E-08	4.61E-06	1.65E-07	1.54E-07	2.00E-09	4.51E-08	4.16E-10	2.83E-07	4.74E-09
DF	3.92E-04	1.01E-05	2.49E-07	1.21E-08	1.52E-06	2.37E-06	9.90E-10	1.28E-10	1.39E-08	1.04E-09	1.58E-07	1.39E-08
DP	1.40E-04	8.54E-06	3.39E-08	2.59E-09	2.49E-07	5.89E-08	3.85E-10	2.59E-11	1.23E-08	8.34E-09	4.22E-08	7.82E-09
MN	1.68E-04	3.79E-06	1.07E-07	6.69E-08	4.31E-06	6.54E-07	9.79E-10	3.88E-10	1.35E-08	2.90E-09	1.77E-07	1.63E-08
OP (07)	2.16E-04	1.53E-06	1.41E-07	9.07E-09	1.32E-06	5.81E-07	1.22E-09	4.52E-10	1.93E-08	1.12E-09	4.47E-08	1.50E-09
OS	6.90E-04	6.93E-06	2.21E-08	1.82E-09	4.01E-07	2.74E-07	1.48E-09	6.12E-11	3.28E-08	1.33E-09	5.26E-08	1.65E-09
PE	8.26E-04	4.12E-05	1.19E-06	1.29E-07	6.24E-08	2.54E-08	4.02E-09	7.21E-10	3.71E-08	1.08E-08	3.88E-08	1.01E-08
RO	2.06E-03	3.15E-04	9.16E-07	8.09E-08	1.90E-07	1.22E-07	1.07E-08	1.37E-09	1.03E-07	5.80E-09	1.03E-07	1.37E-08
SUDBURY (ON)												
CL	1.09E-04	9.84E-07	2.51E-07	3.99E-09	5.36E-08	8.28E-09	8.98E-09	4.44E-10	1.12E-06	2.62E-08	1.31E-07	6.54E-09
CR	7.14E-05	3.32E-07	5.73E-07	1.07E-08	2.84E-07	7.17E-08	3.27E-08	5.05E-10	2.11E-06	1.46E-08	3.23E-07	7.97E-09
HA	2.65E-04	7.93E-07	1.01E-07	2.37E-08	2.76E-07	1.27E-07	2.84E-09	8.02E-10	2.11E-06	3.52E-08	2.90E-07	3.78E-09
KA	6.90E-05	2.31E-07	3.77E-07	2.75E-09	1.05E-06	1.45E-07	3.73E-09	9.59E-11	9.85E-07	2.14E-08	1.38E-07	1.79E-09
KE	4.60E-03	1.60E-04	1.32E-06	2.08E-08	7.33E-07	2.05E-07	8.63E-08	3.76E-09	4.87E-06	3.06E-08	1.85E-07	8.72E-09
MC	4.01E-04	7.12E-07	2.36E-07	5.84E-08	1.40E-07	1.77E-08	1.88E-09	2.24E-10	8.90E-07	1.15E-08	1.08E-07	5.72E-09
PI	2.53E-05	2.02E-06	9.20E-07	1.82E-08	1.82E-07	4.15E-08	3.74E-08	2.94E-10	8.97E-07	9.07E-09	1.03E-07	1.32E-09
RA	7.85E-05	2.12E-06	1.06E-07	9.64E-09	5.35E-08	2.77E-08	4.17E-10	2.35E-11	1.04E-06	5.77E-09	1.13E-07	6.05E-10
RM	3.81E-04	4.57E-07	1.04E-07	2.84E-08	1.10E-07	6.17E-08	1.56E-09	1.72E-10	8.69E-07	1.57E-08	1.63E-07	6.39E-09
SI (07)	1.94E-04	1.71E-07	7.34E-07	3.18E-07	2.45E-07	8.33E-08	1.30E-08	6.83E-10	1.59E-06	1.82E-08	1.61E-07	7.29E-09
TI	8.85E-05	9.17E-07	1.69E-07	6.29E-09	1.14E-07	3.78E-08	1.42E-09	5.35E-11	6.76E-07	8.05E-10	1.00E-07	2.39E-09

Table S2 (cont.)

Water Body	Zn (mol/L)		Cd (mol/L)		Ba (mol/L)		Cl (mol/L)		NO_3 (mol/L)		SO_4 (mol/L)		CO_3 (mol/L)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
ROUYN-NORANDA (QC)														
AR (4.5 m)	4.14E-06	4.04E-08	4.57E-09	5.16E-11	6.85E-08	2.11E-10	3.59E-05	4.48E-06	2.27E-05	1.05E-05	5.13E-04	3.79E-06	2.16E-05	1.25E-05
DF	7.14E-07	6.43E-09	3.34E-09	1.03E-10	4.52E-08	9.71E-10	1.19E-04	1.00E-06	9.28E-06	1.57E-06	2.97E-04	4.58E-06	2.94E-04	7.94E-06
DP	5.85E-08	1.25E-08	2.99E-10	1.11E-10	1.86E-08	9.52E-10	7.61E-06	7.64E-08	2.89E-06	8.27E-07	3.28E-05	1.95E-06	1.95E-04	3.55E-05
MN	5.37E-08	2.05E-08	1.36E-09	3.13E-10	3.40E-08	2.61E-09	7.29E-06	2.15E-07	2.22E-06	6.61E-07	3.18E-05	5.77E-08	2.30E-04	4.96E-05
OP (07)	7.92E-09	5.96E-09	1.63E-10	6.06E-11	5.57E-08	2.07E-09	9.40E-05	4.04E-07	2.12E-06	1.25E-07	6.10E-05	3.61E-07	3.56E-04	3.61E-06
OS	6.45E-08	2.07E-09	4.77E-10	1.03E-11	1.74E-07	2.65E-09	9.25E-04	1.53E-06	5.06E-06	9.25E-07	3.82E-04	1.15E-06	6.30E-04	8.54E-06
PE	1.32E-08	3.75E-09	1.48E-10	4.01E-11	1.31E-07	7.65E-09	1.13E-03	2.31E-05	1.77E-05	2.96E-06	5.32E-04	4.13E-05	7.89E-04	1.23E-04
RO	3.37E-07	2.27E-08	3.91E-09	1.48E-10	1.47E-07	8.09E-09	1.21E-03	3.06E-05	1.42E-04	1.04E-05	1.79E-03	1.12E-04	6.05E-04	4.57E-05
SUDBURY (ON)														
CL	2.51E-07	2.47E-08	1.99E-09	5.91E-10	3.27E-10	1.02E-10	2.74E-04	1.00E-06	3.35E-06	5.92E-07	1.06E-04	5.77E-07	2.21E-05	4.86E-06
CR	2.50E-07	1.09E-08	2.06E-09	8.35E-11	7.99E-10	2.01E-10	6.91E-05	8.26E-06	1.25E-05	1.15E-05	1.16E-04	5.77E-07	3.59E-05	2.30E-05
HA	5.96E-08	5.84E-09	1.43E-09	7.27E-11	3.89E-10	8.96E-11	2.38E-03	2.31E-05	1.32E-05	3.15E-06	1.80E-04	2.08E-06	2.17E-04	2.25E-05
KA	1.15E-07	2.37E-08	4.88E-10	1.61E-11	5.73E-10	4.08E-11	9.68E-06	3.51E-07	4.56E-06	2.39E-06	9.45E-05	1.73E-07	2.86E-05	5.35E-06
KE	1.97E-07	1.46E-08	1.03E-08	1.84E-09	2.25E-07	5.30E-09	3.94E-03	9.87E-05	1.55E-04	6.66E-06	6.17E-03	2.31E-05	5.09E-04	4.05E-05
MC	9.61E-08	1.53E-08	3.21E-10	3.21E-11	2.19E-10	2.55E-11	2.17E-03	5.77E-06	1.55E-05	6.01E-06	1.71E-04	1.00E-06	4.62E-04	1.80E-05
PI	8.58E-08	1.33E-08	1.42E-09	2.35E-11	7.59E-08	1.83E-10	2.11E-05	0.00E+00	5.98E-06	1.80E-07	4.75E-05	3.79E-07	1.00E-05	0.00E+00
RA	7.16E-08	5.57E-10	9.70E-10	1.78E-11	7.84E-08	2.67E-10	3.30E-05	5.77E-08	7.90E-06	1.66E-06	8.24E-05	5.03E-07	6.51E-05	1.44E-05
RM	5.26E-08	3.05E-08	3.28E-10	1.18E-11	3.11E-10	1.18E-10	2.30E-03	3.21E-05	1.86E-05	1.05E-05	1.70E-04	1.73E-06	3.89E-04	2.77E-05
SI (07)	2.44E-07	4.54E-08	2.17E-09	1.43E-10	8.29E-10	3.91E-10	2.48E-03	1.00E-05	1.23E-05	4.51E-06	1.62E-04	1.53E-06	3.07E-05	1.79E-05
TI	9.51E-08	1.13E-08	7.94E-10	6.46E-11	1.59E-10	8.13E-11	1.21E-04	4.16E-06	4.30E-06	3.16E-07	9.00E-05	4.16E-07	2.52E-05	3.22E-06

Table S3. **Comparison between measured and certified mean values (µg/g ± 95% confidence limits) for sediment reference materials: MESS-3 (n=8), PACS-3 (n=6) and CRM 7100 C513 (n=7).**

		As (µg/g ± 95% CI)	Cd (µg/g ± 95% CI)	Co (µg/g ± 95% CI)	Cu (µg/g ± 95% CI)	Mn (µg/g ± 95% CI)	Ni (µg/g ± 95% CI)	Se (µg/g ± 95% CI)	Zn (µg/g ± 95% CI)
PACS-2[a]	Measured (n=6)[d]	20.2 ± 0.9	2.08 ± 0.07	7.0 ± 0.2	279 ± 7	198 ± 11	28.2 ± 6.1	1.10 ± 0.07	321 ± 4
	certified[e]	**26.2 ± 1.5**	**2.11 ± 0.15**	**11.5 ± 0.3**	**310 ± 12**	**440 ± 19**	**39.5 ± 2.3**	**0.92 ± 0.22**	**364 ± 23**
MESS-3[b]	Measured (n=8)[d]	16.1 ± 0.6	0.20 ± 0.01	10.4 ± 0.5	26.6 ± 0.8	249 ± 9	32.1 ± 0.8	0.92 ± 0.84	122 ± 2
	certified[e]	**21.2 ± 1.1**	**0.24 ± 0.01**	**14.4 ± 2.0**	**33.9 ± 1.6**	**324 ± 12**	**46.9 ± 2.2**	**0.72 ± 0.05**	**159 ± 8**
CRM 7100 C513 (2-31)[c]	Measured (n=7)[d]		26.5 ± 1.7	14.6 ± 1.3	444 ± 20	633 ± 80	56 ± 5		
	certified (method 1.0)[f]		**28.2 ± 2.3**	**16.2 ± 1.8**	**475 ± 26**	**645 ± 50**	**59 ± 6**		
	certified (method 1.1)[g]		**27.4 ± 0.9**	**17.9 ± 1.9**	**470 ± 13**	**715 ± 29**	**61 ± 1**		

[a] Marine sediments (Esquimalt Harbour, BC, Canada), National Research Council of Canada (NRC), Ottawa, ON

[b] Marine sediments (Beaufort Sea), National Research Council of Canada (NRC), Ottawa, ON

[c] Freshwater sediments (St. Lawrence River, QC, Canada), COREM and INRS-ETE

[d] Values obtained from partial digestion: HNO_3/H_2O_2 at room temperature

[e] Values obtained from total digestion

[f] Values obtained from partial digestion: HNO_3/H_2O_2/HCl with intervals of heating and cooling (Ministère de l'Environnement et de la Faune du Québec, 1996)

[g] Values obtained from partial digestion: HNO_3/HCl in a block digester (Ministère de l'Environnement du Québec, 1987)

Table S4. **Arsenic concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are As concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments, as well as total dissolved As concentrations (mean ± SD, n=3, nmol/L) in lakewater, from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA														
AR (1.5m)			4.8	2.7										
AR (4.5m)			5.0	0.4			87		228[a]	12	230[a]	26	3.53	0.03
BO			17.8	1.0					139[b]	4	226[a]	3		
DA	33.9	10.8	28.1	3.1					276[a]	2	216[b]	5	20.33	0.32
DF			66.1	8.6					1,108[b]	41	1,320[a]	32	15.41	3.69
DP	5.2	1.2	14.8	2.6	246	63			361[a]	65	244[b]	27	10.79	0.84
FO	12.1	6.7							105[b]	1	153[a]	1	10.37	0.21
MN	12.5	7.1	16.4	1.2	373	52			307[a]	23	395[a]	107	43.17	2.31
OP (07)	10.2	6.2	4.3	1.3					61[a]	3	50[b]	2	6.18	0.26
OP (09-3m)			6.5	4.2										
OP (09-8.5m)	2.9	1.1	9.4	2.7										
OS	35.3	13.9	18.7	7.3			1,325	545	2,654[a]	178	2,869 [a]	282	17.58	0.31
PE	15.8	9.4			201				237[a]	9	209[a]	9	14.09	1.11
RO	28.1	9.3			1,082				1,043[a]	119	1,336[a]	183	24.46	1.32
SUDBURY														
CL			17.5	6.5					597[b]	32	1,108[a]	19	7.15	0.10
CR	19.3	4.9			1,010	575	333		1,048[a]	146	1,221[a]	275	20.54	0.66
HA			36.1	16.1			765	417	1,807[a]	62	1,838[a]	24	14.10	0.10
KA			13.8	6.7					371[b]	9	595[a]	29	9.74	0.21
KE	7.3	2.5			210	87			317[a]	16	256[a]	34	21.50	0.54
MC	8.0	3.2	11.8	4.1	242[a]	65	263[a]	71	475[b]	7	506[a]	14	13.63	0.45
PI			50.0	10.7			2,172	183	1,406[a]	202	1,178[a]	123	11.28	0.04
RA			54.0	18.1			719	216	939[a]	241	777[a]	88	7.97	0.04
RM			21.3	6.5					698[b]	30	1,277[a]	75	15.30	0.70
SI (07)	82.0	38.5	88.1	42.9					1,708[a]	67	1,113[b]	19	15.13	0.21
SI (11)			27.4	17.6										
TI	7.0	3.1	16.7	3.8					241[b]	33	440[a]	34	8.33	0.26

Table S5. **Barium concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Ba concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Ba concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5m)			16.7	11.1												
AR (4.5m)			18.5	5.3			576		331[b]	27	551[a]	71	68.5	0.3	48.8	0.1
DP	4.6	2.1	20.1	10.3	686	101			736[a]	6	750[a]	11	18.6	1.0	16.0	0.8
MN	7.1	2.8	24.0	13.7	891	159			818[a]	20	834[a]	24	34.0	2.6	27.8	2.1
OP (07)	98.3	64.6	42.6	29.4					1,361[a]	36	1,278[a]	51	55.7	2.1	44.9	1.7
OP (09-3m)			35.4	14.5												
OP (09-8.5m)	19.7	12.1	72.7	39.4												
OS	8.0	3.5	3.2	1.3			673	61	793[a]	130	781[a]	139	174.0	2.6	118.0	1.7
PE	40.4	37.4			1,064				902[a]	83	887[a]	19	131.3	8.1	84.2	3.9
RO	6.0	4.4			1,325				722[a]	32	705[a]	29	147.0	7.8	74.7	2.8
SUDBURY																
CL			12.8	7.7					493[b]	14	539[a]	10				
CR	5.5	1.1			475	277	408		540[a]	25	548[a]	20	83.6	0.5	68.9	0.3
HA			16.2	12.1			819	321	910[a]	19	726[b]	14				
KA			12.8	11.4					799[a]	16	769[a]	33				
KE	8.5	4.7			662	145			845[a]	13	687[b]	97	225.0	5.3	75.5	1.5
MC	8.9	2.6	21.3	8.9	1,793[a]	406	935[b]	156	1,291[a]	68	1,063[b]	12				
PI			3.3	0.7			404	97	295[b]	29	376[a]	16	75.9	0.2	67.5	0.2
RA			25.4	8.1			827	186	747[a]	57	725[a]	24	78.4	0.3	66.3	0.2
RM			19.2	12.2					1,224[a]	55	1,055[b]	20				
SI (07)	13.8	7.5	19.7	12.8					366[a]	17	330[b]	3				
SI (11)			2.1	0.8												
TI	8.7	5.7	8.0	1.8					583[a]	46	634[a]	9				

Table S6. **Cadmium concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Cd concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Cd concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5 m)			4.2	2.1												
AR (4.5 m)			1.6	0.1			6.8		12.3^{b}	2.9	61.8^{a}	4.3	4.57	0.06	3.46	0.04
BO			77.6	31.4					21.4^{b}	0.5	27.5^{a}	0.6				
DA	215.9	44.9	45.9	30.3					51.4^{b}	1.0	56.4^{a}	2.0				
DF			167.1	33.9					247.1^{a}	2.8	349.5^{a}	99.6	3.34	0.10	1.87	0.04
DP	293.7	67.2	47.0	23.4	98.5	30.8			52.2^{b}	1.1	65.3^{a}	2.7	0.30	0.11	0.12	0.05
FO	93.5	19.8							32.5^{b}	0.5	34.7^{a}	0.6				
MN	471.5	217.6	4.9	0.8	253.1	103.1			98.6^{a}	0.2	95.0^{a}	12.8	1.36	0.32	0.30	0.08
OP (07)	70.7	32.3	11.2	9.1					7.7^{b}	0.4	9.7^{a}	0.4	0.16	0.06	0.03	0.01
OP (09 - 3m)			21.9	8.1												
OP (09 - 8.5m)	43.0	9.9	14.5	6.7												
OS	92.6		15.8	4.4			542.8	152.9	943.1^{a}	172.4	924.4^{a}	218.1	0.48	0.01	0.23	0.01
PE	44.1	10.9			63.1				76.2^{a}	6.6	85.0^{a}	2.0	0.15	0.04	0.07	0.01
RO	81.0	23.0			452.2				679.8^{b}	47.6	888.2^{a}	75.9	3.91	0.15	1.82	0.04
SUDBURY																
CL			38.4	10.3					48.8^{a}	3.5	51.6^{a}	0.6	2.00	0.59	1.46	0.41
CR	92.9	14.8			73.2	75.9	116.6		18.9^{a}	3.3	21.9^{a}	7.8	1.12	0.03	0.59	0.03
HA			18.1	2.7			31.9	16.4	38.2^{b}	0.3	49.4^{a}	1.6	1.43	0.07	0.72	0.05
KA			3.1	1.9					27.5^{a}	0.4	27.5^{a}	2.0	0.49	0.02	0.27	0.03
KE	1.1	0.5			23.6	7.4			23.0^{a}	0.3	18.7^{a}	3.9	10.27	1.86	3.36	0.57
MC	116.3	8.9	15.4	4.9	534.6^{a}	119.8	89.8^{b}	50.3	48.3^{a}	2.7	51.4^{a}	0.7	0.32	0.03	0.15	0.01
PI			11.1	2.4			22.3	3.9	5.4^{a}	1.3	20.1^{a}	9.3	1.42	0.02	1.20	0.03
RA			28.5	13.4			38.5	12.1	19.7^{a}	2.5	22.4^{a}	1.9	0.97	0.02	0.55	0.03
RM			12.7	8.9					43.0^{b}	1.8	49.2^{a}	0.8	0.33	0.01	0.18	0.00
SI (07)	11.1	5.5	2.1	0.8					15.3^{a}	0.8	9.5^{a}	0.5	2.17	0.15	1.32	0.09
SI (11)			23.1	9.4												
TI	97.3	8.9	24.7	6.5					16.1^{b}	1.3	29.5^{a}	0.4	0.79	0.06	0.54	0.05

Table S7. **Cobalt concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Co concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Co concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5 m)			12.6	6.2												
AR (4.5 m)			6.3	0.7			229		169[b]	7	450[a]	8	154.00	1.73	119.33	1.53
BO			42.2	11.2					294[b]	8	384[a]	9				
DA	15.9	8.8	23.2	3.6					244[a]	3	241[a]	4				
DF			19.8	2.4					604[a]	14	673[a]	97	0.99	0.13	0.55	0.07
DP	1.7	0.5	6.2	1.2	162	6			259[a]	20	245[a]	1	0.38	0.03	0.23	0.02
FO	7.2	2.1							167[b]	1	170[a]	0				
MN	2.3	1.0	4.8	2.0	202	39			230[a]	6	237[a]	12	0.98	0.39	0.43	0.16
OP (07)	15.7	11.1	9.2	4.8					297[a]	8	295[a]	12	1.22	0.45	0.41	0.18
OP (09 - 3m)			7.0	2.1												
OP (09 - 8.5m)	3.7	1.8	11.7	5.2												
OS	14.6	3.4	4.7	1.3			574	254	1,071[a]	103	1,220[a]	123	1.48	0.06	0.31	0.02
PE	11.3	9.7			257				386[a]	33	422[a]	8	4.02	0.72	0.94	0.09
RO	5.6	2.1			497				554[b]	42	675[a]	24	10.69	1.36	3.61	0.40
SUDBURY																
CL			8.2	4.6					533[a]	41	732[a]	23	8.98	0.45	7.29	0.32
CR	21.6	3.3			298	168	164		344[a]	10	385[a]	102	13.57	0.31	9.64	0.30
HA			18.3	9.9					1,136[a]	24	1,216[a]	63	2.84	0.80	1.70	0.49
KA			8.9	3.5					942[a]	13	1,019[a]	48	3.72	0.10	2.69	0.18
KE	8.6	3.6			603	166			1,267[a]	125	1,150[a]	124	86.23	3.76	29.67	1.00
MC	20.3	2.2	37.1	16.1	720[a]	109	712[a]	181	1,671[a]	73	1,674[a]	84	1.88	0.22	0.99	0.11
PI			1.5	0.3			145	59	137[a]	8	246[a]	71	37.43	0.29	32.87	0.31
RA			15.2	7.0			232	60	439[b]	56	539[a]	47	0.42	0.02	0.30	0.02
RM			10.7	5.5					1,282[a]	50	1,744[a]	71	1.56	0.17	0.90	0.11
SI (07)	8.2	6.3	11.1	4.8					324[a]	21	211[b]	4	13.00	0.70	9.63	0.54
SI (11)			4.7	6.0												
TI	10.4	2.8	10.1	8.6					304[b]	43	435[a]	17	1.42	0.05	1.12	0.05

Table S8. **Cu concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Cu concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Cu concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5 m)			748	61												
AR (4.5 m)			1,404	951			6,160		7,100[b]	720	11,830[a]	700	283.67	5.13	203.667	3.512
BO			877	204					650[b]	20	900[a]	40				
DA	1,131	296	799	317					2,190[b]	40	2,300[a]	20				
DF			3,847	348					47,950[a]	2,180	54,620[a]	4,900	158.00	13.53	0.239	0.022
DP	1,295	381	1,231	382	5,570	1,000			3,140[b]	170	3,630[a]	90	42.17	7.84	0.019	0.004
FO	460	103							1,400[a]	20	1,440[a]	40				
MN	1,072	631	510	75	9,020	2,690			11,610[a]	570	11,060[a]	1,850	177.00	16.70	0.030	0.004
OP (07)	800	325	630	233					1,020[b]	40	1,150[a]	50	44.70	1.48	0.002	0.001
OP (09 - 3m)			457	105												
OP (09 - 8.5m)	574	172	634	193												
OS	1,324	4	695	159			66,610	29,310	147,030[a]	2,150	148,830[a]	5,990	52.53	1.65	0.013	0.001
PE	639	165			6,280				6,960[a]	590	7,560[a]	150	38.77	10.03	0.007	0.003
RO	832	121			28,260				45,340[a]	2,110	49,210[a]	1,220	103.00	13.75	0.128	0.048
SUDBURY																
CL			5,058	1,150					28,730[a]	1,540	25,790[a]	1,290	131.00	6.56	2.220	0.125
CR	6,290	1,017			34,850	10,900	72,870		26,760[a]	870	20,270[b]	1,080	353.67	15.14	1.227	0.105
HA			3,801	1,181			42,010	16,100	48,080[b]	610	77,660[a]	3,060	290.00	4.00	0.899	0.158
KA			833	192					11,420[a]	330	11,130[a]	440	137.67	1.53	0.750	0.532
KE	717	161			21,340	2,580			40,290[a]	990	33,770[a]	5,740	185.00	8.89	0.408	0.072
MC	3,441	535	2,035	734	30,770[a]	6,310	14,600[b]	5,250	13,780[a]	1,230	16,180[a]	230	108.00	5.57	0.156	0.025
PI			3,397	1,393			37,500	18,700	12,080[a]	400	15,960[a]	2,800	103.33	1.15	8.830	2.000
RA			4,564	1,331			27,160	9,770	16,400[a]	660	16,430[a]	680	113.00	1.00	0.254	0.039
RM			1,508	1,042					30,170[b]	1,400	35,170[a]	1,590	163.00	6.56	0.562	0.069
SI (07)	2,307	1,026	1,293	576					16,560[a]	1,040	13,960[b]	340	161.67	7.37	6.440	0.616
SI (11)	3,130	399	2,489	598												
TI	5,357	1,924	9,238	3,538					18,700[a]	1,580	19,370[a]	430	100.10	2.48	0.743	0.149

Table S9. **Manganese concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Mn concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Mn concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5 m)			249	20												
AR (4.5 m)			239	45			4,970		3,900[a]	80	4,590[a]	510	7,830	36	6,137	32
BO			3,262	1,436					7,660[a]	80	7,300[a]	70				
DA	2,079	1,040	1,834	475					42,300[a]	480	24,080[b]	200				
DF			650	85					12,310[a]	370	8,770[b]	830	249	12	83	3
DP	169	89	343	103	13,120				23,080[a]	6,610	9,310[b]	130	34	3	7	0
FO	2,400	1,160							15,080[a]	240	10,860[b]	70				
MN	206	109	195	72	26,540				9,270[a]	540	8,640[a]	650	107	67	10	7
OP (07)	3,057	1,043	1,537	1,752					54,010[a]	3,110	19,940[b]	1,340	141	9	16	1
OP (09 - 3m)			1,046	373												
OP (09 - 8.5m)	236	132	532	204												
OS	353	254	142	57			5,700	930	12,060[a]	810	11,210[a]	510	22	2	8	1
PE	762	781			13,120				14,660[a]	770	11,190[b]	1,120	1,193	129	432	2
RO	127	105			13,120				6,730[a]	90	6,490[a]	480	916	81	391	39
SUDBURY																
CL			283	97					1,200[b]	30	1,470[a]	20	251	4	155	6
CR	128	29			3,620	1,590	2,020		2,090[a]	520	1,630[a]	100	407	6	101	8
HA			304	143					17,590[a]	460	4,350[b]	90	101	24	31	9
KA			150	56					8,370[a]	210	4,420[b]	180	377	3	130	44
KE	84	12			2,530	600			3,440[a]	60	2,880[b]	90	1,317	21	465	5
MC	2,476	879	935	644	318,640[a]	61,300	60,550[b]	32,470	63,620[a]	4,900	21,290[b]	260	236	58	76	16
PI			91	15			1,630	380	1,060[b]	80	1,270[a]	70	920	18	725	5
RA			389	116			3,810	550	5,890[a]	1,500	4,600[a]	320	106	10	28	4
RM			318	75					8,310[a]	390	8,950[a]	120	105	29	42	12
SI (07)	62	5	67	17					1,150[a]	80	1,010[b]	10	734	318	465	193
SI (11)			145	5												
TI	128	50	149	32					2,580[a]	180	2,690[a]	30	169	6	79	4

Table S10. **Nickel concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Ni concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Ni concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5 m)			14	6												
AR (4.5 m)			12	1			560		347[b]	14	591[a]	58	45.1	0.4	35.1	0.3
BO			22	2					573[a]	6	588[a]	8				
DA	47	24	63	10					571[b]	7	590[a]	8				
DF			28	5					676[a]	19	720[a]	55	13.9	1.0	6.2	0.4
DP	5	1	11	5	419	13			469[b]	3	498[a]	16	12.3	8.3	5.0	3.6
FO	28	8							579[a]	14	591[a]	7				
MN	7	3	14	6	528	73			654[a]	23	656[a]	7	13.5	2.9	3.2	0.7
OP (07)	34	27	20	10					722[a]	7	740[a]	25	19.3	1.1	3.4	0.5
OP (09- 3m)			14	5												
OP (09 - 8.5m)	18	17	29	19												
OS	14	1	10	2			835	195	1,394[a]	108	1,418[a]	75	32.8	1.3	4.4	0.2
PE	27	21			740				891[a]	72	963[a]	18	37.1	10.8	5.6	0.3
RO	17	11			1,368				1,006[b]	70	1,168[a]	27	103.3	5.8	26.8	1.1
SUDBURY																
CL			126	57					15,455[b]	1,517	22,042[a]	1,270	1,123.3	25.2	867.3	10.7
CR	208	21			9,103	6,648	4,735		12,857[a]	1,045	15,038[a]	3,864	1,546.7	83.3	927.3	62.9
HA			370	222			13,995	4,808	27,282[b]	4,542	44,758[a]	2,184	2,110.0	36.1	1,073.3	46.2
KA			148	86					16,632[a]	1,983	18,106[a]	836	986.0	22.6	610.7	67.7
KE	233	87			13,589	7,524			26,281[a]	854	24,870[a]	2,680	4,873.3	30.6	1,493.3	49.3
MC	296	43	380	126	10,176[a]	1,480	8,667[a]	1,779	26,052[b]	1,113	31,005[a]	253	890.0	11.3	371.7	4.2
PI			45	8			4,207	1,964	4,507[b]	464	7,819[a]	1,683	897.3	9.1	768.7	8.3
RA			253	72			5,593	1,599	9,933[a]	1,124	12,120[a]	1667	1,043.3	5.8	636.7	15.9
RM			223	125					35,337[b]	1,142	51,560[a]	2717	869.0	15.9	424.0	20.1
SI (07)	139	29	259	101					11,169[a]	872	6804[a]	333	1,590.0	17.3	1,146.7	11.5
TI	103	26	128	16					9,186[b]	1,942	14,806[a]	2502	675.7	0.6	486.7	7.4

Table S11. **Selenium concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Se concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved Se concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA														
AR (1.5 m)			90	14										
AR (4.5 m)			50	6			29		38.9[a]	3.0	49.5[a]	1.7	1.4	0.5
BO			45	5					13.4[a]	1.2	12.7[a]	0.7		
DA	28	7	124	21					24.5[a]	1.2	24.8[a]	0.3		
DF			204	48					244.9[b]	8.6	262.1[a]	5.9	5.3	0.4
DP	88	5	218	27	35	4			25.1[a]	0.9	26.2[a]	0.6	1.2	0.4
FO	27	4							24.3[a]	0.6	24.2[a]	0.3		
MN	65	11	110	8	43	5			43.3[a]	1.2	45.9[a]	2.1		
OP (07)	18	1	41	8					14.0[a]	0.2	14.6[a]	0.3		
OP (09 - 3m)			29	9										
OP (09 - 8.5m)	42	10	73	9										
OS	140	8	194	15			311	117	419.9[a]	30.2	435.3[a]	42.7	6.8	1.2
PE	97	12			74				63.3[a]	7.5	72.4[a]	3.3	7.6	1.6
RO	454	68			441				363.8[b]	22.2	444.6[a]	37.9	20.6	1.8
SUDBURY														
CL			154	12					282.4[a]	25.8	239.2[a]	8.3		
CR	156	16			231	32	433		282.8[a]	7.7	286.7[a]	32.8	5.5	0.2
HA			314	22			225	89	289.5[b]	8.4	403.4[a]	33.7		
KA			83	11					85.8[a]	4.3	83.5[a]	2.4		
KE	324	31			375	104			477.0[a]	4.3	292.1[b]	87.4	38.8	2.5
MC	44	9	70	21	98[a]	20	70[a]	6	84.3[a]	4.6	83.9[a]	0.7	2.8	0.2
PI			191	8			221	31	228.9[a]	25.8	238.7[a]	10.2	3.0	0.3
RA			177	17			109	15	98.8[a]	3.5	100.9[a]	7.2	4.3	0.1
RM			146	17					174.2[b]	4.3	197.8[a]	3.5		
SI (07)			197	62					173.2[a]	6.8	157.5[b]	2.9		
SI (11)			210	22										
TI			192	28					165.4[a]	5.9	156.9[a]	3.1		

Table S12. **Zinc concentrations (mean ± SD, n=3-9, nmol/g) in larvae and feces/gut contents of *Chironomus* feeding on oxic and anoxic particles from lakes in Rouyn-Noranda and Sudbury. Also given are Zn concentrations (mean ± SD, n=3, nmol/g) in oxic and anoxic sediments as well as total dissolved and free ion Zn concentrations (mean ± SD, n=3, nmol/L) in lakewater from which *Chironomus* larvae were collected. For each lake, sediment data followed by the same letter do not differ significantly (p<0.05).**

Water body	*Chironomus* feeding on oxic particles (nmol/g)		*Chironomus* feeding on anoxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on oxic particles (nmol/g)		Feces/gut contents of *Chironomus* feeding on anoxic particles (nmol/g)		Oxic sediments (nmol/g)		Anoxic sediments (nmol/g)		Water - total dissolved (nmol/L)		Water - free ion (nmol/L)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
ROUYN-NORANDA																
AR (1.5 m)			1,660	310												
AR (4.5 m)			1,070	30			4,010		7,750[b]	1,950	31,270[a]	2,500	4,143	40	3,187	35
BO			1,160	190					2,450[b]	40	2,840[a]	40				
DA	1,870	810	1,490	170					3,600[b]	70	3,840[a]	40				
DF			5,390	460					38,390[a]	1,360	49,400[a]	9,570	714	6	249	2
DP	3,180	700	2,130	550	7,220	40			9,200[b]	30	10,430[a]	380	59	12	13	3
FO	1,610	250							2,760[a]	60	2,820[a]	40				
MN	3,200	700	1,540	150	4,950	1,170			6,210[a]	130	6,350[a]	550	54	21	5	2
OP (07)	1,020	40	1,120	220					2,220[b]	80	2,420[a]	90	8	6	1	1
OP (09 - 3m)			1,710	430												
OP (09 - 8.5m)	1,590	120	1,420	110												
OS	5,170	2,120	2,680	430			72,600	27,770	123,240[a]	6,030	130,720[a]	8,100	65	2	13	1
PE	3,450	560			8,820				15,800[a]	1,410	16,430[a]	250	13	4	3	0
RO	4,260	1,280			52,820				50,820[b]	6,540	64,190[a]	2,540	337	23	114	10
SUDBURY																
CL			890	130					3,160[b]	250	3,780[a]	10	250	25	163	14
CR	1,220	60			1,990	730	1,740		1,160[a]	90	900[a]	180	101	4	33	3
HA			1,320	280			2,060	790	2,420[b]	30	2,580[a]	30	60	6	22	3
KA			1,080	240					2,560[a]	50	2,780[a]	130	115	24	43	3
KE	1,450	270			3,230	940			4,210[a]	300	3,620[a]	440	197	15	61	3
MC	2,270	60	1,420	240	4,160[a]	380	3,860[a]	460	6,740[b]	100	7,110[a]	100	96	15	32	6
PI			810	60			890	160	420[a]	90	920[a]	360	86	13	68	9
RA			1,280	310			1,680	420	1,460[a]	250	1,570[a]	170	72	1	24	2
RM			1,000	170					4,520[b]	170	4,960[a]	120	53	31	22	13
SI (07)	1,120	170	1,080	170					1,650[a]	90	1,090[b]	40	244	45	160	31
SI (11)			1,350	400												
TI	940	120	1,310	490					1,470[b]	100	2,320[a]	10	95	11	49	5

Table S13. **Correlation coefficients and p-values for linear relationships between the concentrations of trace elements (TE) measured in *Chironomus* larvae feeding on oxic and anoxic particles and the concentrations of trace elements in sediments (oxic and anoxic sediments as well as oxic and anoxic sediments divided by the % of organic carbon (%OC)) and in water (total dissolved ($[TE]_{water}$) and free ion concentration ($[TE^{+}]_{water}$)) of lakes from which they were collected.**

	$[TE]$ *Chironomus* feeding on oxic particles vs. …						$[TE]$ *Chironomus* feeding on anoxic particles vs. …					
	$[TE]_{oxic\ sediments}$	$[TE]_{oxic\ sediments/\ \%\ OC}$	$[TE]_{anoxic\ sediments}$	$[TE]_{anoxic\ sediments/\ \%\ OC}$	$[TE]_{water}$	$[TE^{+}]_{water}$	$[TE]_{oxic\ sediments}$	$[TE]_{oxic\ sediments/\ \%\ OC}$	$[TE]_{anoxic\ sediments}$	$[TE]_{anoxic\ sediments/\ \%\ OC}$	$[TE]_{water}$	$[TE^{+}]_{water}$
As	p=0.027** R^2=0.372	p=0.044** R^2=0.321	p>0.05**	p>0.05**	p>0.05**	n/a	p<0.001** R^2=0.540	p=0.003** R^2=0.462	p=0.004** R^2=0.433	p>0.05**	p>0.05*	n/a
Ba	p>0.05$^{\beta}$	p>0.05	p>0.05$^{\beta}$	p>0.05	p>0.05$^{\beta}$	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Cd	p>0.05$^{\beta}$	p>0.05$^{\beta}$	p>0.05$^{\beta}$	p>0.05$^{\beta}$	p>0.05**	p>0.05**	p>0.05**	p>0.05**	p>0.05**	p>0.05**	p>0.05	p>0.05
Co	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05**	p>0.05	p>0.05	p>0.05	p>0.05**	p>0.05**
Cu	p>0.05$^{\beta}$	p>0.05**	p>0.05 $^{\beta}$	p>0.05**	p>0.05	p>0.05	p>0.05**	p>0.05**	p>0.05**	p>0.05**	p>0.05**	p>0.05**
Mn	p<0.001** R^2=0.737	p<0.001** R^2=0.677	p<0.001** R^2=0.711	p=0.001** R^2=0.633	p>0.05**	p>0.05**	p=0.004** R^2=0.443	p=0.001** R^2=0.506	p=0.002** R^2=0.493	p=0.001** R^2=0.506	p>0.05**	p>0.05**
Ni	p<0.001 R^2=0.930	p<0.001 R^2=0.837*	p<0.001 R^2=0.910	p<0.001 R^2=0.811*	p<0.001 R^2=0.751*	p<0.001 R^2=0.795*	p<0.001** R^2=0.818	p<0.001** R^2=0.792	p<0.001** R^2=0.775	p<0.001** R^2=0.732	p<0.001** R^2=0.824	p<0.001** R^2=0.773
Se	p=0.003 R^2=0.638	p<0.001 R^2=0.784	p<0.001 R^2=0.771**	p=0.003 R^2=0.652*	p=0.043 R^2=0.593	n/a see Fig. S4	p=0.005 R^2=0.423	p=0.021 R^2=0.307	p=0.001 R^2=0.505	p=0.023 R^2=0.301	p>0.05	n/a see Fig. S4
Zn	p<0.001** R^2=0.852	p<0.001 R^2=0.733	p<0.001 R^2=0.672	p<0.001 R^2=0.733	p>0.05	p>0.05	p<0.001** R^2=0.620	p<0.001** R^2=0.591	p<0.001** R^2=0.521	p=0.003 R^2=0.456	p>0.05**	p>0.05**

*Independent data were log_{10}transformed

**Independent and dependant data were log_{10}transformed

$^{\beta}$Spearman Rank Order Correlation

Table S14. **Correlation coefficients and p-values for linear relationships between the concentrations of trace elements (TE) measured in *Chironomus* larvae feeding on oxic and anoxic particles and the concentrations of trace elements in their feces/gut contents. Also given are those for linear relationships between the concentrations of trace elements in feces/gut contents of *Chironomus* larvae feeding on oxic and anoxic particles and the concentrations of trace elements in sediments (oxic and anoxic) and in water (total dissolved) of lakes from which they were collected.**

	***Chironomus* feeding on oxic particles**				***Chironomus* feeding on anoxic particles**			
	$[TE]_{Chironomus}$ vs. $[TE]_{feces/gut\ contents\ Chironomus}$	$[TE]_{feces/gut\ contents}$ vs.			$[TE]_{Chironomus}$ vs. $[TE]_{feces/gut\ contents\ Chironomus}$	$[TE]_{feces/gut\ contents}$ vs.		
		$[TE]_{oxic\ sediments}$	$[TE]_{anoxic\ sediments}$	$[TE]_{water}$		$[TE]_{oxic\ sediments}$	$[TE]_{anoxic\ sediments}$	$[TE]_{water}$
As	p=0.013 R^2 = 0.739	p<0.001 R^2=0.937	p=0.015[β] R^2=0.821	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Ba	p>0.05[β]	p=0.026 R^2=0.662	p=0.028 R^2=0.655	p>0.05	p>0.05	p=0.012 R^2=0.747	p=0.001 R^2=0.777	p>0.05
Cd	p>0.05*	p>0.05**	p>0.05*	p>0.05	p>0.05	p<0.001[δ] R^2=0.963	p<0.001[δ] R^2=0.950	p>0.05*
Co	p>0.05	P=0.002 R^2=0.885	p<0.001 R^2=0.923	p>0.05*	p>0.05	p<0.001 R^2=0.953	p<0.001 R^2=0.990	p>0.05
Cu	p>0.05	p=0.054 R^2=0.558	p=0.052 R^2=0.562	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05
Mn	p=0.031** R^2=0.638	p=0.15** R^2=0.726	p=0.021** R^2=0.691	p>0.05**	p=0.006 R^2=0.879	p=0.013** R^2=0.758	p=0.004 R^2=0.835	p>0.05[5]
Ni	p=0.001* R^2=0.910	p<0.001 R^2=0.928	p=0.002 R^2=0.878	p=0.015 R^2=0.728	p=0.015 R^2=0.807	p=0.016* R^2=0.718	p<0.001 R^2=0.965	p=0.013 R^2=0.738
Se	p<0.001 R^2=0.913	p<0.001 R^2=0.904	p<0.001 R^2=0.939	p>0.05	p=0.031 R^2=0.726	p<0.001** R^2=0.924	p=0.045 R^2=0.585	p>0.05
Zn	p=0.023** R^2=0.677	p<0.001 R^2=0.978	p<0.001 R^2=0.989	p=0.031 R^2=0.640	p=0.004 R^2=0.899	p=0.004 R^2=0.898	p=0.005** R^2=0.824	p>0.05

[β]Spearmann Rank Order Correlation
*Independent data were log_{10}transformed
**Independent and dependant data were log_{10}transformed
[δ]Significant correlations due to high [Cd] in Lake Osisko sediments and in fecal matter of *Chironomus* feeding on anoxic particlesin this lake. Without data from Lake Osisko, there is no correlation between the variables (p>0.05).

Table S15. **Total organic carbon (OC) (%) in oxic and anoxic sediments from lakes in Rouyn-Noranda and Sudbury.**

	$OC_{oxic\ sediments}$ (%)	$OC_{anoxic\ sediments}$ (%)
ROUYN-NORANDA		
AR (4.5 m)	10.9	7.3
BO	5.0	3.7
DA	6.5	6.4
DF	6.8	6.7
DP	6.7	6.6
FO	7.8	7.4
MN	5.6	5.6
OP (07)	2.8	2.5
OS	7.5	7.1
PE	3.8	3.5
RO	5.0	5.1
SUDBURY		
CL	9.9	9.2
CR	15.3	14.2
HA	8.9	7.1
KA	8.1	2.6
KE	5.5	4.3
MC	5.3	4.7
PI	14.1	15.0
RA	8.0	7.7
RM	5.3	4.6
SI	10.6	11.1
TI (07)	7.8	7.2

Figure S2. **Bivariate scatterplots of mean (nmol/g; ±SD; n=2-19) (A) As, (B) Ba, (C) Cd, (D) Co, (E) Cu, (F) Mn, (G) Ni, (H) Se and (I) Zn concentrations in *Chironomus* larvae feeding on oxic particles (●) and on anoxic particles (●) and mean (nmol/L, ±SD; n=3) dissolved concentrations of these trace elements in lakewater from which larvae were collected.**

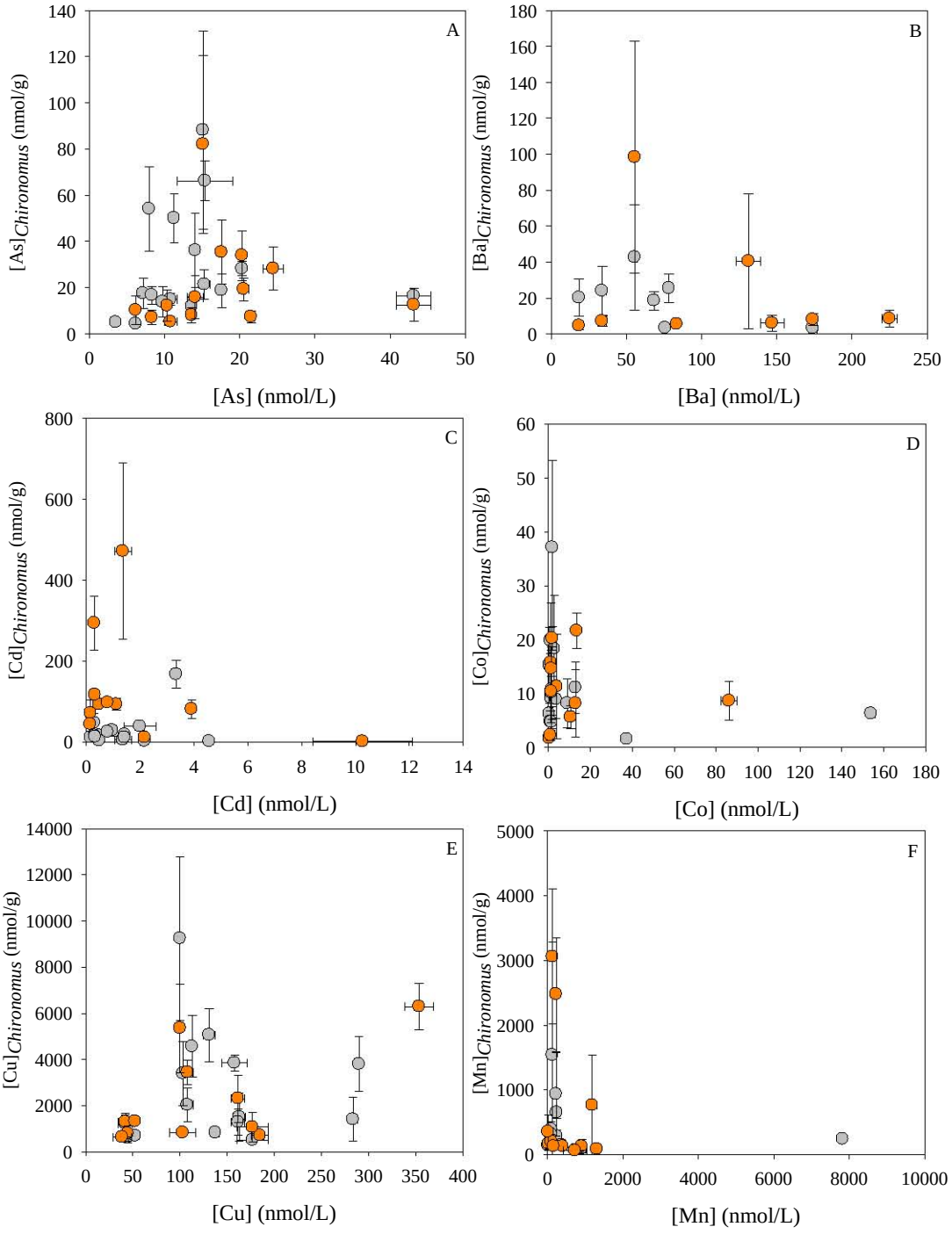


Fig. S2 (cont.)

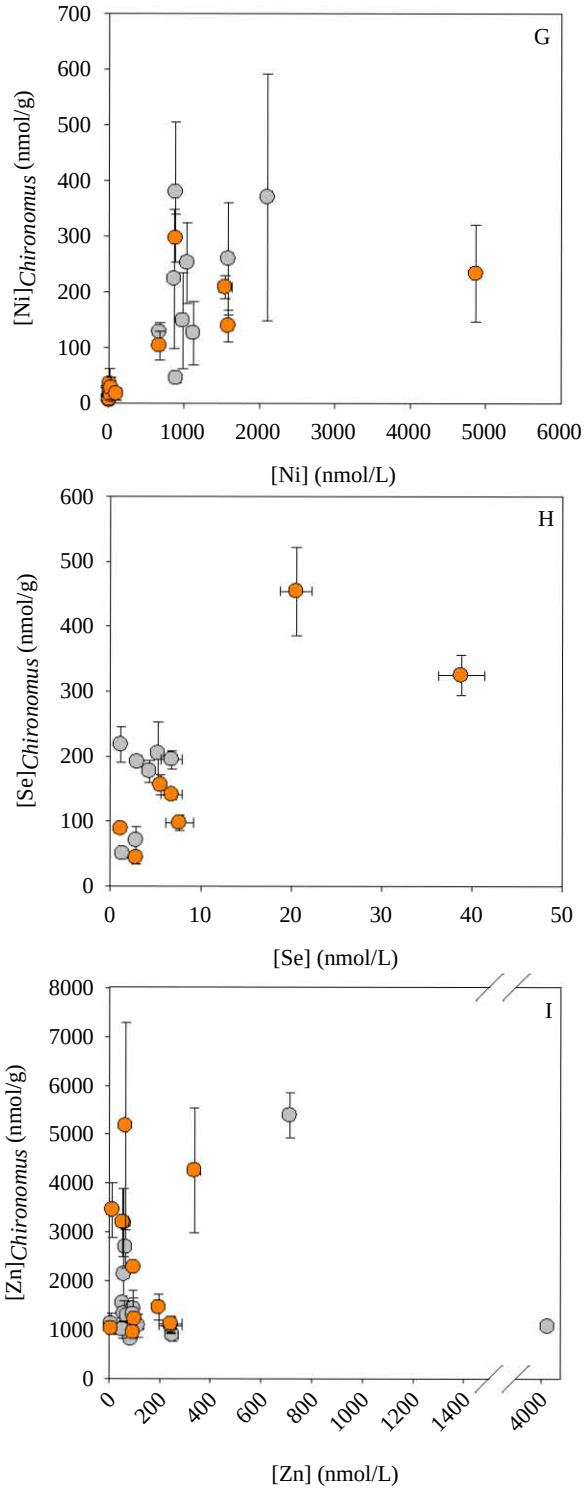

G
[Ni]*Chironomus* (nmol/g)
[Ni] (nmol/L)
H
[Se]*Chironomus* (nmol/g)
[Se] (nmol/L)
I
[Zn]*Chironomus* (nmol/g)
[Zn] (nmol/L)

Figure S3. **Bivariate scatterplots of mean (nmol/g; ±SD; n=2-19) (A) Ba, (B) Cd, (C) Co, (D) Cu, (E) Mn, (F) Ni and (G) Zn concentrations in *Chironomus* larvae feeding on oxic particles (●) and on anoxic particles (○) and mean (nmol/L; ±SD; n=3) free ion concentrations of these trace elements in lakewater from which larvae were collected.**

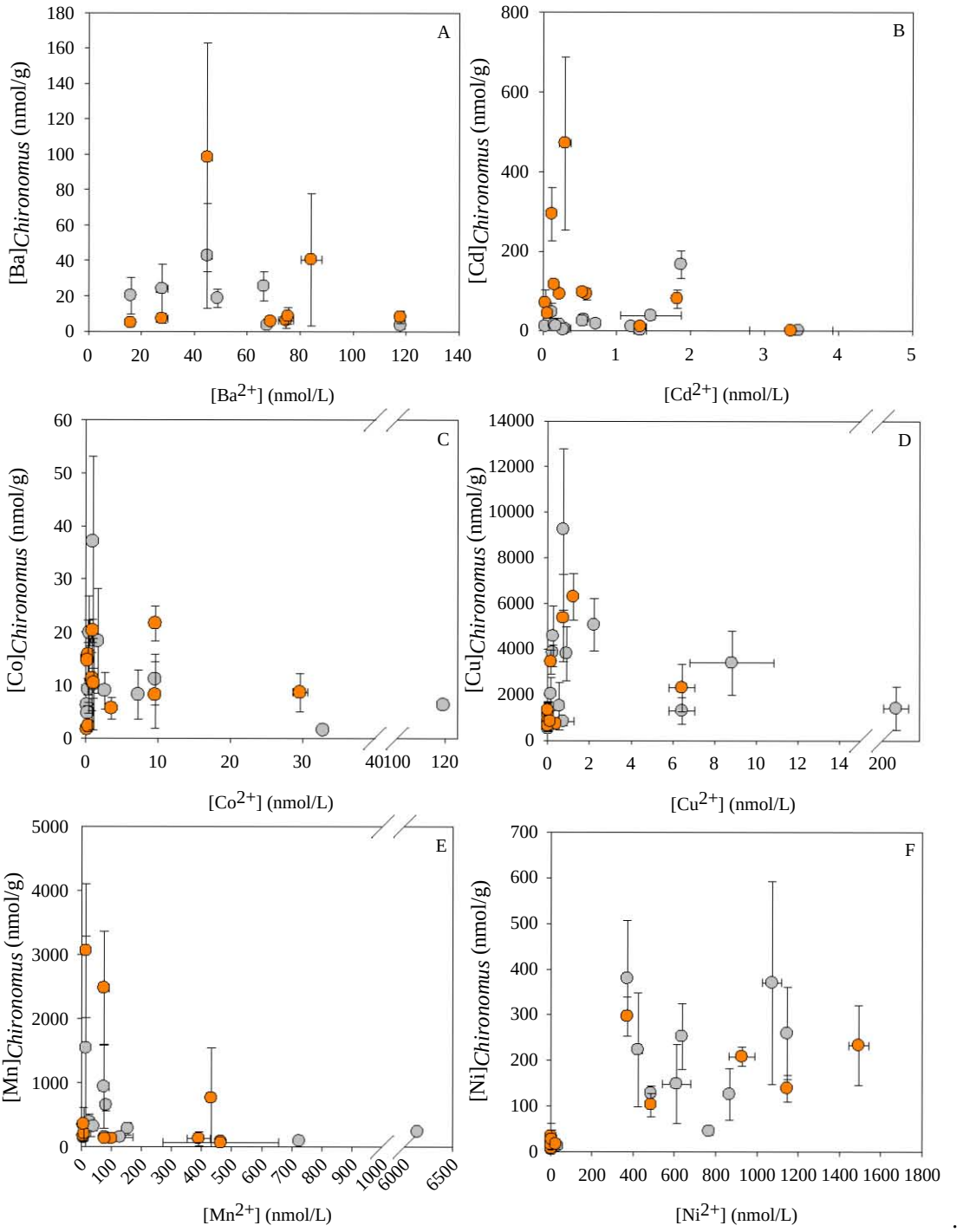


Fig. S3 (cont.)

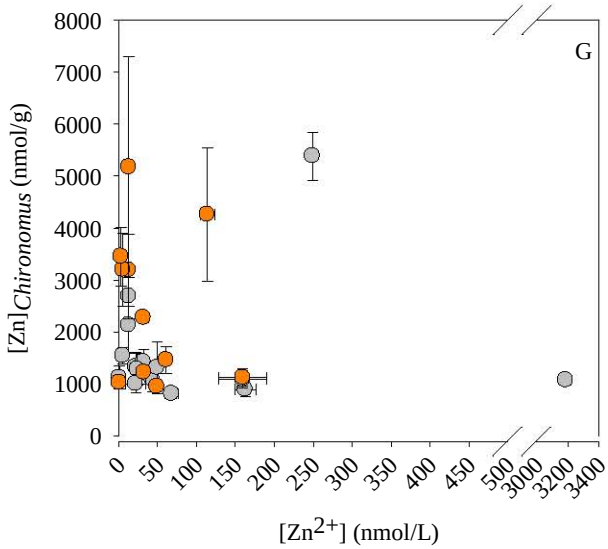

G
8000
7000
6000
5000
4000
3000
2000
1000
0
[Zn]Chironomus (nmol/g)
0
50
100
150
200
250
300
350
400
450
500
3000
3200
3400
[Zn2+] (nmol/L)

Figure S4. **Mean (±SD; n=2-19) Se concentrations in *Chironomus* larvae (nmol/g) feeding on oxic particles (●) and on anoxic particles (○) in relation to mean (±SD; n=3) concentrations of dissolved (A) total selenium (nmol/L), (B) selenite (Se(IV)) (µg/L), (C) organo-Se (µg/L), (D) selenate (Se(VI)) (µg/L), (E) organo-Se plus selenite (µg/L) and (F) organo-Se plus selenate (µg/L) in lakewater. Linear relationship statistics are indicated.**