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ÉCOTOXICOLOGIE LACUSTRE DU SÉLÉNIUM EN RÉGIONS MINIÈRES

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Le véritable voyage de découverte
ne consiste pas à chercher de nouveaux paysages,
mais à avoir de nouveaux yeux.

Marcel Proust

RÉSUMÉ

Le sélénium (Se) est un élément essentiel, mais une concentration interne cinq fois plus élevée que celle nécessaire peut induire des effets toxiques. Son écotoxicologie a été très étudiée, mais plusieurs mécanismes d'accumulation et d'effet ne sont pas compris. Nous avons mesuré les concentrations de trois espèces chimiques du sélénium (sélénite, séléniate et séléniures organiques) dans l'eau d'une quinzaine de lacs des régions de Sudbury, Ontario et Rouyn-Noranda, Québec. Ces mesures ont été comparées avec les concentrations de sélénium ([Se]) dans une chaîne trophique planctonique composée de microplancton (algues et bactéries), de crustacés planctoniques et de larves du diptère *Chaoborus*. Ce sont les concentrations de séléniures organiques dissous qui sont les mieux corrélées avec les [Se] dans cette chaîne trophique, mais l'addition des concentrations de séléniate rend les corrélations plus fortes. Afin de comprendre les mécanismes derrière ces observations, nous avons exposé l'algue verte unicellulaire *Chalmydomonas reinhardtii* à la même concentration de sélénite, de séléniate ou de sélénométhionine, au laboratoire, mais à des concentrations variables de sulfate, d'ions hydrogène et ce, pour plusieurs durées d'exposition. Ces expériences démontrent que l'accumulation par cette algue verte est plus importante lorsqu'exposée au séléniate qu'au sélénite, mais à des concentrations de sulfate très élevées, la prise en charge de sélénite est un peu plus importante. Par contre, l'accumulation de sélénométhionine n'a pas été influencée par les concentrations de sulfate. À des [SO_4] représentatives des lacs étudiés, les [Se] chez l'algue étaient trois fois plus élevées lorsqu'exposée à la sélénométhionine qu'aux espèces inorganiques. Dans toutes les conditions expérimentales, les [Se] chez *C. reinhardtii* étaient faibles comparativement à celles mesurées dans le microplancton naturel des lacs, et ce à de plus faibles concentrations aqueuses. Nous avons donc récolté le microplancton du lac Bédard (lac témoin) au nord de la ville de Québec et exposé ces organismes aux mêmes conditions que l'algue verte. Ces expériences démontrent que les [Se] chez microplancton naturel étaient 30 fois plus élevées que celles de *C. reinhardtii* lorsqu'exposés à la sélénométhionine. Contrairement à l'algue, les [Se] chez le microplancton étaient plus élevées lorsqu'exposé au sélénite qu'au séléniate. De façon générale, pour une concentration donnée de Se dissous, l'accumulation de la forme organique sera supérieure à celle observée pour les formes inorganiques, mais l'accumulation préférentielle des espèces chimiques du sélénium est étroitement liée aux types de micro-organisme étudiés.

Ces données de laboratoire nous ont permis d'estimer les [Se] mesurées chez les organismes pélagiques des lacs étudiés.

Nous n'avons pas observé de bioamplification du Se dans la chaîne trophique pélagique constituée d'invertébrés et de microplancton pélagiques, mais une bioamplification du Se a été observée entre les perchaudes et leur proie. De plus, lorsque les perchaudes se nourrissent davantage de proies venant des sédiments, elles sont exposées à des [Se] plus élevées. Effectivement, nous avons obtenu des régressions négatives entre la signature isotopique de soufre ($\delta^{34}S$) et les [Se] dans les perchaudes et leurs proies suggérant que plus les organismes se nourrissent dans un environnement anoxique, plus ils sont exposés au Se. Les chironomes qui consomment les sédiments (p. ex. *Chironomus*) sont les invertébrés avec les plus fortes [Se]. Nous suggérons donc de les utiliser pour les évaluations du risque environnemental afin d'estimer l'exposition maximale potentielle des perchaudes. L'estimation des [Se] dans les gonades suggèrent un risque de toxicité pour certaines perchaudes des lacs Kelly et Rouyn. Afin d'évaluer si les métaux et le Se causent des effets toxiques à ces perchaudes, nous avons mesuré le stress oxidatif cellulaire dans leur foie.

Des mesures physiologiques hépatiques indiquent que 44% des 50 perchaudes récoltées provenant de 11 lacs subissent du stress oxydatif cellulaire. Ce stress est inversement corrélé avec les [Se] dans le foie indiquant son pouvoir comme antioxydant. Les poissons qui subissent davantage de stress oxydatif ont des proportions de cadmium, cuivre et zinc plus élevées dans leurs compartiments cellulaires sensibles. Nous avons quantifié le débordement subcellulaire de cadmium dans les fractions sensibles et observé qu'il était corrélé avec la peroxydation lipidique. Par contre, la peroxydation lipidique était négativement corrélée avec les concentrations de Se dans le foie. Cette étude est la première qui démontre un effet antagoniste du Cd et du Se en milieu naturel. Puisque le Se peut interagir avec des métaux dans les cellules, nous avons observé chez un autre organisme, l'accumulation et la répartition subcellulaire du Cd et du Se au cours d'une étude de transplantation entre deux lacs.

La transplantation de larves du diptère *Chaoborus* du lac Dasserat au lac Dufault a démontré que le taux d'accumulation de Se est constant durant toute l'expérience, mais qu'un délai d'environ une semaine est observé avant que les larves accumulent le Cd. Par l'utilisation d'un modèle déterministe, nous avons calculé que le changement d'accumulation de Cd était dû à une augmentation de l'efficacité d'assimilation passant de 2,3 à 4,6 %. Le fractionnement

subcellulaire indique une grande différence dans la gestion intracellulaire de ces deux éléments. À la fin de l'expérience, les quantités de Se étaient similaires dans les compartiments cellulaires sensibles et ceux qui détoxiquent les métaux. Au contraire, les quantités de Cd dans les parties sensibles sont toujours restées faibles. Cette expérience démontre comment les larves de *Chaoborus,* en deux semaines, arrivent à gérer les éléments traces intracellulaires comme le fait les larves indigènes du lac Dufault.

Cette thèse présente plusieurs nouveautés concernant l'écotoxicologie du sélénium. Nous avons clarifié l'influence de la spéciation chimique du Se dans l'eau sur l'accumulation par des organismes pélagiques. Les organismes benthiques et leurs prédateurs, quant à eux, sont davantage exposés au sélénium que les organismes se nourrisant dans la colonne d'eau et finalement, cet élément semble protéger les perchaudes du stress oxydatif et de la toxicité du cadmium.

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AVANT-PROPOS

Cette thèse a un format « par articles », c'est-à-dire qu'elle comporte une synthèse qui est un premier chapitre sur les particularités écotoxicologiques du sélénium et les 2e, 3e, 4e et 5e chapitres sont des versions courtes de mes cinq articles écrits et présentés à la suite. Le premier article porte sur les relations entre la spéciation du Se dans l'eau lacustre et l'accumulation du Se par la larve de l'insecte *Chaoborus.* Le deuxième explore l'accumulation de sélénite, de séléniate et de sélénométhionine par l'algue *Chlamydomonas reinhardtii* et le microplancton exposés au Se en laboratoire. Le troisième décrit l'exposition au Se des perchaudes (*Perca flavescens*) de quatre lacs, en la reliant avec son alimentation par les mesures de la signature isotopique de soufre. Le quatrième montre le lien entre les mesures de stress oxydatif chez des perchaudes sauvages et le fractionnement subcellulaire des métaux ainsi que l'exposition de ces poissons au Se. Le dernier article décrit les résultats d'une expérience de transplantation de larves de *Chaoborus* entre deux lacs et les mesures temporelles de l'accumulation du Cd et du Se, leurs fractionnement subcellulaire et les différentes stratégies de détoxication de ces deux éléments. J'ai effectué ces études au complet, de la conception à l'écriture des articles et les activités de la dernière étude ont été partagées à 50% avec Maikel Rosabal-Rodriguez.

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SYNTHÈSE

PREMIER CHAPITRE - INTRODUCTION

1.1. Sélénium et sa découverte

Le sélénium (Se) est un non-métal du groupe des chalcogènes qui possède le numéro atomique 34 du tableau périodique et sa masse molaire est de 78,96 g (Lide, 1994). Il a été découvert en 1818 par le Suisse Berzélius lors de la production d'acide sulfurique. Le tellure, qui est très similaire au Se, fait référence au dieu de la Terre, donc en référence à la déesse de la lune Séléné, Berzélius nomma ce nouvel élément sélénium (Berzélius, 1818). Le soufre est également un chalcogène et est très similaire au sélénium. Ceux-ci se retrouvent souvent au même endroit dans l'environnement, par exemple dans les sulfures de métaux et aussi dans les cellules.

1.2. Le caractère essentiel du sélénium

En 1957, le Se a été identifié comme élément essentiel pour le bon fonctionnement physiologique des mammifères (Schwarz et Foltz, 1957). Depuis, des protéines contenant du Se, les sélénoprotéines, ont été identifiées dans pratiquement tous les règnes (Young et al., 2010). Une trentaine de familles de sélénoprotéines ont été découvertes, mais les rôles de certaines ne sont pas encore connus (Hesketh, 2008). Certaines d'entre elles, comme la glutathion peroxidase et la thioredoxine réductase, participent à la défense des cellules contre le stress oxydatif (Flohé, 1988; Arnér et Holmgren, 2000). D'autres sélénoprotéines servent à réguler le métabolisme de la glande thyroïde (Berry et Larsen, 1992), le statut redox de certaines molécules comme la vitamine C (May et al., 1998) et la synthèse de sélénocystéine. Une sélénoprotéine très étudiée est la sélénoprotéine P. Sa séquence génétique est conservée chez les bactéries, les mammifères et les poissons (Tujebajeva et al., 2000). Il s'agit d'une protéine extracellulaire qui a été identifiée comme participant au transport de Se dans le plasma sanguin des rats (Birk et Hill 2005; Reilly, 2006). Les rats déficients de cette protéine ont des concentrations de Se plus faibles dans le cerveau, les testicules et le fœtus et ont présenté des signes pathologiques dans ces tissus (Birk et Hill, 2005). De plus, la sélénoprotéine P peut lier le mercure,

l'argent, le cadmium, le zinc et le nickel (Yoneda et Suzuki, 1997a; 1997b; Mostert et al., 1998; Sasaku et Suzuki, 1998; Yan et Barret, 1998; Mostert, 2000). Le sélénium peut réduire les risques de contracter un cancer (Schrauzer, 2000), en autre, en diminuant le stress oxydatif cellulaire (Rayman, 2000).

La dose recommandée de Se dans la nourriture pour l'humain est de 55 µg par jour (Institute of Medecine, 2000) et la dose minimale causant un effet chez l'humain est de 1260 µg par jour, ce qui est seulement 22 fois plus que celle qui est recommandée (U.S. EPA, 2002). La dose maximale tolérable a été fixée à 400 µg par jour afin de protéger l'humain (Institute of Medecine, 2000), ce qui est seulement huit fois plus que la dose recommandée. Donc, un des dangers associés au Se est la faible marge entre les doses essentielles et toxiques. D'ailleurs, il est rapporté qu'il y aurait davantage de risques de carence chez l'humain que de risques de toxicité (Winkel et al., 2012). Dans le cas des poissons, des concentrations quatre fois plus élevées que celles qui sont essentielles peuvent causer des déformations (Lemly, 1993). Mentionnons que le règne végétal est beaucoup plus résistant aux concentrations élevées de Se que le règne animal (Wilber, 1980) et que les vertébrés ovipares sont plus sensibles que les mammifères. Les [Se] internes pouvant induire des effets toxiques chez les poissons (Lemly, 1993) peuvent aussi, selon de récentes études, en causer chez des invertébrés (Debruyn et Chapman, 2007).

1.3. Sources naturelles et anthropiques de sélénium

L'atmosphère est une source importante de Se pour les plans d'eau et les sols (Wen et Carignan, 2007). Les sources naturelles de Se qui alimentent l'atmosphère sont l'érosion du sol par le vent, les volcans, les sels océaniques et les biosphères marine et terrestre (Mosher et Duce, 1987; Nriagu et Pacyna, 1988; Nriagu, 1989). Mosher et Duce (1987) ont estimé qu'entre 13 000 et 19 000 tonnes de Se étaient en circulation annuellement dans la troposphère et que 50 à 65 % de cette masse provenait de la contamination naturelle. Cependant, une réévaluation globale plus récente serait nécessaire puisque la contamination anthropique a augmenté due à l'utilisation croissante de charbon en Asie depuis les années 80. À l'époque, 50 % de la contamination anthropique de Se provenait de la combustion du charbon et 20 % de la fonte de métaux comme le cuivre, le plomb, et

le zinc. Mises à part ces sources principales vers l'atmosphère, mentionnons l'incinération de déchets et la production et l'utilisation d'engrais (Wen et Carignan, 2007).

Les concentrations dans le sol sont généralement entre 0,1 $\mu g\ g^{-1}$ et 0,5 $\mu g\ g^{-1}$ (Dhillon et Dhillon, 2003; Lemly, 2004) mais ont une distribution géographique très hétérogène dans la croûte terrestre (Lenz et Lens, 2009) et celles-ci peuvent être aussi élevées que 100 $\mu g\ g^{-1}$ naturellement (Fordyce, 2007). Cette distribution entraîne des risques de carence en Se et de toxicité qui peuvent survenir à proximité (cas de la Chine; Lenz et Lens, 2009). Durant les dernières décennies, il y eut une grande réduction des apports atmosphériques de plusieurs contaminants provenant de l'industrie du charbon et de la fonte des métaux semi-précieux. Les technologies de réduction des apports atmosphériques sont maintenant si efficaces qu'elles peuvent éliminer 99,5 % des émissions particulaires d'une centrale énergétique au charbon (Lemly, 2004). Par contre, cette impressionnante efficacité peut avoir des répercussions sur d'autres systèmes puisque ces particules doivent se retrouver ailleurs. Des amoncellements énormes de cendres de charbon contenant des concentrations impressionnantes (50-300 $\mu g\ g^{-1}$) de Se sont entreposés à même des terrains vagues (Lemly, 2004). La production annuelle de cendres de charbon aux États-Unis était évaluée à environ 120 millions de tonnes (US EPA, 1998). Ces cendres sont souvent disposées sur de l'argile, mais avec les années, l'argile craque et des écoulements contenant de concentrations de sélénium entre 50 et 200 $\mu g\ L^{-1}$ peuvent se rendre aux plans d'eau environnants (Lemly, 2004). Le caractère alcalin des cendres du charbon augmente le risque de contamination des écoulements d'eau puisque les oxyanions du Se (sélénite et séléniate) se dissous plus facilement à pH élevé (Lemly, 2004). Le meilleur exemple d'environnements touchés par l'industrie du charbon et la subséquente contamination en Se est probablement le cas du lac Belews, en Caroline du Nord. Sa contamination dans les années 70 a entrainé des pathologies graves chez les poissons et la disparition d'une importante partie de la communauté piscivore (20 espèces; Lemly, 2002).

Les mines de charbon, de phosphate et de métaux (cuivre, nickel, or, uranium, zinc) ont été responsables de plusieurs événements de contamination majeure qui ont

entraîné des impacts néfastes sur des populations de poissons ou d'oiseaux aquatiques à travers le monde (Lemly, 2004). Lemly (2004) prétend que l'activité croissante de raffinage de métaux risque d'augmenter les concentrations de Se dans l'environnement et donc les risques reliés à sa toxicité.

Un autre type de contamination par le sélénium provient de l'irrigation des terres arables semi-arides (Luoma et Rainbow, 2008). Les facteurs climatiques et géographiques contribuant au potentiel de trouver du Se dans l'eau d'irrigation en agriculture sont : (1) la présence de schiste riche en matière organique, (2) un climat qui génère plus d'évaporation que de précipitation et (3) un drainage du sol qui est limité par une couche imperméable de sous-surface (p. ex : argile; Presser, 1999). Prenons l'exemple de la vallée San Joaquin (centre ouest), en Californie. L'eau d'irrigation est caractérisée par un pH alcalin, des concentrations élevées en sels, en éléments traces, en éléments azotés et pauvres en pesticides. Elle s'écoule vers les terres de basse altitude et si elle n'est pas évacuée, elle s'évapore et laisse derrière les éléments qu'elle contient, ce qui empêche les plantes d'avoir une bonne croissance. Les gestionnaires de ces terres agricoles ont donc décidé, au début des années 80, d'écouler l'eau chargée en éléments par des canaux. Cette eau s'est finalement retrouvée dans la réserve faunique Kesterson où, en 1983, les oiseaux aquatiques se sont retrouvés intoxiqués par le Se et plus de 60% de leur progéniture étaient gravement déformés (Presser et Ohlendorf, 1987).

1.4. La toxicité du sélénium

Les manifestations cliniques d'une toxicité due au sélénium chez l'humain s'appellent, en anglais, *selenosis* ou *alkali disease* (Goldhaber, 2003). Les symptômes d'effets toxiques chez l'humain comprennent la perte de cheveux, la décoloration et déformation des ongles, l'haleine à odeur d'ail, la diarrhée, la fatigue, des nausées et, dans les cas extrêmes, des lésions de la peau et du système nerveux (Yang et al., 1983; MacFarquhar et al., 2010). Parmi les vertébrés, les ovipares sont les plus sensibles aux effets toxiques du sélénium. Le principal effet toxique chez les animaux aquatiques (poissons et oiseaux) est une déformation des juvéniles (effet tératogène) dès leur éclosion due au transfert de Se de la mère au juvénile durant la vitellogenèse (Chapman et al., 2010). Certains invertébrés peuvent aussi être affectés par de faibles concentrations de Se. Effectivement,

une [Se] interne de 3 µg g^{-1} chez *Chironomus decorus* peut réduire sa croissance de 15 à 40 % (Debruyn et Chapman, 2007). Par contre, *Chironomus dilutus* peut accumuler des [Se] impressionnantes de plus de 100 µg g^{-1} (Gallego-Gallegos et al., 2012).

Certaines études démontrent que des concentrations de Se trop élevées chez les animaux entrainent une substitution du souffre par le sélénium au niveau de la structure tertiaire des protéines (liaison S-S), pour ainsi déformer les protéines et perturber le développement des tissus et le fonctionnement des enzymes (Sunde, 1984; Maier and Knight, 1994; Spallholz et Hoffman, 2002). Par contre, quelques études récentes tendent à expliquer la toxicité du sélénium par un stress oxydatif plutôt que par une substitution du soufre (Chapman et al., 2010). Par contre, le Se est aussi connu comme étant un antioxydant très puissant. Le glutathion (GSH), une molécule aussi très abondante dans les cellules pour protéger contre le stress oxydatif, réduit le peroxyde d'hydrogène en eau avec l'enzyme glutathion peroxydase. Cette dernière enzyme catalyse la réaction et nécessite le sélénium pour être active. Pendant cette réaction, le glutathion est oxydé et deux molécules se lient pour former le disulfure de glutathion (GSSG). Un article rétrospectif concernant des études physiologiques effectuées sur des canards colverts (*Anas platyrhynchos*) écrit par Hoffman (2002) indique que dans la majorité des cas de canards colverts exposés au sélénium, le ratio GSH/GSSG diminue, indiquant un stress oxydatif. Certaines espèces chimiques de sélénium, principalement le sélénium lié à des groupements méthyles peuvent réagir avec le glutathion et générer un stress oxydatif (Spallholz et Hoffman, 2002). Par contre, étant donné son caractère d'antioxydant puissant, des concentrations très élevées doivent être nécessaires pour induire un stress oxydatif. Le mécanisme de toxicité exact n'est pas encore connu.

Plusieurs études de toxicité ont été effectuées avec des algues, mais les concentrations nécessaires pour induire des effets toxiques sont nettement plus élevées que celles normalement retrouvées dans le milieu naturel (< 0,2 µg L^{-1}; Luoma and Rainbow, 2008,) ou même contaminé (0,2-10 µg L^{-1}; Lemly, 2004; Conde et Sanz Alaejos, 1997). Le sulfate peut d'ailleurs protéger les algues de l'accumulation de séléniate et de sélénite (Morlon et al., 2005; Fournier et al., 2010; Geoffroy et al., 2007). En augmentant les concentrations de sulfate de 8 à 80 µmol L^{-1}, la concentration causant une diminution de la densité de 50% (EC_{50}) passe de 0,4 à 3,1 µmol L^{-1} (32 et 245 µg L^{-1},

respectivement; Fournier et al. 2010). Le séléniate s'avère moins toxique que le sélénite chez *Chlamydomonas reinhardtii*. Pour une concentration de sulfate similaire de 80 µmol L^{-1}, la EC_{50} de séléniate est de 80 µmol L^{-1} (6317 µg L^{-1}; Morlon et al., 2005). Bien que les algues soient résistantes au Se, c'est davantage les concentrations internes à une [Se] dans l'eau donnée que l'on doit surveiller afin de protéger les niveaux trophiques supérieurs.

Comme le mentionne les études de Lemly (1993), une [Se] de 3 µg g^{-1} chez les proies des poissons devrait être celle maximale afin de les protéger, mais d'autres études suggèrent une concentration maximale de 11 µg Se g^{-1} pour éviter l'apparition d'effets toxiques. La protection des poissons est bien entendu importante, mais un article de rétrospective de Debruyn et Chapman (2007) vérifie si la gamme de [Se] de 3 à 11 µg g^{-1} (Lemly 1993) permet aussi de protéger les invertébrés. Il s'avère que des concentrations internes entre 3 et 11 µg de Se g^{-1} chez des invertébrés peuvent entraîner des effets toxiques (Debruyn et Chapman, 2007) et est près (environ 12 µg g^{-1}) de la concentration entrainant une diminution de la croissance de *Daphnia*. Comme l'étude de Malchow et al. (1995) le démontre, des larves de l'insecte *Chironomus decorus* nourries avec des algues ayant une [Se] > 2,1 µg g^{-1} ont vu leur croissance réduite malgré une [Se] interne faible de 2,6 µg g^{-1}. Il est donc très important, lors de tests de toxicité, de mesurer les concentrations internes de Se des organismes testés afin d'y lier la toxicité et de bien prédire l'accumulation chez les niveaux trophiques supérieurs (Stewart et al., 2004).

1.5. Spéciation et mesure du sélénium dans l'eau

Il existe une grande abondance d'espèces chimiques de sélénium qui peuvent être regroupées en quatre groupes : le sélénium inorganique (sélénite, séléniate, séléniures minérales, sélénium élémentaire), le sélénium méthylé et volatile (dimethylséléniure, methylselenol, etc.), les intermédiaires biochimiques (séléno-urée, sélénophosphate, etc.) et les sélénoprotéines (comprenant la sélénométhionine, la sélénocystéine, la sélénoprotéine P; Chapman et al., 2010). Parmi toutes ces espèces chimiques, le sélénium peut être présent sous des états d'oxydation allant de –II à VI : le sélénium élémentaire (Se(0)), les séléniures (Se(-II) à Se(II); Wallshlager et Feldmann, 2010), le sélénite (Se(IV)) et le séléniate (Se(VI); Chapman et al., 2010).

Les espèces chimiques dissoutes les plus abondantes dans les eaux oxygénées sont le sélénite (SeO_3^{2-}) et le séléniate (SeO_4^{2-}; Conde et Sanz Alaejos, 1997). Les séléniures organiques (Se(-II) à Se(II); Wallshlager et Feldmann, 2010) et le Se élémentaire (Se(0)) sont davantage présents en milieux anaérobies et plus abondants dans les sédiments (Martin et al., 2011). Le sélénite et le séléniate absorbés par les organismes sont réduits à des états d'oxydation inférieurs pour ensuite être sous une forme non liée dans le cytosol ou incorporés aux acides aminés dans les cellules (Wrench and Campbell, 1981; Bottino et al., 1984; Besser et al., 1994). L'excrétion, la mort ou la lyse des cellules peuvent libérer les séléniures organiques dans l'environnement. Contrairement à la spéciation de plusieurs métaux régis par des lois d'équilibre thermodynamique, la spéciation du sélénium dépend davantage des réactions biogéochimiques du plan d'eau (Chapman et al., 2010). Ainsi, un temps de rétention plus élevé d'un plan d'eau comme un lac ou un marais augmentera les proportions de séléniures organiques dans l'eau (Presser and Luoma, 2010).

Le sélénium est un des rares éléments dont il est possible de déterminer sa spéciation à des concentrations environnementales. Plusieurs techniques comme la spectrométrie en fluorescence atomique (AFS) et la spectrométrie en absorption atomique (AAS)) ne peuvent que mesurer le Se(IV), soit en génération d'hydrure ou par fluorométrie à l'aide d'un fluorophore (Hawkes et Kutnink, 1996; Gao et Wang, 2000). Ces techniques nécessitent généralement plusieurs prétraitements d'échantillon afin de convertir les différentes espèces en Se(IV) (D'Ulivo, 1997). Ces prétraitements peuvent entraîner des pertes ou des changements de spéciation. La technique de Chen et al. (2005) peut diminuer les pertes et les changements de spéciation. L'appareil de détection est un AFS couplé avec un générateur d'hydrure (HG-AFS). Premièrement, les [Se(IV)] sont mesurées dans un sous-échantillon d'eau acidifiée avec de l'acide chlorydrique ([HCl] finale de 3 M). Un deuxième sous-échantillon deux fois plus volumineux est ensuite exposé à des rayons ultra-violets (UV) pendant deux heures afin d'oxyder les séléniures organiques en Se(IV) et de mesurer les concentrations de séléniures organiques additionnées à celles de Se(IV). La moitié de ce dernier volume doit finalement être chauffée aux micro-ondes pendant 15 minutes afin de transformer le Se(VI) en Se(IV) et ainsi mesurer les [Se total]. Par soustraction, les concentrations de ces trois espèces

chimiques peuvent être connues. Afin de mieux distinguer les séléniures, il est possible de soumettre l'échantillon en chromatographie liquide afin de les séparer avant l'analyse en couplage (article rétrospectif de Chen et Belzile, 2010) avec un spectromètre de masse à plasma couplé par induction (ICP-MS) ou un spectromètre en fluorescence atomique (AFS). L'AFS est à privilégier pour les analyses dans les eaux naturelles car sa limite de détection est faible (12 ng L^{-1}) contrairement à celle de l'ICP-MS (200 ng L^{-1}). Par contre, en utilisant l'AFS couplé avec la chromatographie, il faut transformer les espèces en sélénite avant l'analyse car seulement cette espèce chimique peut former des hydrures.

Le sélénium provenant de sources anthropiques se retrouvent d'abord dans les plans d'eau sous les formes dissoutes inorganiques. Ensuite, plus le temps de résidence de l'eau est long (p. ex. un marais), plus le sélénium est absorbé et réduit par les organismes de l'espèce chimique séléniate à l'espèce sélénite, puis finalement aux espèces organiques (Cutter, 1989; Zhang et Moore, 1996) qui sont les formes connues comme étant absorbées plus efficacement par les êtres vivants (Wang et Lovell, 1997). Les séléniures organiques en milieu aérobie peuvent se retransformer en sélénite, mais le retour naturel à l'espèce plus oxydée, le séléniate, à partir du sélénite, peut prendre des centaines d'années (Cutter et Bruland, 1984) à cause de l'équilibre thermodynamique qui favorise le Se(IV) comme espèce chimique la plus stable. Donc, des proportions de séléniate plus élevées peuvent indiquées une source récente de sélénium dans un environnement aquatique particulier (Gao et al., 2007).

1.6. Accumulation de Se dans les réseaux trophiques lacustres

L'entrée de sélénium dans les réseaux trophiques aquatiques se fait principalement chez les producteurs primaires (algues; Stewart et al., 2010) et les bactéries (Baines et al., 2004) car les animaux absorbent très peu de sélénium de la voie aqueuse (Luoma and Rainbow, 2008). Donc, les communautés microplanctoniques ou microbenthiques, dans le cas du périfilm, sont un assemblage de micro-organismes à la base des réseaux trophiques et une source de Se pour les consommateurs primaires, mais est-ce que l'exposition au Se diffère entre ces deux types de réseaux trophique? Il est possible que oui, car le sélénium dans la colonne d'eau est davantage sous formes oxydées (sélénite et séléniate; Luoma and Presser, 2009) et dans les sédiments, en proportion plus grande sous

formes réduites (séléniures; Martin et al., 2011). Cette spéciation et le type de micro-organismes présents dans ces deux compartiments influenceront les concentrations de Se retrouvées à la base des chaînes alimentaires. Le type de micro-organismes influencera les concentrations de sélénium car selon le taxon d'algue, par exemple, l'accumulation de Se sera très différente (Baines and Fisher, 2001). Ce phénomène est probablement dû à la variation des types de transporteurs anioniques entre les taxa.

Après être absorbé par les producteurs primaires, le sélénium est transféré vers les niveaux trophiques supérieurs des chaînes alimentaires. Généralement, les animaux ont une très grande efficacité d'assimilation du Se, c'est-à-dire qu'ils vont assimiler plus de 70% du sélénium présent dans leur nourriture (Luoma and Rainbow, 2008). Ceci combiné avec une perte physiologique généralement faible entraîne la possibilité, comme pour le méthyl-mercure, que le sélénium se bioamplifie dans les chaînes alimentaires, suggérant un plus haut risque pour les organismes en haut des chaînes trophiques.

Malgré le consensus par rapport à l'absorption plus importante des espèces chimiques organiques dissoutes du sélénium par les producteurs primaires, les études ne sont pas claires quant à quelle espèce inorganique, le séléniate ou le sélénite, est plus ou moins bioaccumulée. Une lacune de connaissances sur l'influence du sulfate et du pH avec les deux espèces inorganiques aux sites d'entrée des membranes biologiques pourrait expliquer les résultats contradictoires. Des organismes pélagiques comme le plancton pourraient servir de biomoniteurs afin de comparer leur [Se] avec la spéciation du sélénium dans l'eau en milieu naturel. Des études au laboratoire avec des algues pourraient aussi aider à comprendre l'influence des comptétiteurs chimiques.

Les organismes à la base du réseau trophique benthique et ceux à la base du réseau trophique pélagique sont très différents biologiquement et ces différences peuvent faire varier leur [Se] et l'exposition des animaux s'y nourrissant. Un outil qui permet de différencier les modes d'alimentation benthique et pélagique est la signature isotopique de soufre (Croisetière et al., 2009). Effectivement, si un poisson se nourrit davantage de proies benthiques, sa signature isotopique de soufre, comme ses proies, sera plus négative que s'il se nourrissait de proies pélagiques.

Le sélénium est essentiel pour la majorité des organismes vivants et est un antioxydant bien connu et très puissant (Rayman, 2000). Cette caractéristique est, entre

autre, liée à son caractère essentiel dans l'enzyme glutathion peroxydase (Flohé, 1988) qui permet, avec le glutathion, la réduction du peroxyde d'hydrogène (H_2O_2) cellulaire en eau. Plusieurs fonctions physiologiques des sélénoprotéines ne sont pas expliquées comme l'importante protection contre les effets toxiques des métaux. Par contre, une dose de Se environ cinq fois plus élevée que la dose essentielle peut entraîner des effets toxiques chez les poissons (Lemly, 1993). La faible marge entre essentialité et toxicité est une autre caractéristique du sélénium qui lui confère un risque élevé.

1.7. Analyse de risque écologique relié au sélénium

Le risque environnemental que pose le sélénium est au deuxième rang en importance, après le mercure, mais ce constat n'est pas largement connu (Luoma et Rainbow, 2008). Effectivement, les [Se] peuvent, comme celles du mercure, augmenter vers le haut des chaînes alimentaires. Cette bioamplification entraîne des risques plus élevés pour les prédateurs et les humains. De plus, des [Se] dissous très faibles peuvent entraîner une accumulation importante si ce Se dans l'eau est transformé en Se organique par les organismes. L'évaluation du risque du Se est complexe et doit être faite avec une logique écotoxicologique multidisciplinaire qui comprend, entre autres, de l'écologie, de la chimie, de la physiologie, de la toxicologie et de l'hydrologie. Les critères de concentrations de Se dans l'eau pour la protection de la vie aquatique sont très variables entre les pays (Canada, Colombie-Britannique: 2 $\mu g\ L^{-1}$, Beatty et Russo, 2014; É.-U. : 5 $\mu g\ L^{-1}$, US EPA, 2002; Europe : pas de critère, Luoma et Presser, 2009), ce qui indique l'importance qu'ont les études scientifiques afin d'établir une valeur cible qui protège adéquatement la vie aquatique. La plupart des méthodes en toxicologie classique, par exemple une exposition aqueuse à des [Se] élevées pour induire un effet, ne considèrent pas le comportement propre au Se qui est très différent des autres éléments traces. Lorsqu'un écosystème est contaminé en Se, le cycle particulier du Se entraîne la formation graduelle de séléniures organiques hautement biodisponibles et des conséquences qui sont difficilement réversibles (Lemly, 2004).

1.8 Régions minières à l'étude

Le Se peut être présent en grande concentration dans le minerai où il y a des sulfures de métaux divers. Les concentrations de Se dans le minerai de cuivre (20-82 µg de Se/g) peuvent être plus importantes que dans le minerai de charbon (0,4-24 µg de Se/g; Shamberger, 1981). En termes de quantité, le minerai de la région de Sudbury était la plus grande source de sélénium de l'Amérique du Nord (Nriagu et Wong, 1983). À Rouyn-Noranda, à environ 300 km de Sudbury, la contamination par le Se a été peu étudiée. Les deux seules études publiées sur la contamination en Se dans la région de Rouyn-Noranda sont celles de Speyer (1980) sur l'effet antagoniste du Se et du Hg chez le brochet du Nord (*Esox lucius*) du lac Dufault et de Glooschenko et Arafat (1988) sur l'utilisation de la mousse *Sphagnum fuscum* comme biomoniteur de contamination en Se. Une mesure effectuée dans le bassin de sédimentation de la fonderie Horne de Rouyn-Noranda a permis de constater une concentration de Se de 300 µg L^{-1} (D. Ponton, donnée non publiée) et cette eau s'écoule dans le lac Rouyn, dans la rivière Kinojévis et vers les lacs Kinojévis et Caron. Ceci dit, les réductions des émissions de soufre par les fonderies au cours des dernières décennies ont probablement réduit du même coup, les émissions de sélénium.

OBJECTIFS

Les objectifs de cette thèse sont :

(1) Connaître, en milieu naturel, quelles espèces dissoutes du sélénium sont corrélées avec les concentrations de cet élément dans une chaîne trophique planctonique composée de microplancton, de crustacés planctoniques et de la larve du diptère *Chaoborus*.

(2) Comparer l'accumulation des différentes espèces chimiques du sélénium chez une algue verte unicellulaire à différentes concentrations de sulfate et d'ions hydrogène et effectuer des expériences similaires avec le microplancton naturel d'un lac non contaminé.

(3) Par l'utilisation des isotopes stables de soufre, démontrer l'influence du comportement alimentaire de la perchaude (*Perca flavescens*) et de ses proies, sur leur exposition au sélénium.

(4) En prélevant le foie de perchaudes de 11 lacs, mesurer l'influence des métaux et du sélénium sur le stress oxydatif cellulaire.

(5) Décrire l'accumulation temporelle de Cd et de Se chez des larves du diptère *Chaoborus* transférées d'un lac peu contaminé à un lac hautement contaminé et déterminer si les larves s'adaptent rapidement en séquestrant ces deux éléments traces dans les mêmes compartiments cellulaires que les larves indigènes du lac fortement contaminé.

DEUXIÈME CHAPITRE

ACCUMULATION DE SÉLÉNIUM PAR DES ORGANISMES PÉLAGIQUES

2.1. Spéciation du sélénium dans l'eau et son accumulation par une chaîne trophique planctonique

Nous avons mesuré les concentrations de séléniate (Se(VI)), de sélénite (Se(IV)) et de séléniures organiques (Se-Org) dans une quinzaine de lacs des régions de Sudbury et de Rouyn-Noranda et avons comparé les concentrations de ces espèces chimiques avec l'accumulation de Se dans le microplancton < 64 µm, dans les crustacés planctoniques (principalement des copépodes) et dans les larves zooplanctivores de la mouche fantôme *Chaoborus* (quatrième stade larvaire; Moore 1988) Cette chaine alimentaire est représentative de plusieurs lacs puisque les larves du diptère *Chaoborus* ont une très grande répartition géographique (Saether, 1972). Elles ont été utilisées comme biomoniteur d'exposition au cadmium et au nickel dans les lacs de Sudbury et Rouyn-Noranda (Hare et al., 2008; Ponton et Hare, 2009). Les détails des concentrations dissoutes des différentes espèces chimiques du Se, de la caractérisation des lacs (positionnement géographique, pH, carbone organique dissous, $[SO_4]$), ainsi que les principales corrélations faites entre la spéciation du Se et l'accumulation chez *Chaoborus* sont présentées dans le premier article de cette thèse (Ponton et Hare, 2013, *Environmental Pollution*). Cette étude est la première qui met en relation la spéciation chimique du Se avec l'accumulation dans un organisme d'eau douce en milieu naturel. L'accumulation des différentes espèces chimiques de sélénium dans les maillons trophiques inférieurs à *Chaoborus* est expliquée dans le deuxième article de cette thèse (Ponton et al., 2015a; à soumettre à *Environmental Chemistry*).

Les proportions des trois espèces chimiques varient beaucoup entre les lacs étudiés. De manière générale, les proportions de séléniate sont très faibles mis à part certains lacs plus acides. Les concentrations de séléniate sont aussi généralement faibles. On retrouve des proportions plus grandes de séléniate surtout en aval des bassins de décantation de résidus des fonderies (lacs Kelly et Rouyn), en aval des résidus miniers

acides (lacs Arnoux et Dasserat) et dans un lac de tête acide (lac Pine) dont le pH varie selon les années, (2007 : 4,7; 2009: 5,2; 2010 : 5,7; 2011 : 5,1) probablement selon l'intensité des activités des fonderies de Sudbury. Effectivement, une baisse importante des activités et des émissions a été observée en 2008 lors de la crise économique (Vale, 2011), ce qui a pu entraîner l'augmentation du pH dans ce lac. Bien que les émissions d'oxydes de soufre et d'azote aient diminué par les fonderies, la formation d'acides dans l'atmosphère et dans les précipitations peut toujours affecter le pH de certains lacs comme celui-ci, qui a un pouvoir tampon plus faible.Les proportions plus élevées de séléniate par rapport à celles des deux autres espèces chimiques (Se organique et sélénite) dans les lacs Arnoux, Dasserat, Kelly, Pine et Rouyn comparativement aux proportions des deux autres espèces chimiquesautres lacs peuvent être reliées à une entrée récente de sélénium dans ceux-ci puisque le séléniate peut être réduit en sélénite, mais l'oxydation du sélénite en séléniate peut prendre des centaines d'années en conditions naturelles (Cutter, 1989; Luoma and Rainbow, 2008). Quant au sélénite, il peut être réduit en séléniures organiques par les organismes et être réoxydé en sélénite, mais plus le temps de rétention d'un plan d'eau est long, plus les proportions de séléniures organiques seront élevées (Luoma et Presser, 2009). Malheureusement, nous n'avons pas mesuré cette variable dans les lacs. Le pH peut aussi influencer les concentrations et les proportions de sélénite puisque cette espèce chimique se dissous plus facilement à pH élevé, comme le montre la Figure 2.1. Donc, lorsque le pH de l'eau qui percole dans le sol du bassin versant d'un lac est élevé ou lorsque le pH de l'eau du lac est élevé, le sélénite est moins lié aux particules et davantage sous forme dissoute. L'augmentation des [Se(IV)] dissous en fonction du pH (corrélation non présentée) entraîne aussi une augmentation des proportions de sélénite en fonction du pH (Figure 2.1). Puisque le Se organique et le sélénite sont les espèces chimiques les plus abondantes, l'augmentation des proportions de sélénite en fonction du pH entraîne la relation inverse avec les proportions de sélénium organique. Les proportions et les concentrations de ces deux espèces chimiques sont en moyennes similaires dans nos lacs, mais varient beaucoup entre eux. Par exemple, dans le lac Crooked, nous avons mesuré environ 90 % du sélénium dissous sous forme organique alors que cette proportion peut être inférieure à 20 % dans un autre lac (Figure 2.1).

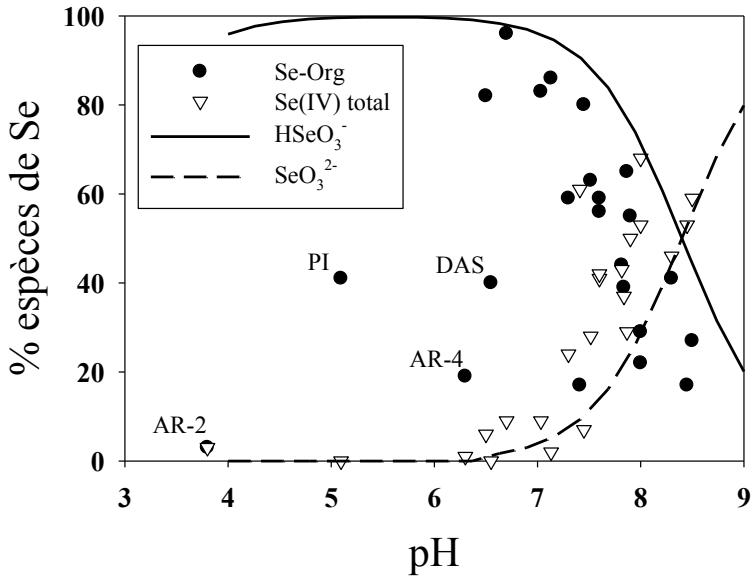

Figure 2.1 : Proportions (%) de sélénite et de séléniures organiques (Se-Org) en fonction du pH de différents lacs des régions de Sudbury et de Rouyn-Noranda. Les lacs plus acides (Arnoux, Dasserat et Pine) sont présentés avec des lettres. Les deux formes inorganiques de sélénite ($HSeO_3^-$ et SeO_3^{2-}; lignes pleine et pointillée, respectivement) sont présentées à titre informatif.

Nous avons premièrement comparé les concentrations des différentes espèces chimiques de Se avec l'accumulation de Se chez la larve de *Chaoborus* (premier article de cette thèse). Bien que les concentrations totales de Se dissous soient corrélées avec les [Se] chez cette larve, les concentrations de séléniures organiques dissous sont celles qui rendent la régression forte. Les concentrations des espèces inorganiques dissoutes seules ou combinées ne sont pas corrélées avec l'accumulation de Se chez *Chaoborus*. En excluant les points représentants les [Se] chez les larves de *Chaoborus* des lacs Kelly et Rouyn, l'addition des concentrations dissoutes de sélénite à celles de Se organique n'améliore pas la régression. Par contre, l'addition des concentrations dissoutes de séléniate à celles de Se organique améliore légèrement la régression (Ponton et Hare 2013). En ajoutant les données des lacs Kelly et Rouyn, les concentrations dissoutes de séléniures organiques additionnées à celles de séléniate sont bien corrélées avec l'accumulation de Se chez les trois niveaux trophiques pélagiques étudiés. Par contre, dans le lac Kelly, les concentrations dissoutes de ces deux espèces chimiques surestiment

les [Se] chez ces organismes (Figure 2.2). Dans le lac Kelly (accumulation la plus élevée), la concentration de sulfate est très élevée et près de 4 mM. Il est bien connu que le sulfate peut diminuer l'entrée de séléniate chez des algues (Williams et al. 1994; Fournier et al. 2010; Neumann et al. 2003), donc seulement les séléniures organiques dissous semblent être absorbés par les organismes à la base de cette chaîne alimentaire. Afin de représenter cette hypothèse, nous avons illustré dans la Figure 2.2 les concentrations dissoutes de séléniures organiques (carrés noirs) et celles des deux espèces chimiques additionnées (triangles noirs).

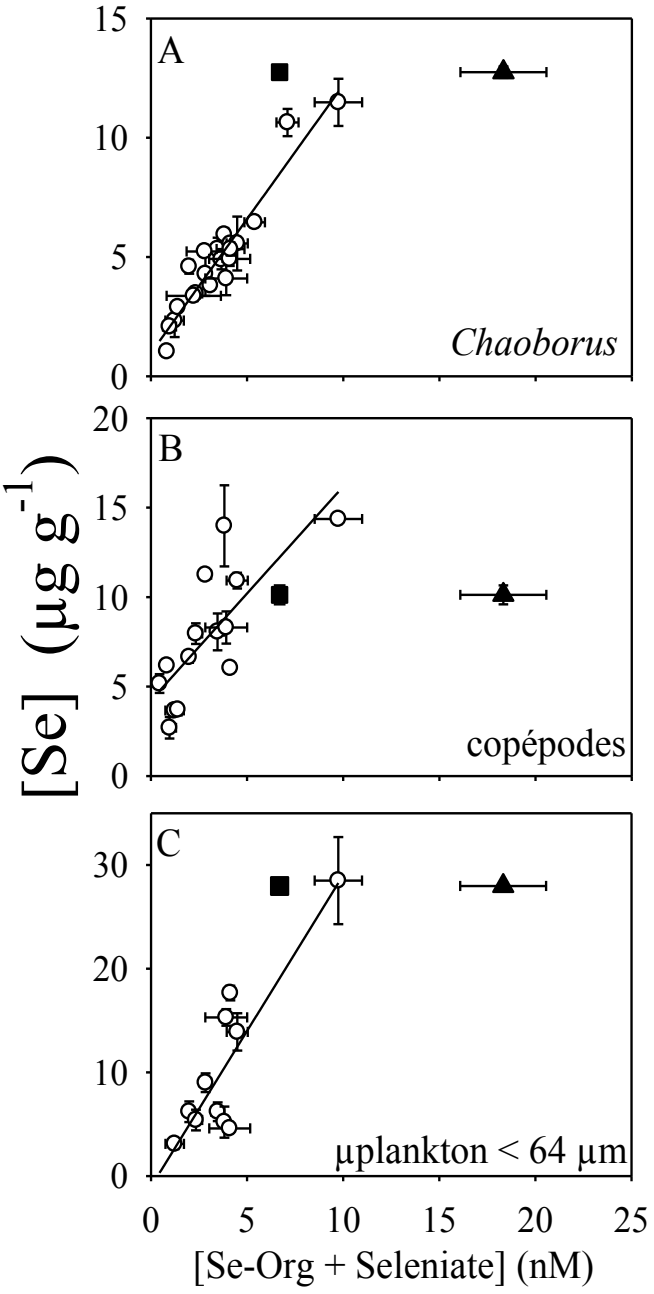

Figure 2.2 : Accumulation de sélénium (µg g^{-1} poids sec (PS); ± écart-type (ET)) par trois membres (microplancton < 64 µm, copépodes et *Chaoborus*) d'une même chaîne alimentaire en fonction des concentrations de séléniures organiques additionnées à celles de séléniate ([Se-Org + séléniate]; nM). Les données du lac Kelly (symboles noirs) ne sont pas incluses dans les régressions. Les carrés représentent seulement les concentrations de Se-Org et les triangles la somme des deux espèces chimiques.

2.2. Facteurs de transfert trophique dans la chaîne alimentaire planctonique et calcul d'une concentration aqueuse de Se recommandée pour la protection de la vie aquatique

Afin d'observer s'il y a bioamplification du Se dans cette chaîne alimentaire, nous avons calculé les facteurs de transfert trophique (FTT = $[Se]_{consommateur}/[Se]_{nourriture}$). Le FTT du Se entre *Chaoborus* et ses présumées proies, les copépodes, est de 0,6 ± 0,2 (n = 13) et celui entre les copépodes et le microplancton est de 1.0 ± 0.7 (n = 13). Le premier indique l'absence de bioamplification (FTT < 1) du Se entre *Chaoborus* et ses proies mais celui entre les copépodes et le microplancton ne permet pas de conclure s'il y a augmentation ou diminution des [Se] entre ces deux maillons trophiques. Le quatrième stade larvaire de *Chaoborus* est planctivores (Moore, 1988) et selon Rasmussen et al. (2008), les jeunes perchaudes de l'année de Rouyn-Noranda le seraient aussi. De plus, des analyses d'isotopes stables d'azote nous suggèrent cette observation. Nous avons donc comparé les [Se] de ces jeunes perchaudes avec les [Se] chez *Chaoborus* (Figure 2.3). Il y a un rapport des [Se] de 2,7 entre ces deux prédateurs ($[Se]_{P.\ flavescens}/[Se]_{Chaoborus}$ = 2,7 ± 0,9; n = 18), donc en multipliant ce rapport avec le FTT de *Chaoborus* ($[Se]_{Chaoborus}/[Se]_{zooplancton}$ = 0,6 ± 0,2; n = 13), nous annulons les termes "$[Se]_{Chaoborus}$" et obtenons le FTT entre les perchaudes et le zooplancton. Cette estimation donne un FTT de 1,6 et l'étude de l'alimentation des perchaudes de différentes grosseurs (Chapitre 3) nous a permis de confirmer que le FTT des perchaudes d'une grande gamme de taille est bien de 1,6 ± 0,7 (n = 50), et ce, avec différents types de proie. Cette moyenne bien qu'elle présente un écart-type élevé, suggère une bioamplification du Se entre les perchaudes et leurs proies et un risque d'exposition au Se plus élevé pour les perchaudes que pour les larves de *Chaoborus*. Afin d'être exposé à une [Se] totale inférieure à celle produisant des effets chez les poissons (8-9 µg g^{-1}; DeForest et al., 1999), les jeunes perchaudes de l'année devraient se nourrir de zooplancton ayant des [Se] inférieures à 5,3 µg Se g^{-1} (FTT de 1,6), ce qui correspond à la même [Se] dans le microplancton (FTT de 1) et une [Se-Org + Se(VI)] d'environ 2,5 nM (0,2 µg L^{-1}; Figure 2.2C) . Cette [Se] dans l'eau est 25 fois inférieure à la recommandation de l'US EPA (5 µg L^{-1}) pour protéger la vie aquatique (US EPA, 2002) et correpond à la [Se] dans l'eau suggérée par Luoma et

Rainbow (2008). La [Se] de 5,3 µg g^{-1} chez les crustacés planctoniques permettrait aussi de protéger plusieurs invertébrés des effets toxiques du Se (Debruyn et Chapman, 2007).

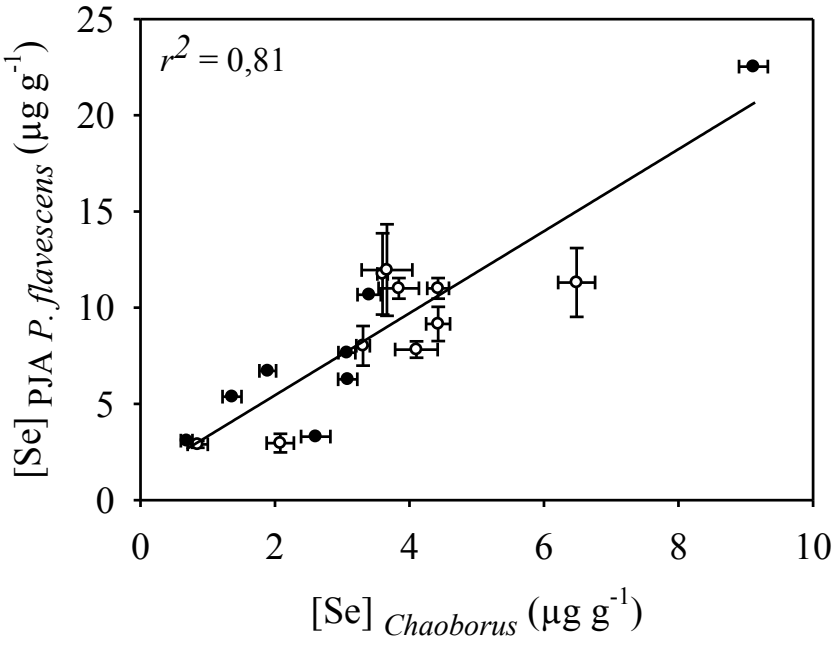

Figure 2.3 : Régression linéaire entre les concentrations (µg g^{-1} PS; ± ET) de Se chez les jeunes perchaudes de l'année ([Se] PJA *P. flavescens*) et les concentrations de Se chez *Chaoborus* ($[Se]_{Chaoborus}$). Certaines données de [Se] chez les perchaudes ont été prises de Belzile et al. (2006; points blancs) et d'autres, de perchaudes récoltées en 2010 (points blancs).

Comme l'absorption de Se dans l'eau s'effectue surtout par les producteurs primaires (Chapman et al., 2010), calculer le coefficient de répartition (K_d) entre le microplancton et l'eau ($[Se]_{microplancton}$ (µg g^{-1})/$[Se]_{eau}$ (µg mL^{-1})) permet de connaître le potentiel d'accumulation d'un certain plan d'eau. Le K_d moyen des lacs des deux régions minières étudiées est de 32,840 ± 12,712 (± ET; gamme de 14,179 à 54,141; n = 12) ce qui correspond à un des plus élevés observés dans la littérature (Presser et Luoma, 2010). Il est de plus comparable aux valeurs observées dans la baie de San Francisco (K_d entre 3,000 et 40,000) et la baie de Newport en Californie (K_d entre 6,933 et 42,715; Presser et Luoma, 2010). Ces K_d élevées sont probablement dus à la proportion élevée de séléniures organiques dans ces plans d'eau (Presser et Luoma, 2010; Figure 2.1). Afin de déceler les mécanismes d'accumulation engendrant les trois régressions présentées à la Figure 2.2,

de l'importance relative des trois espèces chimiques sur l'accumulation et de l'influence du pH et de la compétition avec le sulfate sur l'accumulation, une étude au laboratoire avec des micro-organismes a été effectuée.

2.3. Accumulation des espèces chimiques de sélénium chez l'algue verte *Chlamydomonas reinhardtii* et influence du sulfate, du pH et du temps d'exposition

Selon nos résultats observés sur le terrain (Figure 2.2) et la littérature scientifique, le sulfate (SO_4; Fournier et al., 2010; Morlon et al., 2006; Neumann et al., 2003) et le pH (Riedel et Sanders, 1996; Morlon et al., 2006) semblent jouer des rôles dans l'accumulation des espèces inorganiques du sélénium. Le phosphate peut aussi réduire l'accumulation de sélénite, mais les concentrations de phosphate mesurées dans nos lacs (< 6,2 µg L^{-1}) sont possiblement trop faibles pour avoir une influence, du moins lorsque l'on compare ces concentrations en phosphore à celles nécessaires pour inhiber la prise en charge de sélénite chez l'algue verte *Chlamydomonas reinhardtii* (Morlon et al., 2006). Nous avons exposé l'algue *Chlamydomonas reinhardtii* à la même concentration (63 nM; 5 µg L^{-1}) de séléniate, de sélénite et de sélénométhionine à différentes concentrations de [SO_4] et d'ions hydrogène ([H^+]) pendant différentes périodes d'exposition. Cette concentration de Se représente le critère de concentration aqueuse prévenant les effets chroniques en eau douce aux É.-U. (US EPA, 2002), mais correspond à un plan d'eau hautement contaminé (Luoma and Rainbow, 2008; section 2.2). Mentionnons que les différentes molécules de sélénium utilisées ont des poids différents pour la même quantité de Se, mais nous avons mesuré la masse de Se et non celle des molécules complètes afin d'établir les concentrations de 5 µg L^{-1}. La composition du milieu d'exposition correspond à celle des lacs étudiés. Veuillez consulter le deuxième article de cette thèse (Ponton et al., 2015a; à soumettre dans *Environmental Chemistry*) pour plus de détails.

L'accumulation de séléniate par *C. reinhardtii* atteint des concentrations impressionnantes de 225 µg g^{-1} (poids sec) sans SO_4 et il faut 1 µM de SO_4 afin de réduire cette concentration de 50% (Figure 2.4A). Aux concentrations plus élevées de SO_4 (petit graphique dans la Figure 2.4A), les [Se] de *C. reinhardtii* deviennent inférieures à la limite de détection (0,2 µg g^{-1}) à 2 et 4 mM de SO_4. L'importante

accumulation de séléniate, et ce même à des concentrations de sulfate élevées, peut expliquer pourquoi nous observons une meilleure corrélation entre l'accumulation par la chaîne trophique étudiée et les [Se] dans l'eau lorsque nous additionnons les concentrations de séléniate à celles des séléniures organiques (Figure 2.2). Les concentrations de SO_4 mesurées sur le terrain variaient entre 0,030 et 4,23 mM. Le séléniate peut représenter un très haut risque pour les consommateurs si les [SO_4] sont faibles, mais heureusement, la présence de ces deux éléments (le soufre et le sélénium) est souvent corrélée dans l'environnement et dans nos lacs (Tableau 1; Ponton et Hare, 2013).

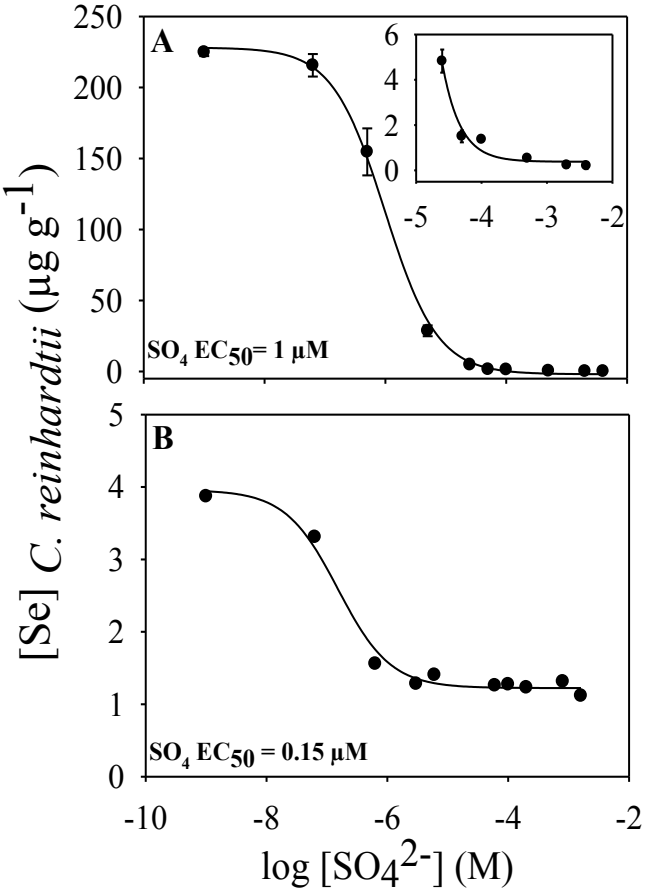

Figure 2.4 : Concentrations de Se chez *Chlamydomonas reinhardtii* (µg g^{-1} PS; ± ET) exposé au séléniate (panneau A) et au sélénite (panneau B) pendant 12 heures à une concentration de Se dissous de 63 nM, en fonction des concentrations de sulfate (M). Le traitement sans sulfate est représenté par la concentration 10^{-9} M. Le pH est de 7,5.

Sans SO_4 et à pH 7,5, la [Se] chez *C. reinhardtii* est de 4 µg g^{-1} lorsqu'exposé pendant 12 heures à 63 nM de sélénite. Une [SO_4] de 0,15 µM a réduit de 50% cette accumulation (Figure 2.4B). Aux concentrations les plus élevées de SO_4 (2 et 4 mM), *C. reinhardtii* accumule toujours des concentrations significatives de sélénite d'environ 1,3 µg g^{-1} par rapport aux algues non exposées (< 0,2 µg g^{-1}). Donc, la prise en charge de séléniate est plus élevée que celle de sélénite et moins influencée par les concentrations de sulfate (Figure 2.4A). À des concentrations très élevées de SO_4 ($10^{-3,5}$ M et plus), la prise en charge de sélénite va prédominer par rapport à celle de séléniate. Ces observations suggèrent que le sélénite et le séléniate empruntent des transporteurs membranaires différents. Contrairement aux espèces inorganiques de Se, la prise en charge de sélénométhionine n'a pas été influencée par les [SO_4] et après 30 heures, à un pH de 7,5, les [Se] chez *C. reinhardtii* étaient de 4 µg g^{-1} (Figure 2.5).

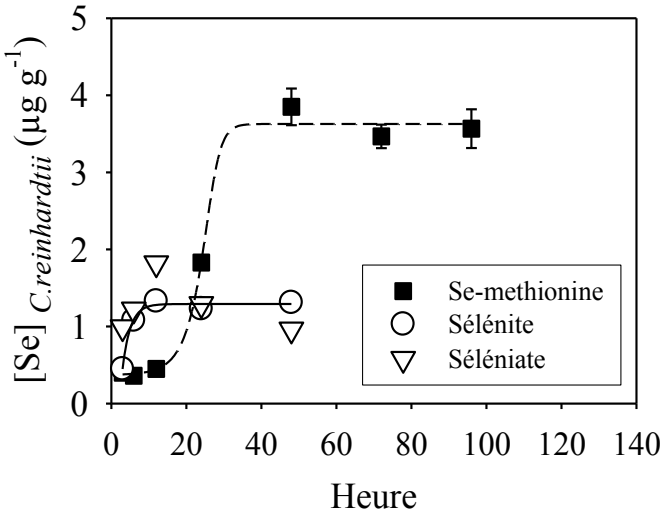

Figure 2.5 : Accumulation temporelle par *Chlamydomonas reinhardtii* (µg g^{-1} PS; ± ET) de sélénite (cercles), de séléniate (triangles) et de sélénométhionine (carrés) à une même concentration de 63 nM et une [SO_4] de 100 µM. Le pH est de 7,5.

Les faibles [Se] chez *C. reinhardtii* sont surprenantes comparativement à celles mesurées chez le microplancton des lacs des régions de Sudbury et de Rouyn-Noranda (jusqu'à 30 µg g^{-1} à moins de 2 µg L^{-1}). Suite à des expositions de 12 heures à 63 nM de sélénométhionine (comme pour les espèces inorganiques), les [Se] de *C. reinhardtii*

étaient seulement de 1,2 µg g^{-1}. Donc, nous avons préexposé l'algue à une [Se-Met] de 0,2 µg L^{-1} pendant trois cycles de croissance afin d'observer si des transporteurs membranaires de sélénométhionine pouvaient être synthétisés pendant cette préexposition et augmenter la subséquente prise en charge pendant l'exposition normale à 5 µg L^{-1}. Cette préexposition n'a pas modulé la prise en charge subséquente et ne suggère pas une synthèse de transporteurs. Cette période initiale sans détection d'accumulation par l'algue verte suggère que la prise en charge est si faible que nous la détectons seulement lorsque les algues cessent leur croissance et leur mitose dans le milieu d'exposition.

L'exposition de *C. reinhardtii* à des pH de 7 à 9 a modifié la prise en charge de sélénométhionine (Figure 2.6). Celle-ci est deux fois plus élevée aux pH 8,5 et 9 qu'aux pH 7,0 et 7,5. Par contre, le pH n'a pas influencé la prise en charge de séléniate (Figure 2.6A). Une augmentation du pH de 5 à 7 a doublé la prise en charge du complexe hydrophobe de Cd (Lavoie et al., 2012). Étant donné la lenteur de l'entrée de sélénométhionine (Figure 2.5) et la faible accumulation, le transport passif de la sélénométhionine est peu probable. Il faut environ 30 heures afin que les algues soient à l'état stationnaire avec les [Se-Met] dans l'eau alors que pour les espèces inorganiques, il faut environ 6 heures. Après 30 heures, l'accumulation de Se-Met est de 4 µg g^{-1} (Figure 2.5) comme elle l'est à pH 9 après 12 heures (Figure 2.6). Il serait intéressant de comparer ces résultats avec la prise en charge de sélénométhionine par une autre algue verte.

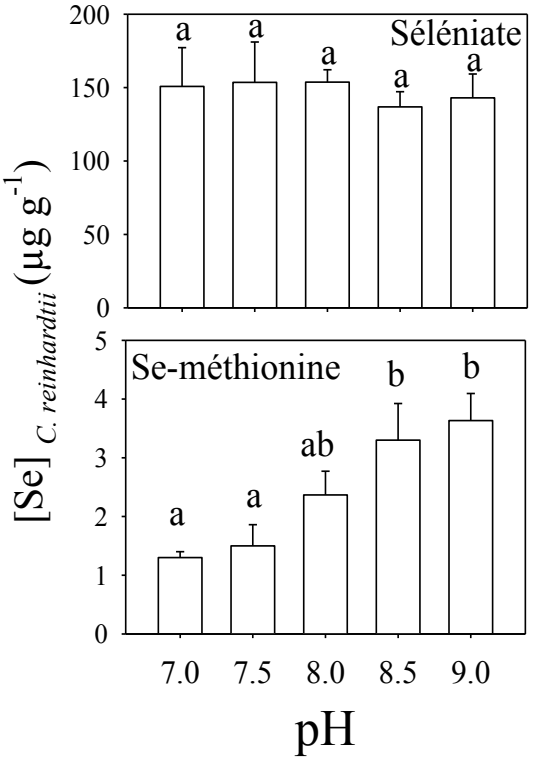

Figure 2.6 : Concentration de Se chez *Chlamydomonas reinhardtii* (µg g^{-1} PS; ± ET) exposé à 63 nM de séléniate (panneau A) ou de sélénométhionine (panneau B) à différents pH et sans sulfate. Différentes lettres représentent des différences significatives ($p < 0,05$).

Nous avons aussi mesuré l'influence du pH sur l'accumulation de sélénite par *C. reinhardtii*. Le sélénite a une spéciation inorganique variable à des pHs naturels. Comme le montre la Figure 2.1 (lignes pointillées), lorsque le pH augmente de 7 à 9, la proportion de SeO_3^{2-} augmente et celle de la forme protonée ($HSeO_3^-$) diminue. À pH 8,4 (pK_a), environ 50% des deux formes de sélénite sont présentes. Les résultats présentés à la Figure 2.7 suggèrent que *C. reinhardtii* accumule seulement la forme non-protonée du sélénite (SeO_3^{2-}). Effectivement, la [Se(IV)] totale était de 63 nM, mais par les calculs thermodynamiques (MINEQL+), nous avons présenté les [Se] chez *C. reinhardtii* en fonction des [SeO_3^{2-}]. Cela confirme que le pH peut effectivement avoir un effet sur l'accumulation de sélénite. Ce phénomène devrait être plus important dans les environnements aquatiques où le pH est élevé et souvent contaminé par le sélénium comme : (1) dans les estuaires et océans (pH 8.2), (2) en aval des eaux d'irrigation de terres agricoles semi-arides (cas de la Californie; Luoma and Presser, 2009); (3) en aval

des résidus de centrales thermiques au charbon (Lemly, 1993; Lemly, 2004) et (4) en aval des bassins résidus miniers chaulés (cette étude). Effectivement, l'accumulation passe de 2,5 µg g^{-1} (pH 7,5) à environ 20 µg g^{-1} (pH 9). Plusieurs études en eaux salées mentionnent que le sélénite est davantage accumulé que le séléniate (Luoma and Rainbow, 2008) par les micro-organismes à la base des chaînes alimentaires. Il est possible que ces conclusions soient dues aux pHs plus élevés de l'eau de ces études et aux concentrations élevées en sulfate qui, à certain point, n'influencent plus la prise en charge de Se(IV), du moins dans notre étude (Figure 2.4B).

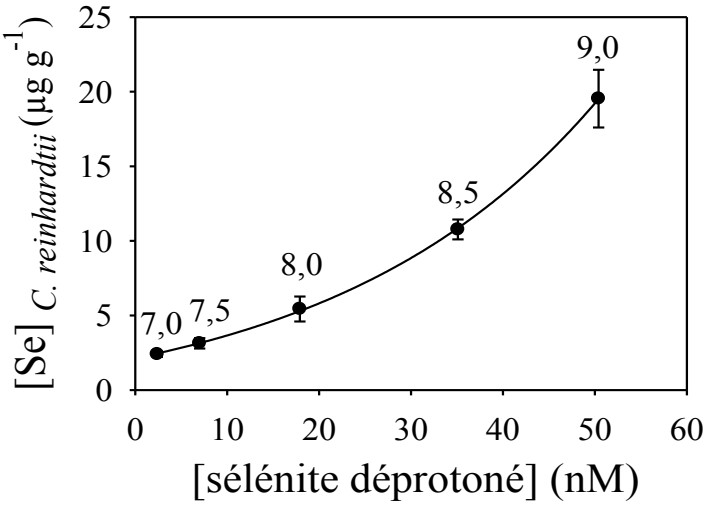

Figure 2.7 : Accumulation de Se par *Chlamydomonas reinhardtii* (µg g^{-1} PS; ± ET; *n* =3) exposé au sélénite (63 nM) à différents pH (indiqués par les chiffres au-dessus des symboles) en fonction des concentrations de sélénite non-protoné (SeO_3^{2-}; nM).

2.4. Accumulation de sélénium par le microplancton en laboratoire

Les [Se] chez *C. reinhardtii* étaient très faibles (4 µg g^{-1}) après une exposition à 63 nM de sélénométhionine comparativement aux [Se] mesurées chez le microplancton des lacs étudiés (jusqu'à 30 µg g^{-1} ; $[Se]_{eau}$ ~13 nM; Figure 2.2). Afin de confirmer si ce résultat était dû à l'algue verte utilisée ou à la forme chimique de notre sélénium organique (Se-Met), nous avons récolté le microplancton < 64 µm (algues et bactéries) du lac Bédard, à 70 km au nord de la ville de Québec (Forêt Montmorency), et exposé ces organismes aux mêmes conditions que *C. reinhardtii*. La Figure 2.8 démontre que les [Se] chez le microplancton sont beaucoup plus élevées (jusqu'à 110 µg g^{-1}) que celles de *C.*

reinhardtii (4 µg g^{-1}) lorsqu'exposés en conditions similaires à 63 nM de sélénométhionine. Le biovolume algal du microplancton était à plus de 70% composé de Chrysophyceae, de Dinophyceae et d'Euglenophyceae (voir Ponton et al. 2015a) avec un faible pourcentage (10%) dans la famille des Chlorophyceae ou se trouve *C. reinhardtii*. Nous avons mesuré par cytométrie en flux une grande abondance de bactéries (15,500 ± 3580 cellules/µL) comparativement à celle des algues (2,5 cellules/µL), mais les biovolumes d'algues et de bactéries étaient similaires. Par contre, en terme de surface, les bactéries présentaient une surface membranaire beaucoup plus grande. Baines et al. (2004) ont observé que 34 à 67 % de l'accumulation de sélénite en milieu naturel était bactérienne, ce qui pourrait expliquer les différences d'accumulation entre les organismes de nos expériences en laboratoire.

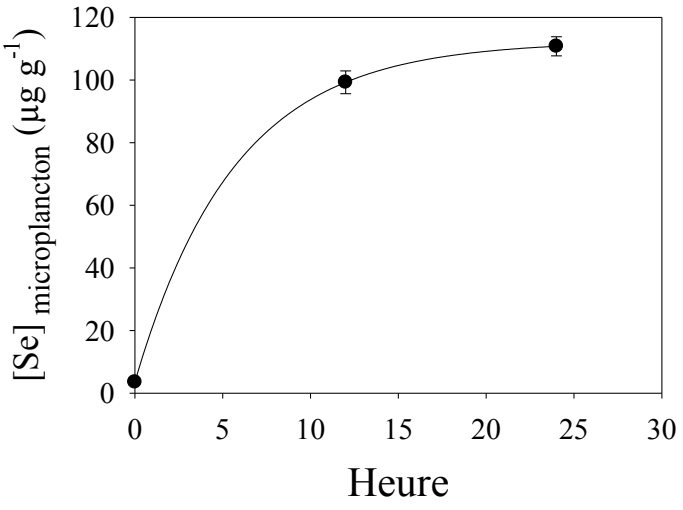

Figure 2.8 : Accumulation temporelle de Se par le microplancton < 64 µm du lac Bédard (µg g^{-1} PS; ± ET; n = 3) exposé à 63 nM de sélénométhionine dans le même milieu d'exposition que les algues sans sulfate.

Nous avons aussi exposé le microplancton au sélénite et au séléniate à différentes concentrations de sulfate (Figure 2.9). Ces deux expériences démontrent que les [Se] accumulées sont plus élevées lorsque le microplancton est exposé au sélénite qu'au séléniate et que l'accumulation de sélénite n'est pas influencée par les [SO_4] chez ces organismes. Il serait intéressant d'observer la phylogénie des transporteurs de sélénium chez différents groupes de micro-organisme afin d'observer si, avec l'apparition de

l'oxygène au cours de l'Histoire, les micro-organismes ont évolué avec des transporteurs d'oxyanions du Se plutôt qu'avec ceux de séléniures organiques. Effectivement, nos résultats diffèrent beaucoup entre les expériences de prise en charge par *C. reinhardtii* et le microplancton et reflètent les importantes différences de prise en charge de sélénite par différents taxons d'algues (Baines and Fisher, 2001).

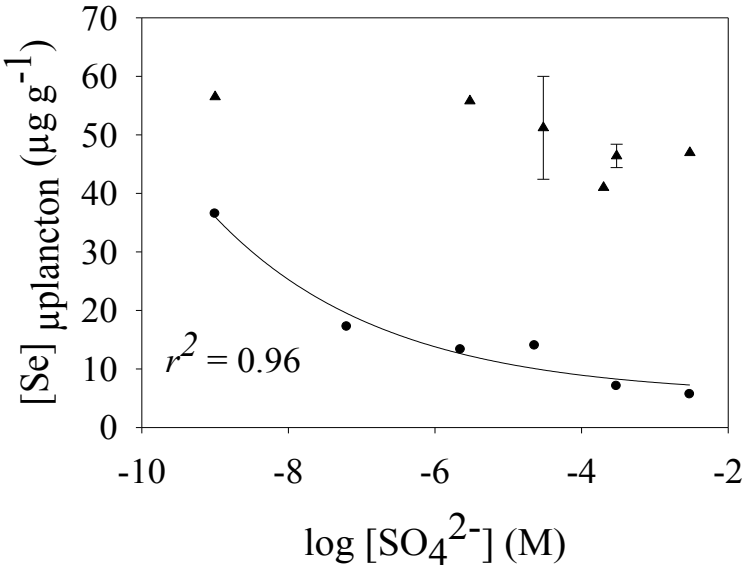

Figure 2.9 : Accumulation par le microplancton du lac Bédard (µg g^{-1} PS; ± ET; *n* =3) exposé à 63 nM de sélénite (triangles) et de séléniate (cercles noirs) pendant 12 heures. Mêmes conditions que l'algue *C. reinhardtii* à différentes concentrations de sulfate. Le traitement sans sulfate est représenté comme 10^{-9} M. Le pH est 7,5.

2.5. Estimation de l'accumulation de Se par la chaine trophique lacustre selon les données de laboratoire

Afin de bien comparer nos résultats au laboratoire avec les données de terrain, nous avons combiné nos données dans un modèle qui estime les [Se] dans le microplancton à l'état stationnaire. La [Se] chez le microplancton d'un lac est exprimée par:

$$[\mathrm{Se}]_{\mathrm{microplankton}} = K_{d(\mathrm{IV})}[\mathrm{Se(IV)}] + \frac{(5.4 + 0.63e^{(-0.43[SO_4])})[\mathrm{Se(VI)}]}{63} + K_{d(-\mathrm{II})}[\mathrm{Se(-II)}] \qquad (1)$$

Les coefficients de répartition (K_d) sont les mêmes que ceux de la section 2.2 (Presser and Luoma, 2010), mais obtenus à partir des données de laboratoire, et ce, pour

chaque espèce chimique. Les coefficients pour le sélénite ($K_{d(IV)}$) et la sélénométhionine ($K_{d(-II)}$) sont obtenus par la division des [Se] accumulées par le microplancton au laboratoire (nmol g^{-1}) pour une espèce chimique donnée (Se(IV) : Figure 2.9 ou Se-Org : Figure 2.8) et la concentration de cette espèce chimique dans le milieu d'exposition (63 pmol mL^{-1}). Le K_d pour le séléniate a été remplacée par l'équation présentée dans la Figure 2.9 et celle-ci divisée par 63 pmol mL^{-1}. Il est possible que cette équation ne fonctionne pas pour de plus hautes [Se] dissous, mais celles observées généralement sont inférieures (Conde et Sanz Alaejos, 1997), comme dans les lacs étudiés lors de cette étude. Les [Se(IV)], de [Se(VI)], de [Se-Met] et de [SO_4] dans l'équation 1 sont celles mesurées dans les lacs (Ponton et Hare, 2013; Tableau 1). Nous avons ensuite utilisé les facteurs de transfert trophique (FTT des copépodes et de *Chaoborus*) afin d'estimer les [Se] dans les larves de *Chaoborus*. La Figure 2.10 montre que nos estimations s'accordent avec les [Se] réelles mesurées (proximité de la ligne 1 :1). Ces estimations nous indiquent que 75 % de l'accumulation provient du sélénium organique et qu'environ 25 % proviendraient du sélénite. L'ajout du sélénite dans l'estimation améliore les prédictions principalement dans le lac Rouyn. Les concentrations de séléniate étaient trop faibles et celles de sulfate trop élevées dans nos lacs pour que le modèle prédise une accumulation de séléniate l'accumulation par le microplancton du lac Bédard au laboratoire.

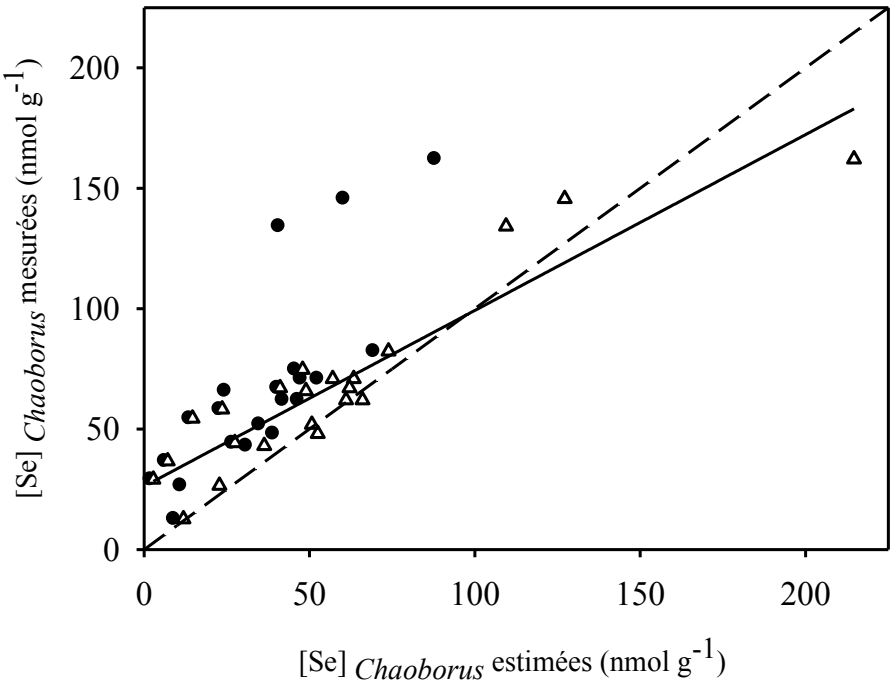

Figure 2.10 : Concentrations de Se mesurées chez *Chaoborus* (µg g^{-1} PS) en fonction des concentrations de Se estimées selon l'équation 1. Les points noirs sont les estimationsconsidérant les [Se] organique dissous dans le modèle et les points blancs sont celles considérant les [Se] organiques et de sélénite. La ligne pointillée représente une relation 1:1.

TROISIÈME CHAPITRE

INFLUENCE DE L'ALIMENTATION SUR L'ACCUMULATION DE SÉLÉNIUM CHEZ DES PERCHAUDES (*PERCA FLAVESCENS*) SAUVAGES

3.1. Relation entre les concentrations de sélénium, la taille et l'alimentation de *Perca flavescens*

Nous avons récolté des perchaudes (*Perca flavescens*) de différentes tailles dans quatre lacs (Osisko, Dufault, Rouyn et Kelly) et prélevé leur contenu stomacal et un échantillon de muscle antérieur dorsal. De plus, nous avons récolté des proies benthiques et pélagiques sur le lieu de récolte des perchaudes qui correspondaient à celles observées dans les contenus stomacaux. Nous avons mesuré le ratio isotopique de souffre ($\delta^{34}S$) et les [Se] dans ces échantillons. La signature isotopique de soufre permet de savoir si un organisme se nourrit davantage d'organismes venant de la colonne d'eau ou du compartiment benthique (Croisetière et al., 2009). Les détails de cette étude sont présentés dans le troisième article de cette thèse (Ponton et Hare, 2015; révisions demandées par *Environmental Science and Technology*).

Les [Se] dans les perchaudes augmentent avec leur poids jusqu'à 25 g (poids total humide; Figure 3.1) dans deux (Dufault et Kelly) des quatre lacs et ensuite diminuent (lac Dufault) ou plafonnent (Kelly). Dans les lacs Osisko et Rouyn, nous n'avons pas observé de relations significatives entre les [Se] et la taille. L'identification des proies retrouvées dans les contenus stomacaux indique que les jeunes perchaudes < 10 g peuvent être planctivores (six poissons de trois lacs; 40%), mais aussi benthivores (neuf poissons de quatre lacs; 60%). Par exemple, une perchaude de 1 g dans le lac Kelly avait 22 Chironomidae dans son estomac et une autre de 8 g, de ce même lac, avait seulement des crustacés planctoniques. Ces résultats visuels nous indiquent que malgré leur petite taille, les jeunes perchaudes peuvent aussi se nourrir de proies plus grosses que le zooplancton, contrairement aux résultats de Rasmussen et al. (2008). Cette variabilité de l'alimentation est aussi visible par la grande gamme de valeurs des signatures isotopiques de soufre des plus petites perchaudes. Plus les perchaudes grandissent, moins grande est cette variabilité puisqu'elles arrêtent progressivement de se nourrir de zooplancton pour

exclusivement consommer des proies benthiques, ce qui est avantageux du point de vue de bilan en énergie de prédation (Rassmussen et al., 2008).

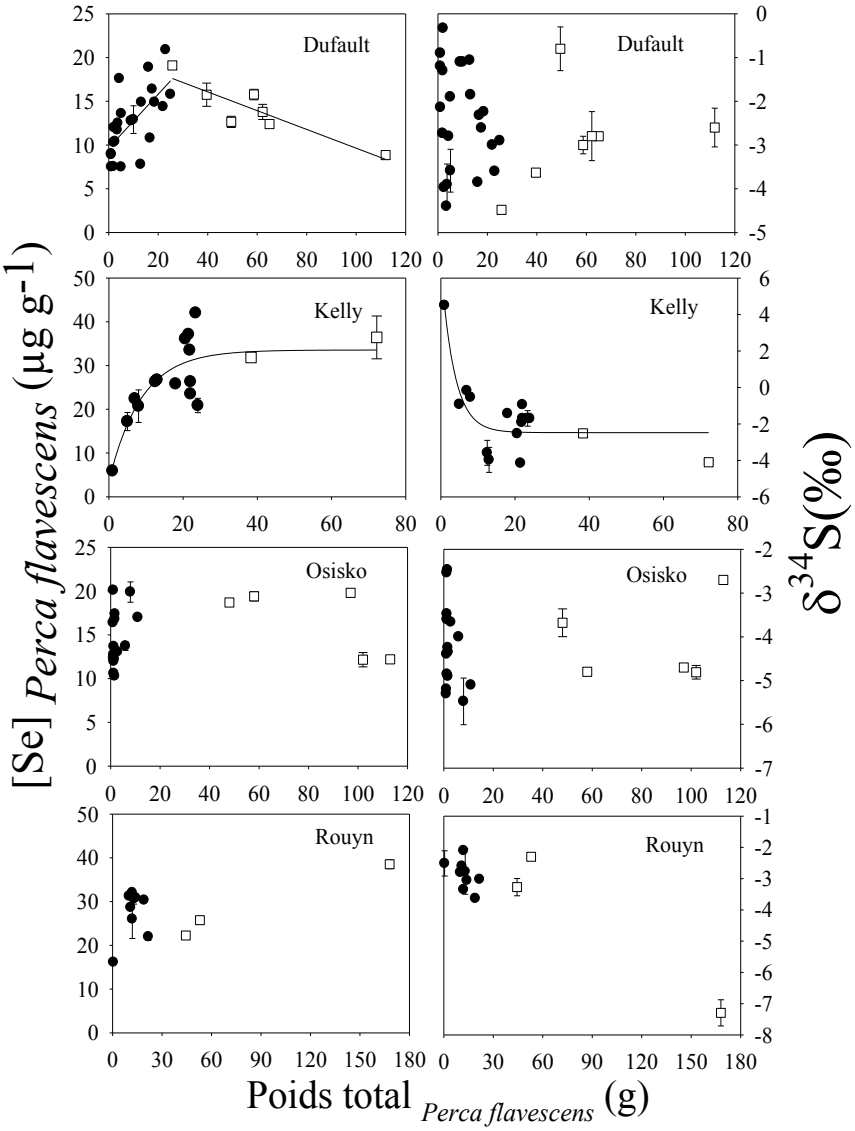

Figure 3.1 : Concentrations de sélénium (µg g^{-1} PS; ± ET; panneaux de gauche) et signature isotopique de soufre ($\delta^{34}S$; ± ET; panneaux de droite) du muscle antérieur dorsal des perchaudes récoltées dans quatre lacs (Osisko, Dufault, Rouyn et Kelly) en fonction de leur poids total humide (g). Les carrés blancs sont les perchaudes de plus de 25 g. Les écarts-types sont les variabilités analytiques (n = 3).

Les perchaudes de plus de 25 g restent benthivores ou changent leur alimentation pour de petits poissons planctivores ou benthiques. Dans le lac Kelly, les perchaudes > 25 g ne changent pas leur alimentation et restent benthivores dû à l'abondance de ce type de proie comparativement aux petits poissons. Dans le lac Rouyn, un petit poisson benthivore a été observé dans l'estomac de l'une des plus grosses perchaudes, mais celle-ci continuait toujours à manger des proies benthiques, ce qui se traduit par une des [Se] les plus élevées (38 $\mu g\ g^{-1}$). Les plus grosses perchaudes récoltées dans le lac Kelly sont aussi benthivores et ce comportement alimentaire se traduit aussi par les [Se] les plus élevées observées. Dans le lac Osisko, la forte abondance de perchaudes fait en sorte qu'il y ait une forte compétition interspécifique pour la nourriture ce qui entraine une grande diversité de type d'alimentation pour une même taille. Les signatures isotopiques de soufre et l'identification des proies indiquent que la taille n'est pas un bon outil pour connaître l'alimentation des perchaudes et du fait même, l'exposition au Se.

3.2. Concentrations de sélénium chez les proies de *Perca flavescens*

Les [Se] les plus élevées chez les proies échantillonnées sont celles des larves des diptères de la famille des Chironomidae, ensuite celles des invertébrés épibenthiques (e.g. *Hyalella*, *Anisoptera*) et les concentrations les plus faibles sont celles des poissons planctivores et du zooplancton (Figure 3.2). Donc, plus les organismes dépendent d'une source de nourriture venant des sédiments, plus leurs [Se] sont élevées. Cette affirmation est vérifiable par les signatures isotopiques de soufre qui sont plus faibles chez les larves de *Chironomus* à plus élevées selon cette même classification de proies (épibenthiques < poissons planctoniques < zooplancton; Figure 3.2).

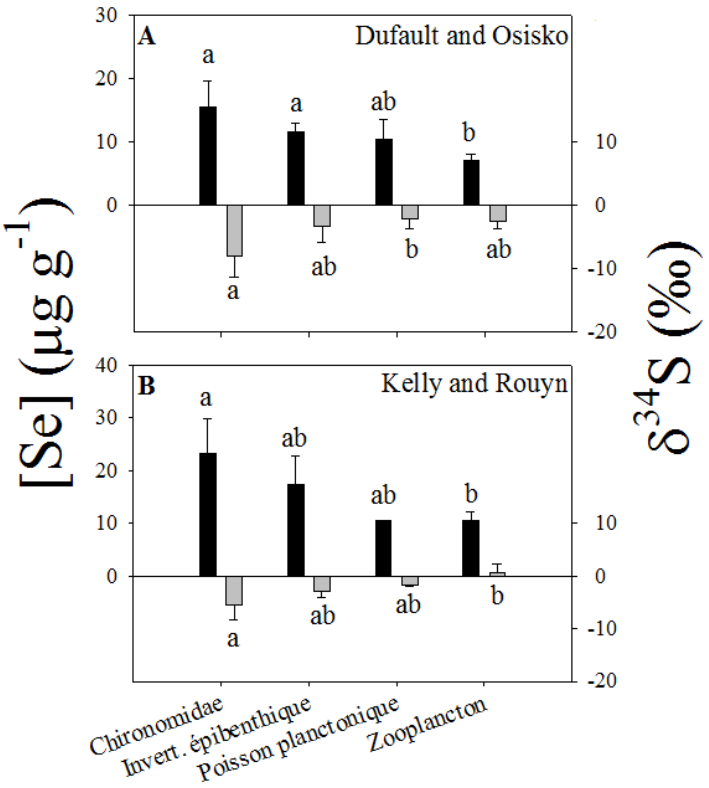

Figure 3.2: Concentrations de sélénium (µg g^{-1} PS; ± ET; barres noires) et signatures isotopiques de soufre ($\delta^{34}S$; ‰; ± ET; barres grises) des proies des perchaudes venant des quatre lacs étudiés. Les proies des lacs Dufault et Osisko et celles des lacs Kelly et Rouyn ont été regroupées selon les résultats statistiques d'un modèle général linéaire.

3.3. Relations entre les concentrations de sélénium et la signature isotopique de soufre

Les [Se] dans les perchaudes et dans leurs proies sont inversement proportionnelles avec les signatures isotopiques de soufre (Figure 3.3). Ces relations sont dues au fait que les signatures isotopiques de soufre sont plus négatives en milieu anoxique que dans les environnements oxygénés. En milieu marin, les bactéries sulfato-réductrices sont responsables de la majorité du fractionnement isotopique de soufre qui peut être aussi important que -70‰ (Brunner et Bernasconi, 2005). Par contre, dans les lacs étudiés, puisque les [SO_4] sont faibles dans les sédiments (Croisetière et al., 2009), il est suggéré que ce serait davantage la diffusion préférentielle de l'isotope le plus léger qui serait

responsable du fractionnement isotopique de soufre de la colonne d'eau jusqu'aux sédiments plus profonds (Peterson et Fry 1987).

Le Se dans les sédiments est principalement (70% du Se total) sous formes réduites (Se(-II) à Se(II)). Celles-ci peuvent être organiques ou élémentaire (Se(0); 30 à 40% du Se total, respectivement; Martin et al., 2011). Selon Schlekat et al. (2000), cette dernière forme est moins absorbée par le bivalve *Potamocorbula amurensis* que la forme organique. La formes oxydée du Se dans les sédiments est le sélénite et représente environ 30% du Se total (Martin et al. 2011). Dans la colonne d'eau, le sélénium peut aussi être présent sous des formes réduites organiques, mais en plus grandes proportions sous formes oxydées (sélénite et séléniate), généralement moins biodisponibles. Ceci explique en partie pourquoi l'accumulation de Se par les organismes pélagiques est plus faible que par les organismes benthiques, mais des études plus précises sont nécessaire afin de répondre à cette question.

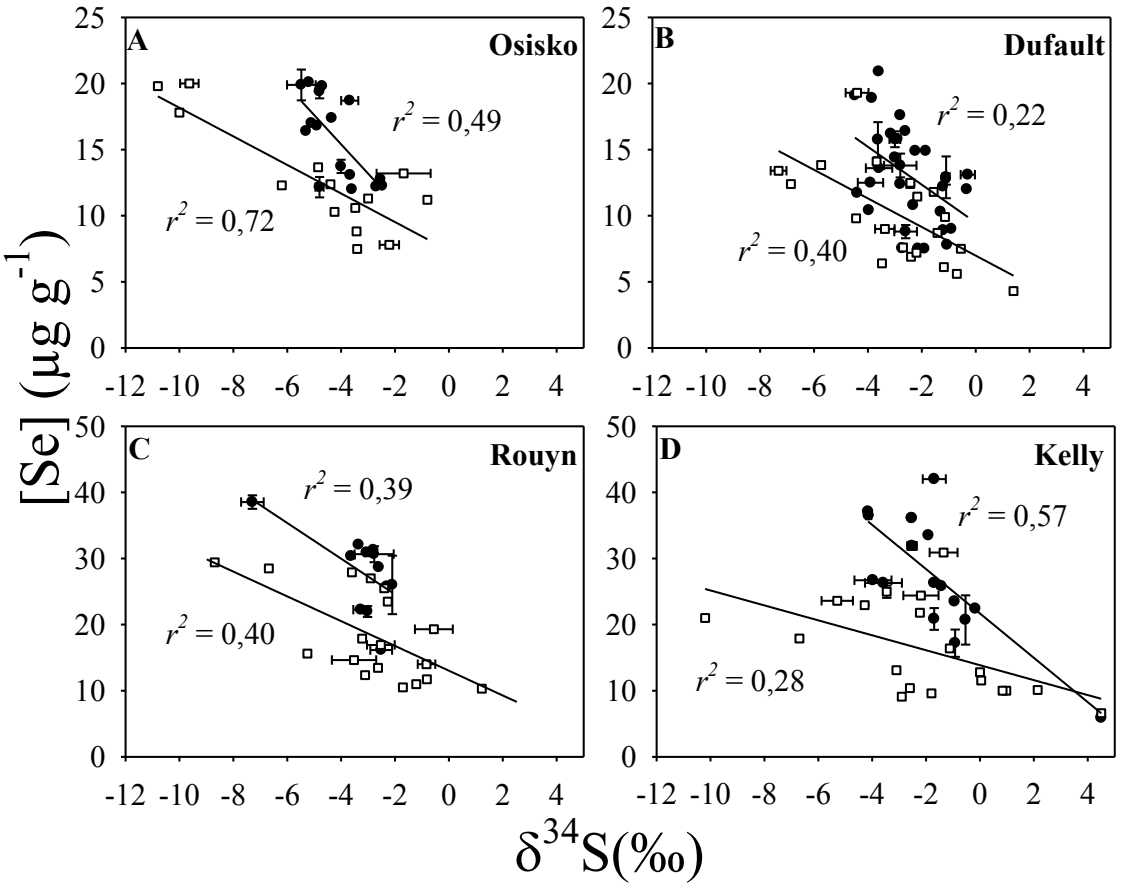

Figure 3.3 : Concentrations de sélénium (µg g^{-1} PS; ± ET) en fonction des signatures isotopiques de soufre ($\delta^{34}S$; ‰; ± ET) chez les perchaudes (points noirs) et leurs proies (carrés blancs) des quatre lacs étudiés. Les écarts-types sont les variabilités analytiques.

3.4. Facteur de transfert trophique et bioamplification de sélénium

Nous avons divisé les [Se] chez des perchaudes de différentes tailles avec les [Se] des proies prélevées dans leur estomac (Figure 3.4). Ce FTT est de 1,6 ± 0,7 et similaire à celui obtenu chez les plus jeunes perchaudes (section 2.2) et indiquent une bioamplification entre ces deux niveaux trophiques (Figure 3.4; points au-dessus de ligne 1 :1). Ce facteur de transfert trophique est un des plus élevés observés entre différentes espèces de poissons et leurs proies selon la revue de la littérature de Presser et Luoma (2010). Étant donné que les proies les plus contaminées sont les larves de chironomes, nous suggérons de les utiliser comme biomoniteur de sélénium et d'appliquer le facteur de 1,6 afin de connaître l'exposition maximale des perchaudes d'un lac donné. Dans un

plus large spectre environnemental, l'analyse du Se chez un organisme se nourrissant dans les sédiments d'un environnement aquatique donné, pourrait être utilisé comme biomoniteur d'exposition maximale au Se.

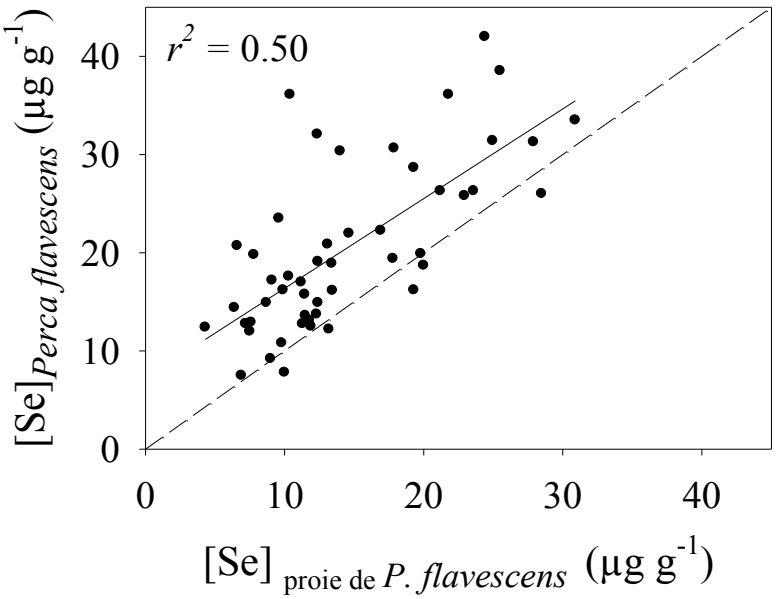

Figure 3.4 : Concentrations de sélénium chez les perchaudes (µg g^{-1} PS) des quatre lacs en fonction des concentrations de sélénium chez leurs proies (µg g^{-1} PS) récoltées à même leur estomac. La ligne pointillée est la relation 1:1.

3.5. Estimation du risque de subir un effet tératogène dû aux concentrations de sélénium

Les mesures de [Se] dans différents tissus et organes de perchaudes du lac Lohi (Sudbury; Supplementary Figure S1; Ponton et Hare, 2015) nous indiquent que les [Se] dans les gonades des perchaudes représentent 62 % des [Se] retrouvées dans le muscle. Ces mesures nous permettent d'estimer les [Se] des gonades des perchaudes des quatre lacs étudiés. Selon la littérature (DeForest et al., 2011), une [Se] > 20 µg g^{-1} dans les ovaires représenteraient un risque de reproduction pour une grande variété d'espèces de poissons. Selon nos estimations, une [Se]$_{muscle}$ > 31 µg g^{-1} suggérerait une [Se] dans les ovaires > 20 µg g^{-1}. Dans les lacs Kelly et Rouyn, 38% et 33% des perchaudes, respectivement, seraient à risque de subir des effets reproductifs. Dans les deux autres lacs, les [Se] dans le muscle ne dépassent pas 21 µg g^{-1} ([Se]$_{gonade}$ < 13 µg g^{-1} P.S.), ce qui

suggère l'absence de risques reproductifs. Étant donné que ces quatre lacs sont parmi les plus contaminés en Se dans ces deux régions, le Se ne devrait pas être une source d'effet majeur pour les poissons.

QUATRIÈME CHAPITRE

STRESS OXYDATIF INDUIT PAR LES FAIBLES CONCENTRATIONS DE SÉLÉNIUM ET LE CADMIUM CHEZ DES PERCHAUDES (*PERCA FLAVESCENS*) SAUVAGES

4.1. Stress oxydatif des populations de perchaudes

Nous avons prélevé le foie de 50 perchaudes dans 11 lacs des régions de Sudbury et Rouyn-Noranda. Celles-ci ont été récoltées à l'aide d'une seine à partir du rivage et mises dans une glacière avec de l'eau aérée du lac. Nous les avons ensuite sacrifiées et prélevé leur foie qui a été gelé instantanément dans l'azote liquide. Au laboratoire, nous avons séparé les foies individuels en cinq parties pour l'analyse : (1) du malondialdéhyde (MDA), (2) du glutathion (GSH), (3) du disulfure de glutathion (GSSG), (4) du fractionnement subcellulaire des éléments traces, des concentrations de métallothionéine et (5) des concentrations totales en éléments traces. Veuillez consulter le quatrième article de cette thèse (Ponton et al., 2015b; à soumettre à *Environmental Science and Technology*) pour plus de détails.

La figure 4.1A montre que nos deux mesures du risque de stress oxydatif sont inversement corrélées puisque, lorsque le ratio GSH/GSSG est faible, les perchaudes subissent davantage de peroxydation lipidique comme l'indiquent les concentrations plus élevées de MDA. Le glutathion (GSH) est la principale molécule responsable de la réduction du stress oxydatif (Mannervick et al., 1976). À l'aide d'une méthode statistique par itération, nous avons établi un seuil (0,51 µmol MDA g^{-1} de protéine) au-delà duquel les perchaudes subissent une peroxydation lipidique significative par rapport aux autres individus. Ces individus sont représentés par les symboles ouverts dans les figures de cette section de la thèse. Les cellules en bonne santé ont plus de 85% de leur glutathion sous la forme réduite (GSH) ou moins de 15% sous la forme du disulfure (GSSG). Une autre façon de présenter les [MDA] en fonction du glutathion est d'utiliser les pourcentages de disulfure de glutathion (GSSG) ou de GSH (GSH + GSSG = 100%). La Figure 4.1B présente donc les [MDA] en fonction de la proportion de GSSG et démontre

que lorsque les pourcentages de GSSG sont plus élevés que 20%, les perchaudes ont un risque très élevé de subir une peroxydation lipidique.

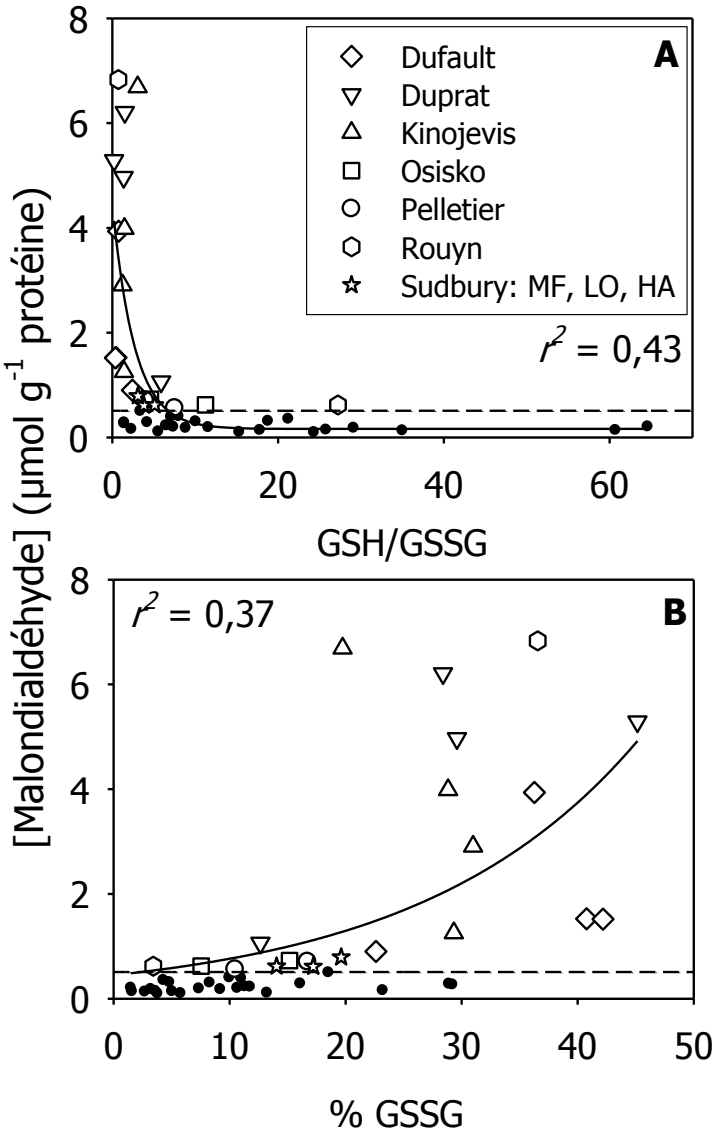

Figure 4.1 : Concentrations de malondialdéhyde (µmol g^{-1} de protéine) en fonction du ratio de glutathion et son disulfure (GSH/GSSG; panneau A) et en fonction des proportions de GSSG (%; panneau B) pour les foies de perchaudes récoltés dans 11 lacs (données individuelles). Les symboles blancs représentent les perchaudes ayant une [MDA] plus grande que 0,51 µmol g^{-1} protéine. La ligne pointillée est le seuil de 0,51 µmol MDA g^{-1} protéine. Les perchaudes ayant une [MDA] plus grande que ce seuil sont identifiées selon leur lac d'origine.

Pour réduire le peroxyde d'hydrogène en eau le glutathion nécessite l'enzyme dépendante du sélénium nommée glutathion peroxydase et cette réduction entraîne la formation d'eau et le disulfure de glutathion (GSSG), indicateur d'un plus grand risque de stress oxydatif (Figure 4.1). La Figure 4.2 présente que la proportion de disulfure de glutathion est inversement corrélée avec les concentrations de sélénium dans la fraction cellulaire stable à la chaleur. Puisque la forme organique du Se la plus physiologiquement active est la sélénocystéine (Stadtman, 1996), il est logique que la corrélation soit significative avec cette fraction puisque la sélénocystéine, comme son homologue, la cystéine, est résistante à la chaleur. À de faibles concentrations de sélénium, nous émettons l'hypothèse que l'activité de la glutathion peroxydase, comprenant dix sélénocystéine (Chambers et al., 1986), est réduite et que par conséquent, les concentrations de peroxyde d'hydrogène augmentent, ainsi que le stress oxydatif.

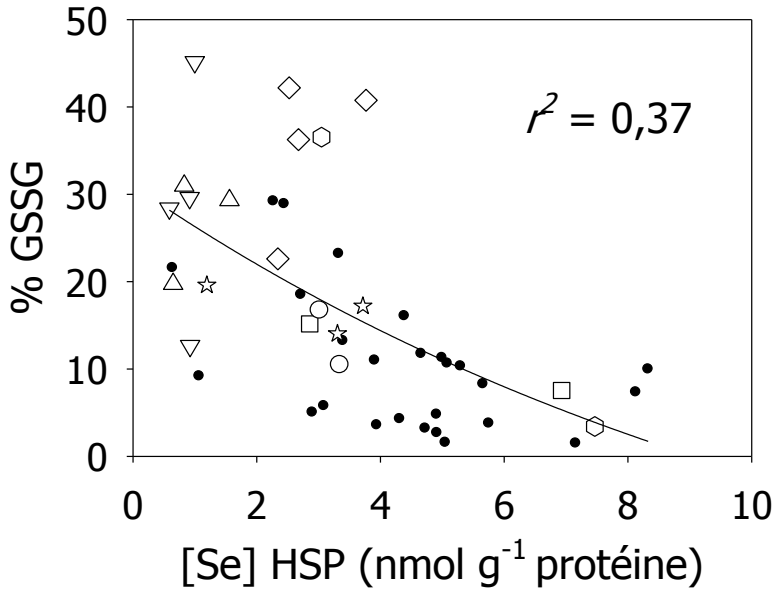

Figure 4.2 : Proportions du disulfure de glutathion (% GSSG) en fonction des concentrations de sélénium dans la fraction cellulaire stable à la chaleur ([Se] HSP; nmol g^{-1} de protéine) des foies de perchaudes récoltées dans 11 lacs. Les symboles blancs représentent les perchaudes ayant une [MDA] plus grande que 0,51 µmol g^{-1} protéine. Voir Figure 4.1 pour l'identification des lacs.

4.2. Concentrations de métaux dans les différents compartiments subcellulaires des perchaudes

Nous avons mesuré les concentrations de métaux dans les organelles, les protéines cytosoliques dénaturées à la chaleur (HDP; p.ex. enzymes) et les protéines cytosoliques stables à la chaleur (HSP) comprenant les protéines séquestrant les métaux comme la métallothionéine. Les deux premiers compartiments sont considérés comme sensibles et la présence de métaux non essentiels comme le cadmium dans ceux-ci peut entraîner des effets toxiques. La Figure 4.3 présente les concentrations de Cd, Cu, Ni et Zn dans les compartiments sensibles en fonction des concentrations dans les protéines stables à la chaleur. Les poissons subissant du stress oxydatif (symboles ouverts) ont des proportions de cadmium, de zinc et de cuivre (dans une moindre mesure) dans les compartiments sensibles qui sont supérieures à celles observées dans les individus ne subissant pas de stress oxydatif (régressions faites avec les individus ne subissant pas de stress). Dans le cas du Cu, ce débordement subcellulaire (*spillover*) est seulement observable chez quelques individus des lacs Kinojévis et Dufault et démontre la forte affinité du Cu pour la MT. Il ne semble pas y avoir de débordement subcellulaire de Ni. Donc, on peut se poser la question suivante : Est-ce les métaux qui causent le stress ou le stress qui cause le débordement subcellulaire (*spillover*)?

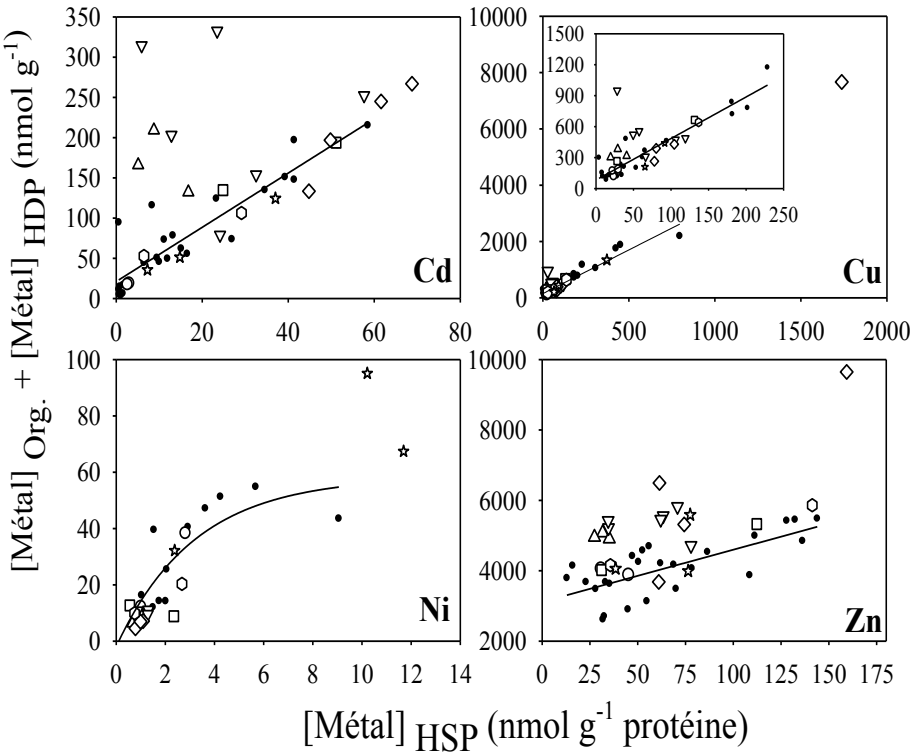

Figure 4.3 : Concentrations de Cd, Cu, Ni et Zn dans les fractions cellulaires sensibles (organelles ($[M]_{Org}$) + protéines se dénaturant à la chaleur ($[M]_{HDP}$); nmol g^{-1} PS) en fonction des concentrations de ces mêmes métaux dans la fraction cellulaire stable à la chaleur ($[Métal]_{HSP}$; nmol g^{-1} protéine) des foies individuels de perchaudes récoltées dans 11 lacs. Les symboles blancs représentent les perchaudes ayant une [MDA] plus grande que 0,51 µmol g^{-1} protéine. Voir Figure 4.1 pour l'identification des lacs.

Puisque le débordement subcellulaire est observable sur tout le gradient et non seulement aux fortes concentrations de métaux, on ne peut conclure, *a priori*, que les métaux causent le stress oxydatif. Par contre, des études démontrent que lorsque des molécules oxydantes comme les espèces réactives de l'oxygène de même que le GSSG sont en concentrations élevées dans les cellules, celles-ci peuvent oxyder la métallothionéine et ainsi diminuer son affinité pour les métaux et conséquemment, entraîner un *spillover* (Jacob et al., 1998). Ce phénomène peut être bénéfique lorsque le *spillover* mobilise des métaux essentiels comme le zinc, par contre, la mobilisation du cadmium, qui est non essentiel, peut causer davantage de stress oxydatif. Afin de quantifier le débordement subcellulaire, nous avons utilisé l'équation suivante :

$$\Delta[\mathrm{M}] = [\mathrm{M}]_{\mathrm{HDP}} + [\mathrm{M}]_{\mathrm{org.}} - (a_{\mathrm{M}}[\mathrm{M}]_{\mathrm{HSP}} + b_{\mathrm{M}}) \qquad (2)$$

où Δ[M] (nmol g^{-1}) est la différence entre les concentrations d'un métal (M) dans les fractions sensibles (premier et second termes) d'un poisson et la concentration de ce même métal dans les fractions sensibles d'un poisson ne subissant pas de stress (réprésenté par les régressions). Le troisième terme de l'équation 2, sous la forme d'une équation linéaire (a*x* + b), représente la [M] dans les fractions sensibles d'un poisson ne subissant pas de stress en fonction de la concentration de métal dans les protéines stables à la chaleur ($[M]_{HSP}$). Les valeurs a_M et b_M sont respectivement la pente et l'ordonnée à l'origine des régressions présentées dans la Figure 4.3. La Figure 4.4 présente la relation entre les valeurs de Δ[Cd] et de Δ[Zn] obtenues par l'équation 2. Ce graphique démontre une relation exponentielle entre le débordement subcellulaire de zinc et de cadmium. Donc, l'oxydation des protéines séquestrant les métaux diminue leurs affinités pour le zinc et le cadmium, mais la relation semble suggérer une affinité plus grande du Cd que du Zn pour ces protéines (Waalkes, 1984).

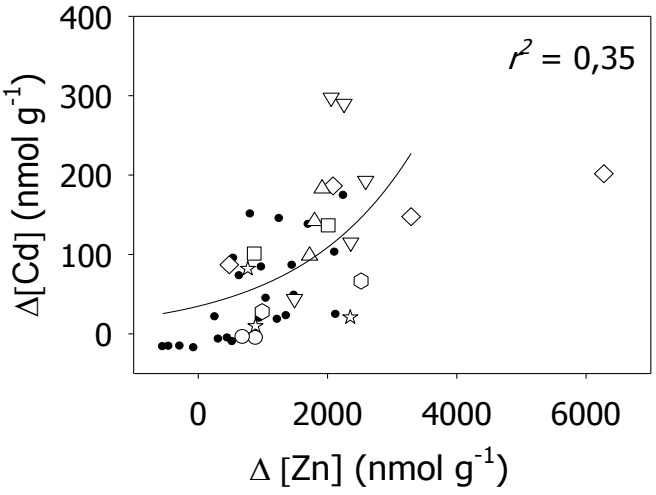

Figure 4.4 : Delta cadmium (Δ[Cd]; nmol g^{-1} PS) en fonction du delta zinc (Δ[Zn]; nmol g^{-1} PS) calculés selon l'équation 2 pour les perchaudes des 11 lacs étudiés. Les symboles blancs représentent les perchaudes ayant une [MDA] plus grande que 0,51 µmol g^{-1} protéine. Voir Figure 4.1 pour l'identification des lacs. La valeur extrême de Δ[Zn] n'est pas incluse dans la régression.

4.3. Effet antagoniste du cadmium et du sélénium sur la peroxydation lipidique

Nous avons observé une relation exponentielle entre la peroxydation lipidique ([MDA]) et les Δ[Cd] (Figure 4.5A), ce qui confirme que les effets toxiques chez les populations de perchaudes, principalement de Rouyn-Noranda, sont dus au cadmium comme le mentionnent les études examinées dans Campbell et al. (2008) pour la perchaude et l'étude de Borgmann et al. (2004) concernant l'amphipode *Hyalella azteca*. Par contre, les [Cd] totales dans le foie n'expliquent pas la toxicité et ce serait plutôt le Δ[Cd] ou le débordement subcellulaire qui augmente le stress oxydatif. Nous avons aussi observé que la peroxydation lipidique est plus faible lorsque les [Se] sont plus élevées (Figure 4.5B). Cette étude est la première qui démontre l'effet antagoniste du Cd et du Se en milieu naturel. Nous suggérons donc que, sans considérer le Cd, les faibles [Se] induisent une augmentation du stress oxydatif, qui entraîne une diminution de l'affinité des protéines séquestrant les métaux. Le débordement subcellulaire de Cd dans les fractions cellulaires sensibles augmente le stress oxydatif induit par les faibles [Se].

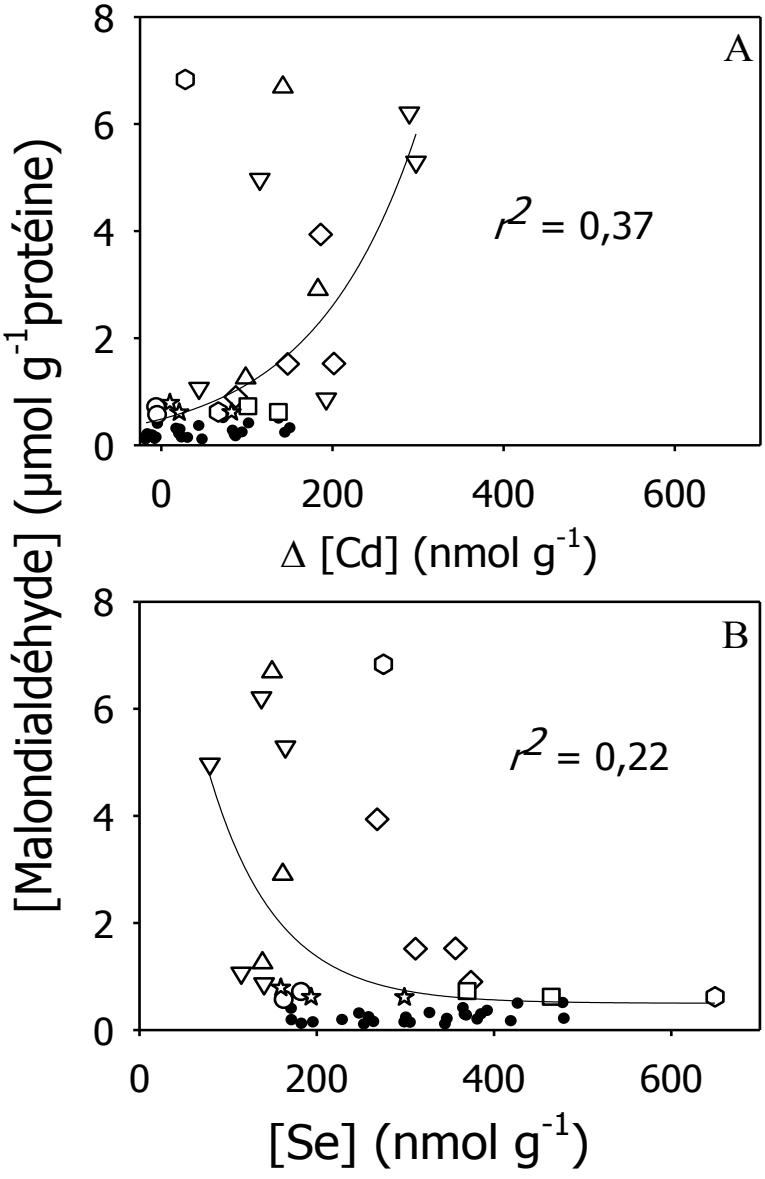

Figure 4.5: Concentrations de malondialdéhyde ([MDA]; µmol g^{-1} protéine) en fonction du delta cadmium (ΔCd; nmol g^{-1} PS; panneau A) et des concentrations de sélénium totales dans le foie ([Se]; µg g^{-1} PS; panneau B) des perchaudes venant des 11 lacs étudiés. Les symboles blancs représentent les perchaudes ayant une [MDA] plus grande que 0,51 µmol g^{-1} protéine. Voir Figure 4.1 pour l'identification des lacs.

CINQUIÈME CHAPITRE

FRACTIONNEMENT SUBCELLULAIRE ET ACCUMULATION DE CADMIUM ET DE SÉLÉNIUM CHEZ LA LARVE DE L'INSECTE *CHAOBORUS PUNCTIPENNIS*

5.1. Concentrations de Cd et de Se chez des larves de *Chaoborus* de différents lacs

Nous avons récolté des larves de l'insecte *Chaoborus* (quatrième stade larvaire; Moore, 1988) dans le lac Dasserat (lac peu contaminé) pour les transférer à l'intérieur de mésocosmes installés dans le lac Dufault (lac très contaminé) pour une période de 16 jours. Des proies (crustacés planctoniques) de taille convenable (Croteau et al., 2003) ont été ajoutées aux mésocosmes chaque deux jours. Des échantillons ont été prélevés temporellement afin de mesurer l'accumulation et effectuer le fractionnement subcellulaire de Cd et de Se. Pour plus de détails, veuillez consulter le cinquième article de cette thèse (Rosabal et al., 2014; publié dans *Environmental Science and Technology*).

La Figure 5.1 présente les [Cd] et de [Se] chez les larves du diptère *Chaoborus punctipennis* de différents lacs des régions de Sudbury et de Rouyn-Noranda. Les points blancs représentent les concentrations de Cd et de Se des larves des lacs Dasserat et Dufault et indiquent la pertinence de les utiliser pour l'expérience de transplantation. La proximité de la régression et la ligne 1:1 (pointillée) suggère qu'il y a une corrélation entre l'accumulation de ces deux éléments.

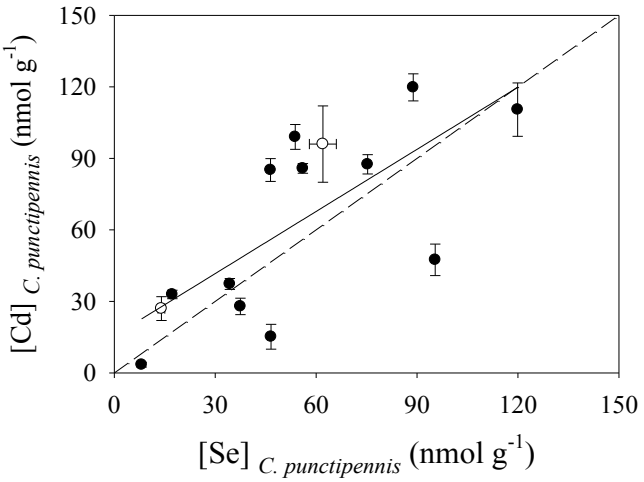

Figure 5.1 : Concentrations de Cd et de Se (nmol g^{-1} PS; ± ET) chez *Chaoborus punctipennis* de différents lacs des régions de Sudbury et de Rouyn-Noranda. Les points blancs identifient les concentrations des deux lacs à l'étude (Dasserat et Dufault).

5.2. Accumulation temporelle de cadmium et de sélénium

La quantité de Se dans les larves a augmenté graduellement pendant les 15 jours de transplantation jusqu'à devenir comparable ($p > 0,05$) à la quantité de Se dans les larves indigènes du lac Dufault (Figure 5.2A). Dans le cas du cadmium, une période de 9 jours d'exposition a été nécessaire avant que l'accumulation soit significative et ensuite, pendant la dernière semaine, la quantité de Cd par larve a augmenté et atteint celle des larves indigènes du lac Dufault (Figure 5.2B). Nous expliquons ce changement de vitesse d'accumulation de Cd par une augmentation de l'efficacité d'assimilation après le 6^{e} jour dû à la synthèse de métallothionéine dans les cellules du tractus digestif (Wang et Rainbow, 2008). Effectivement, à l'aide de calculs déterministes (Thomann, 1981), nous avons estimé que l'efficacité d'assimilation est passée de 2,3 à 4,6 %, ce qui explique très bien le changement d'accumulation de Cd (ligne pointillée correspondant aux points expérimentaux dans la Figure 5.2B). Puisque la croissance a été relativement stable pendant l'expérience et que l'augmentation des [Se] l'a aussi été, nous ne pouvons émettre l'hypothèse que le changement du taux d'ingestion est responsable du changment de vitesse d'accumulation du Cd. Les [Se] et les [Cd] sont restées les mêmes au cours de l'expérience (Figures 5.2C et D) car l'augmentation des quantités de Se et Cd

correspondaient à une augmentation de la biomasse des larves (Supp. Figure S2; Rosabal et al., 2014). Les concentrations plus faibles au jour 0 sont dues à un artefact expérimental car nous avons sélectionné, sans le vouloir, les plus grosses larves pour les échantillons au temps 0. Par contre, ceci n'a pas été le cas pendant les autres jours d'échantillonnage.

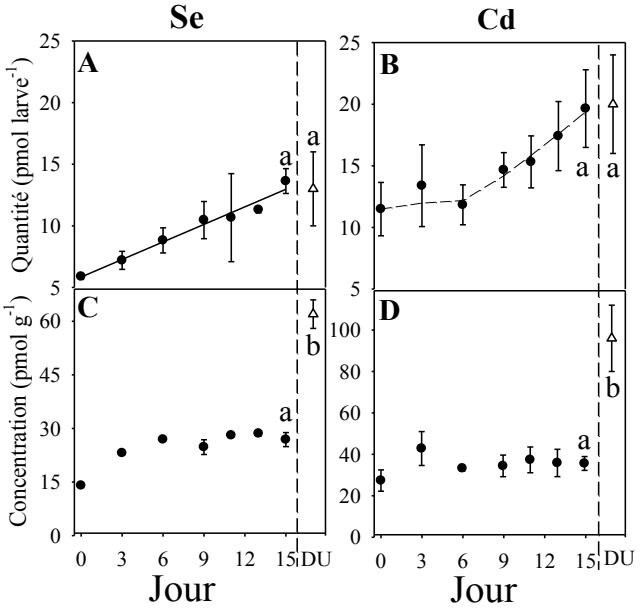

Figure 5.2 : Quantité de Se et de Cd (pmol larve^{-1}; ± ET; panneau A et B) et concentrations de Se et Cd (pmol g^{-1} PS; ± ET; panneaux C et D) chez les larves de *Chaoborus punctipennis* en fonction des jours de transplantation dans le lac Dufault. Les lettres représentent des différences significatives entre le jour 15 et les larves indigènes du lac Dufault (triangles blancs). La ligne pointillée correspondant aux points expérimentaux a été modélisée selon une efficacité d'assimilation de 2,3 % du jour 0 à 6 et de 4,6 % du jour 6 à 15 (voir Rosabal et al. 2014).

5.3. Fractionnement subcellulaire de cadmium et de sélénium chez *Chaoborus*

Le fractionnement subcellulaire de Cd démontre que cet élément est bien lié aux protéines et peptides, comme par exemple la métallothionéine, dans la fraction stable à la chaleur des cellules (Figure 5.3C). Dans les fractions sensibles des cellules (organelles, mitochondries, enzymes), nous avons mesuré une faible proportion du Cd total. (SEN ~ 20%; Figures 5.3C, D). Au jour 16, la quantité de Cd moyenne dans la fraction HSP des larves du lac Dasserat introduites dans le lac Dufault était significativement plus élevée que celle observée dans les larves du lac Dasserat (Figure 5.3B). Par contre, la quantité de

Se dans cette même fraction n'est pas significativement plus grande qu'au début de l'expérience ($p = 0,07$), mais similaire à celle observée dans les larves du lac Dufault ($p < 0,05$). Le Se, qui est un élément essentiel, se retrouve réparti à peu près également entre les différentes fractions des cellules. À la fin de l'expérience, environ les mêmes proportions de sélénium se retrouvaient dans les compartiments sensibles et ceux responsable de la détoxication ($p < 0,05$; Figure 5.3B). La gestion cellulaire de ces deux éléments traduit bien leur essentialité (Se) ou l'absence de fonction physiologique (Cd). Effectivement, le Se a plusieurs fonctions physiologiques, dont la réduction du stress oxydatif et les mitochondries sont connues comme générant une grande quantité de radicaux libres de l'oxygène. La seule donnée temporelle qui n'atteint pas ($p > 0,05$) les valeurs observées chez les larves indigènes du lac Dufault est celle de la quantité de Se dans les mitochondries, ce qui peut refléter la fonction antioxydante du Se chez les larves indigènes et la protection de cette fraction sensible des cellules.

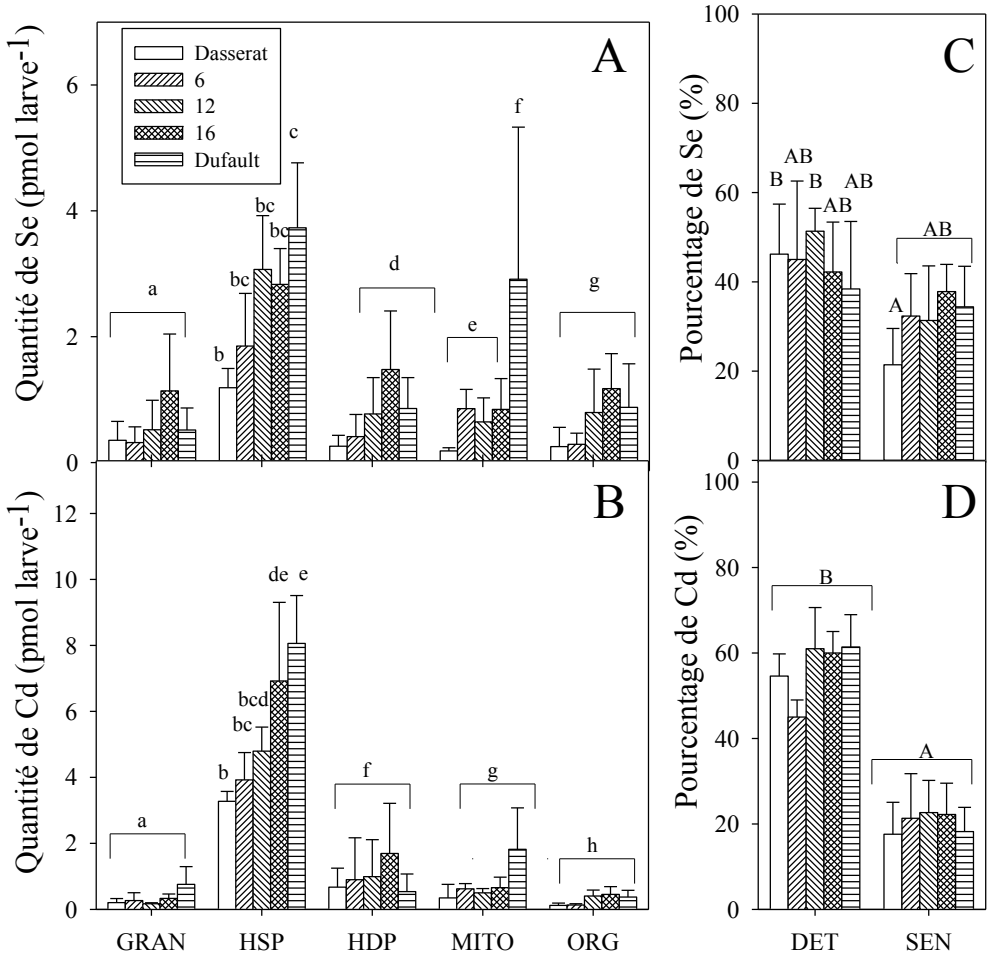

Figure 5.3 : Quantités de sélénium (panneau A) et de cadmium (panneau B) dans chaque fraction cellulaire au cours de l'expérience (voir légende dans le panneau A) et pourcentages de sélénium (panneau C) et de cadmium (panneau D) dans les compartiments détoxiquant les métaux (DET) et sensibles aux métaux (SEN) au cours de l'expérience. Les différentes lettres minuscules présentent des différences significatives temporelles et les lettres majuscules des différences entre les compartiments DET et SEN.

5.4. Relations entre le cadmium et le sélénium dans les cellules de *Chaoborus*

Les quantités de Se augmentent graduellement au cours de l'expérience, alors que celles de Cd augmentent après le 6^{e} jour, ce qui entraîne une régression exponentielle entre les quantités des deux éléments dans les larves entières (Figure 5.4A) et dans les compartiments détoxiquant les métaux (Figure 5.4B). L'augmentation de l'efficacité d'assimilation de cadmium entre les jours 6 et 16 est probablement due à la synthèse de métallothionéine dans les cellules du tractus (Wang and Rainbow, 2008). L'interaction entre le Cd et le Se dans les cellules n'est pas encore très bien connue, mais comme on le mentionne dans le chapitre précédent, la diminution du stress oxydatif dû au sélénium peut augmenter l'affinité des protéines séquestrant les métaux (HSP) et par le fait même, augmenter l'efficacité d'assimilation.

D'autres hypothèses existent concernant l'interaction du Se avec les métaux. Des études indiquent que l'exposition au Se augmente l'accumulation de Cd chez le crabe (Bjerregaard, 1988) et dans certains organes du rat (Trabelsi et al., 2013). De plus, à une exposition élevée, des nanoparticules de séléniure de cadmium (CdSe) peuvent être biosynthétisées par des rats (Trabelsi et al., 2013) et des levures (Li et al., 2013). Finalement, la sélénoprotéine P peut participer à la séquestration de différents métaux (Young et al., 2010), et ce, de façon aussi importante que la métallothionéine chez la souris (Garcia-Sevillano et al., 2014). Nous avons observé que les concentrations de Cd et de Se présentent un rapport très près de l'unité dans les larves de divers lacs de la région de Sudbury et Rouyn-Noranda (Figure 5.1) et au cours de notre expérience (Figure 5.4), mais la répartition subcellulaire indique que la formation de complexes de Cd et de Se est peu probable. Puisque les [Cd] et de [Se] chez *Chaoborus* sont corrélées avec les [Cd^{2+}] et de [Se-Org] dans l'eau, respectivement (Hare et al., 2008; Ponton et Hare, 2013), ces deux formes chimiques de Cd et de Se dans l'eau devraient être corrélées et avoir un rapport molaire près de l'unité entraînant une relation près de l'unité chez les organismes.

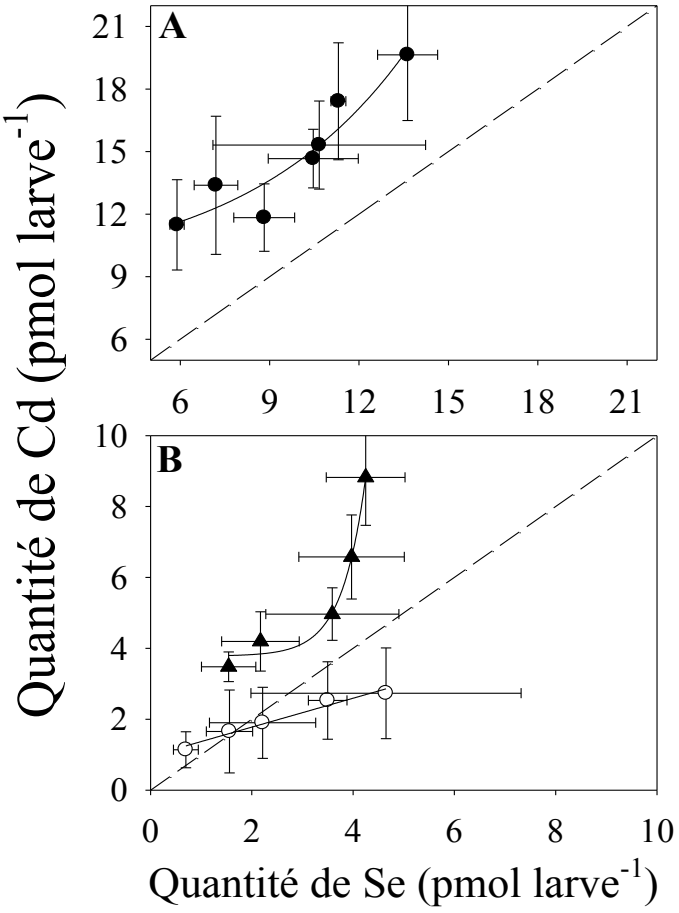

Figure 5.4 : Quantité (pmol larve^{-1}) de Cd en fonction de celles de Se dans les larves entières (panneau A) et dans les compartiments cellulaires (panneau B) détoxifiant les métaux (HSP + granules; triangles noirs) et sensibles aux métaux (HDP + organelles; points blancs). Les lignes pointillées représentent la ligne 1:1. Ces relations ont aussi une dimension temporelle puisque le gradient suit les jours d'exposition.

CONCLUSIONS

Cette thèse présente plusieurs nouveautés dans le domaine de l'écotoxicologie du Se. Nous avons pour la première fois, relié la spéciation du sélénium dans l'eau avec l'accumulation dans une chaîne trophique planctonique naturelle. Les larves de l'insecte *Chaoborus* peuvent être utilisées efficacement comme biomoniteur de l'exposition en Se des organismes pélagiques lacustres. Des études en laboratoire avec une algue verte et des micro-organismes sauvages nous ont permis d'observer et de comparer la prise en charge du sélénite, du séléniate et de la sélénométhionine selon différentes conditions chimiques. Le type de micro-organismes étudié et les concentrations en sulfate semblent être les deux facteurs qui modulent le plus l'accumulation de Se. Les résultats indiquent que le sélénium organique est l'espèce chimique à surveiller puisque des concentrations de 0,2 $\mu g\ L^{-1}$ peuvent être problématiques pour les poissons et cette concentration dans l'eau est 25 fois inférieure à celle recommandée par l'US EPA afin de protéger la vie aquatique. Contrairement au prédateur *Chaoborus*, nous avons observé une bioamplification du Se entre les perchaudes et leurs proies. Le changement d'alimentation de la perchaude entraîne un changement d'exposition au Se puisque les proies benthiques ont des concentrations de Se plus élevées que les proies pélagiques. La larve de *Chironomus* serait l'organisme lacustre idéal comme biomoniteur d'exposition maximale des poissons. Parmi les lacs les plus contaminés en Se, comme les lacs Kelly et Rouyn, les [Se] dans les gonades des perchaudes indiquent un risque de subir des effets tératogènes dus au Se. Effectivement, dans ces lacs, le nombre de poissons récoltés pour un même effort était plus faible. Par contre, nous avons observé que de faibles [Se] dans le foie diminue la défense contre le stress oxydatif cellulaire. Le stress oxydatif est relié à un débordement subcellulaire du Cd dans les compartiments cellulaires sensibles qui augmente la peroxydation lipidique. Les études concernant les effets des métaux devraient, idéalement, prendre en compte le sélénium présent en milieu naturel ou au laboratoire. Finalement, nos résultats de répartition subcellulaire de Cd et de Se chez *Chaoborus* démontrent que ces deux éléments sont séquestrés différemment dans les cellules et reflète leur absence de fonctions physiologiques, ou son essentialité, respectivement.

PERSPECTIVES

- Les relations observées entre les [Se] et les signatures isotopiques de soufre, ainsi que les résultats obtenus par Isabelle Proulx (Proulx, 2014) indiquent que les larves du diptère *Chironomus* qui se nourrissent en milieu plus anoxique accumulent plus de Se que les espèces de *Chironomus* qui se nourrissent en surface des sédiments. La spéciation du sélénium dans les sédiments et celle dans l'eau interstitielle de différentes profondeurs des sédiments pourraient être comparées avec les [Se] accumulée par des organismes benthiques afin de comprendre quels facteurs influencent la spéciation et la biodisponibilité du Se.
- La signature isotopique du soufre et l'exposition au Se devraient être mises en relation chez différentes espèces de poissons ayant différent mode de nutrition (p.ex., benthivore, planctivore).
- Les séléniures organiques représentent plus de 90% du Se total dissous dans certains lacs. La spéciation du sélénium dans l'eau de ces lacs devrait être étudiée par chromatographie afin de connaître précisément les formes organiques dissoutes.
- Malgré de nombreuses études sur la protection qu'offre le Se contre les effets des métaux dans les cellules, ce phénomène de protection n'est pas encore compris clairement.

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PREMIER ARTICLE

RELATING SELENIUM CONCENTRATIONS IN A PLANKTIVORE TO SELENIUM SPECIATION IN LAKEWATER

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Capsule

Our field results suggest that larvae of the phantom-midge *Chaoborus* would be effective sentinels for monitoring selenium (Se) concentrations in lake planktonic food-webs.

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Abstract

We measured selenium (Se) speciation in the waters of 16 lakes located near two major metal smelters and compared it to Se concentrations in a potential biomonitor, the planktivorous insect *Chaoborus*. We used this sentinel because planktonic algae and crustaceans, which are lower in the trophic chain leading to *Chaoborus*, are more difficult to separate and identify to species, whereas many fish species are not obligate planktivores. Percentages of selenate and organo-Se were generally higher in acidic lakes, whereas those of selenite were usually greater in alkaline waters. *Chaoborus* Se concentrations varied widely among lakes and, with the exception of a single high-sulfate lake, were significantly and highly correlated with those of dissolved organo-Se plus selenate (Se(VI)). We suggest that *Chaoborus* larvae would be highly effective for monitoring the Se-exposure of planktonic food webs in lakes.

Introduction

For most animals, Se is a double-edged sword; although essential, the concentration necessary to sustain health is only 2 to 3 times lower than that which is toxic (Janz et al., 2010). Assessing the impact and bioaccumulation of Se in aquatic systems is complicated by the fact that it can occur as several chemical species; that is, selenite, Se(IV), selenate, Se(VI), and a variety of organic forms, Se(-II) to Se(+II) (Maher et al., 2010; Wallschläger and Feldmann, 2010; Winkel et al., 2012). Organisms in aquatic systems take up some Se species more readily than others, although there is a lack of consensus as to their relative bioavailability. Thus, in laboratory studies algae are reported to either take up: organic Se (organo-Se) more rapidly than inorganic Se (Besser et al., 1993; Fournier et al., 2006; Riedel et al., 1991), organo-Se and inorganic Se at similar rates (Baines et al., 2001), or selenate in preference to organo-Se (Neumann et al., 2003). Such differences are crucial for aquatic food webs because Se concentrations in consumers tend to be "set" by Se uptake at the base of the food web (Presser and Luoma, 2010; Stewart et al., 2010). Overall, Se speciation and bioaccumulation are better understood in marine systems than in fresh waters (Stewart et al., 2010).

We set out to measure the relative importance of various Se species in lakewater and to estimate their availability to planktonic food webs. Since dissolved Se is taken up by planktonic algae and bacteria, but less so by plankton-feeding invertebrates or fish (Luoma and Presser, 2009; Stewart et al., 2010), it would be logical to measure Se concentrations in organisms at the base of the food web. However, in practical terms, this is very difficult because plankton hauls include a variety of non-living inorganic and organic particles as well as living algae and zooplankton. The alternative is to measure Se in plankton-feeding animals. Indeed, herbivores are reported to assimilate the majority of the Se present in algae (Rainbow and Wang, 2001; Schlekat et al., 2004) and their Se is efficiently passed on to predatory invertebrates (Dubois and Hare, 2009) and higher consumers such as fish and aquatic birds (Baines et al., 2002; Presser and Luoma, 2010; Stewart et al., 2010).

An ideal animal for evaluating Se availability in planktonic food webs in lakes is one that depends solely on plankton for food (most adult freshwater fish do not) and that is large enough to be easily isolated in plankton samples (most micro-crustaceans are not). In addition, the animal should be tolerant of trace elements, such that it is present in even highly contaminated lakes, as well as being widespread, common and easily identified to species (Luoma and Rainbow, 2008). Larvae of the phantom midge *Chaoborus* are reported to meet these criteria and for this reason have proven their usefulness for monitoring dissolved cadmium (Hare and Tessier, 1996) and nickel (Ponton and Hare, 2009) in lakewater. Since *Chaoborus* larvae are reported to take up substantial proportions of these metals from their planktonic food (Munger et al., 1999; Ponton and Hare, 2010), this is likely the case for Se as well. Indeed, most aquatic animals are reported to take up the majority of their Se from the diet (Luoma and Presser, 2009; Stewart et al., 2010). One potential caveat is that some *Chaoborus* species spend the day in sediment to avoid predation by fish (Carter and Kwik, 1977; Hare and Carter, 1986). However, since larvae are not active while in sediment (Gosselin and Hare, 2003) their Se must come from planktonic prey. We note that metal concentrations in *Chaoborus* larvae are reported to respond rapidly to those in their surroundings (Cd - Croteau et al., 2001; Ni - Ponton and Hare, 2010), which suggests that their Se concentrations are also likely to be in approximate steady state with those in their prey.

To achieve our goals, we collected water and *Chaoborus* larvae from 16 lakes situated at various distances from two metal smelters in eastern Canada and measured Se concentrations in these media. Since Se is an essential element for aquatic animals (Janz et al. 2010), we first determined if Se concentrations in *Chaoborus* vary among lakes or if this insect maintains its Se concentrations at a constant level. We then compared concentrations of total dissolved Se to those in *Chaoborus* larvae to determine if they were correlated. Lastly, we measured Se speciation in lakewater using techniques that are sensitive enough to allow accurate determinations of Se in uncontaminated lakewater (Chen et al., 2005a,b). Using these data, we determined which dissolved Se species best predicted Se concentrations in *Chaoborus* larvae.

Methods

Study sites and sampling dates

We collected water samples and *Chaoborus* larvae in late May and early June at a total of 17 stations in 16 lakes (Table 1) located on the Precambrian Canadian Shield in the mining areas of Sudbury, Ontario, and Rouyn-Noranda, Quebec. We sampled 6 of the study lakes in both 2010 and 2011 to measure inter-annual variations in spring Se concentrations. Selenium inputs to our study lakes are likely to vary depending on their proximity to smelters, mine tailings (Lakes Arnoux, Rouyn and Kelly) and sewage treatment systems (Kelly Lake and Lake Rouyn).

Chaoborus sampling

We collected *Chaoborus* larvae at night by hauling a 164-µm mesh-aperture plankton net horizontally in the water column. Larvae were held in lakewater at field temperatures for transport to the laboratory where they were sorted according to species (Saether, 1972). Where numbers permitted, 5 samples of each *Chaoborus* species were prepared by pooling 10-20 similar sized fourth-instar (Carter and Kwik, 1977) larvae in each sample. These samples were placed on acid-washed, preweighed, pieces of Teflon sheeting held in acid-washed microcentrifuge tubes and frozen at -20 ^{o}C. The maximum delay between collection and freezing was 1 day.

Lakewater sampling

We used diffusion samplers to collect lakewater by *in situ* filtration. In the laboratory, the acid-cleaned, Plexiglas, diffusion samplers were filled with ultrapure water and covered with a 0.2 μm pore-size polysulfone membrane (Gelman HT-200) before being sealed individually in acid-rinsed plastic bags. In each lake, three diffusion samplers were anchored 1 meter below the surface, that is, in the epilimnion where *Chaoborus* larvae feed on zooplankton (Carter and Kwik, 1977; Hare and Carter, 1986). Equilibration times in lakewater varied depending on the volume of sampler cells, that is, from 3 d (4 mL cells) to 10 d (125 and 250 mL cells; our unpublished data showed that 5 d is sufficient). Water samples were removed immediately upon retrieval of the samplers.

Lakewater pH was measured in the water samples and at the lake surface. A 4 mL aliquot was removed for dissolved organic carbon (DOC) determination by piercing the peeper membrane with a NaOH washed (0.005 M) plastic-tipped pipette then injecting the contents into a dark glass bottle that had been washed in NaOH, rinsed, heated to 400 ^{o}C and rinsed again with ultrapure water (ELGA; [DOC] < 0.005 mg L^{-1}). Samples (4 mL) for anions (Cl^{-}, NO_3^{-}, SO_4^{2-}) were removed using a pipette with an unused plastic tip that had been rinsed in ultrapure water. These samples were injected into new High Density Polyethylene (HDPE) bottles (4 mL capacity) that had been rinsed with ultrapure water. The water to be used for Se speciation measurements (a minimum of 30 mL) was stored in acid-washed HDPE or polypropylene bottles, on ice, for a maximum of 10 days to prevent changes in selenium speciation. Wang (1994) showed that there were no measureable losses of selenite, selenate or selenomethionine following storage of purified tap-water for 15 days at 4 ^{o}C. Since most bacteria and algae exceed the pore size of the sampler membrane (0.2 μm), they could not have influenced Se speciation in the water samples.

Selenium measurements in insect larvae

Frozen *Chaoborus* larvae were freeze-dried (FTS Systems) and weighed using a microbalance (Sartorius M2P PRO 11). Dried larvae were placed in acid washed HDPE bottles where they were digested for 2 days in concentrated HNO_3 (Aristar grade; 100 μL per mg sample dry weight) followed by 1 day in concentrated 30% (w/w) hydrogen

peroxide (Trace Select Ultra grade; 40 µL per mg sample dry weight); digestate volume was brought up to 1 mL per mg sample dry weight using ultrapure water. Certified reference material (lobster hepatopancreas, TORT-2, National Research Council of Canada) was subjected to the same digestion procedure. Selenium of mass 82 g mol^{-1} (^{82}Se) was measured using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Thermo Elemental X Series) and interferences with bromine ($^{81}Br + {}^{1}H$) were corrected using a standard curve of several Br concentrations. Selenium in the reference material (5.7 ± 0.1 (SD) µg g^{-1}; $n = 4$) was within the certified range (5.6 ± 0.7 (95% CI) µg g^{-1}). The detection limit of the ICP-MS for Se was 200 ng L^{-1}.

Selenium speciation in lakewater

We measured Se speciation in lakewater by hydride generation of selenite followed by detection using atomic fluorescence spectrometry (HG-AFS; Millenium Excalibur System, PS Analytical) in the laboratory of N. Belzile and Y. Chen at Laurentian University in Sudbury, Ontario. These researchers (Chen et al., 2005a,b) developed the speciation method, which they describe as being simple, precise and not subject to interference due to other elements. It is based on the transformation of the various Se species into selenite, which is then measured by HG-AFS. Quality assurance and quality control data for measurements of Se speciation in lakewater are given in Chen et al. (2005a,b).

First, selenite is measured by acidifying a 10 mL subsample of lakewater with HCl to a final concentration of 3.0 M (Cutter, 1978). A second subsample (20 mL) is acidified using HNO_3 and HCl to a final matrix of 1 and 2%, respectively (volume/volume), and exposed to ultraviolet radiation (wavelength of 300 nm) for 2 hours in a quartz tube sealed with Parafilm. Irradiated samples are cooled on ice for 1 hour and then acidified to 3.0 M with HCl. This UV treatment creates OH radicals that oxidize organo-Se to selenite, and oxidation of selenite to selenate is prevented by HCl. Chen et al. (2005b) describe in detail the photochemical mechanisms involved and show that four types of organo-Se are completely transformed to selenite using this method. Subtracting the first measurement (Se(IV)) from the second (Se(IV) + organo-Se) gives the organo-Se concentration.

The remaining 10 mL of UV-treated sample is acidified to 3.0 M with HCl and submitted to a microwave digestion in closed vials (MicroSYNTH, Milestone; MLS-easyWAVE 3.5.4.0 software) at 110 ^{o}C for 15 minutes to reduce selenate to selenite. The samples are then cooled on ice before measuring total Se. Using this method, Chen et al. (2005a) reported 91% recovery of selenate spiked into lakewater. The concentration of selenate is obtained by subtracting selenite + organo-Se (post UV) from the concentration of total Se (post microwave). For both subtractions, mean values less that the detection limit (21 ng L^{-1}; 3 times the SD of the mean [Se] in 10 blanks) were set as half the detection limit, that is, 10.5 ng L^{-1}.

Other analyses

Concentrations of anions (Cl, NO_3, SO_4) in lakewater were measured by ion chromatography (Dionex, system ICS-2000; AS-18 column). Dissolved organic carbon was measured by a Shimadzu TOC-5000A after transformation to CO_2.

Results and discussion

Selenium concentrations and speciation in lakewater

Total dissolved Se concentrations in lakewater varied by almost two orders of magnitude among our study lakes (Table 1). Values in the Rouyn-Noranda area varied from 35 to 1623 ng L^{-1}, whereas those in the Sudbury area varied from 167 to 3063 ng L^{-1} (Table 1). These wide ranges confirm the existence of strong Se gradients in both regions (Belzile et al., 2009; MDDEP, 2011). Highest values were measured in lakes influenced by drainage from both metal smelters and municipal sewage-treatment plants, that is, Kelly Lake (Sadiq et al., 2002) and Lake Rouyn (MDDEP, 2011). However, since sewage is not considered to be a major source of Se (Lemly, 2004; Maher et al., 2010; Winkel et al., 2012), the high dissolved Se values for these two lakes are likely the result of effluents produced by metal smelting and refining. Indeed, Se concentrations in the decantation pond at the metals smelter in Rouyn-Noranda are reported to reach 106,000 ng L^{-1} and these waters drain into Lake Rouyn (MDDEP, 2011).

Selenium concentrations in uncontaminated to moderately-contaminated lake waters are reported to range from 30 to 1,520 ng L^{-1} (Conde and Sanz Alejos, 1997), and

aqueous Se concentrations above 200 ng L^{-1} are considered to be contaminated (Luoma and Rainbow, 2008). Based on this threshold value, Se-contaminated lakes occur in both of our study areas.

Since several Se species are usually present in aquatic systems, and because algae at the base of the food chain can take up the various Se species at different rates (Riedel et al., 1991; Neumann et al., 2003) we measured Se speciation in our study lakes. We note that in two consecutive springs (2010 and 2011; Table 1) the concentrations of each Se species were significantly correlated between years and that the overall relationship between years for the three species was [Se] species 2010 = 1.12*[Se] species 2011 (r^2 = 0.93; $p < 0.0001$; $n = 20$; regression not shown), which suggests that year to year changes in springtime Se speciation were minor.

Table 1. Mean (±SD; n = 3) pH and concentrations of dissolved: organic carbon (DOC), sulfate (SO_4), selenite, organo-Se, selenate, and total Se in water collected from lakes in the mining regions of Rouyn-Noranda (RN) and Sudbury (Sud.). Also shown are mean Se concentrations (±SD; n = 1-5) in *Chaoborus* larvae collected from these lakes: n.p.: not present, [a] *Chaoborus albatus*, [f] *Chaoborus flavicans*, [p] *Chaoborus punctipennis.* Lakes are listed in order of increasing total dissolved Se concentrations.

Year	Region	Lake	Location	pH	DOC mg L^{-1}	SO_4 mg L^{-1}	selenite ng L^{-1}	organo-Se ng L^{-1}	selenate ng L^{-1}	Total Se ng L^{-1}	Se *Chaoborus* µg g^{-1}
2010	RN	Arnoux 2	48°14'N, 79°18'W	3.8	0.1 ± 0.1	42.2 ± 0.2	< 21	< 21	25 ± 5	35 ± 5	n.p.
2010	RN	Duprat	48°20'N, 79°07'W	7.6	2.9 ± 0.2	3.2 ± 0.2	40 ± 10	55 ± 3	< 21	91 ± 31	[p] 1.0 ± 0.2
2011	RN	Arnoux 4	48°14'N, 79°21'W	6.3	3.6 ± 0.1	33.0 ± 1.1	< 21	< 21	86 ± 33	107 ± 38	[p] 2.3 ± 0.7
2011	RN	Dasserat	48°14'N, 79°22'W	6.6	4.8 ± 0.0	27.4 ± 0.1	< 21	37 ± 1	73 ± 15	122 ± 16	[p] 2.9
2011	Sud.	Tilton	46°22'N, 81°04'W	7.1	3.2 ± 0.4	7.3 ± 0.1	< 21	136 ± 6	< 21	167 ± 11	[p] 4.6 ± 0.3
2011	Sud.	Lohi	46°23'N, 81°02'W	7.5	3.1 ± 0.1	8.4 ± 0.1	< 21	159 ± 13	25 ± 15	195 ± 26	[p] 3.5 ± 0.2
2010	Sud.	McFarlane	46°25'N, 80°57'W	8.0	3.6 ± 0.2	16.4 ± 0.2	152 ± 35	65 ± 27	< 21	225 ± 18	[f] 2.1 ± 0.2
2011	Sud.	Pine	46°22'N, 81°02'W	5.1	2.1 ± 0.2	7.0 ± 0.1	< 21	82 ± 23	142 ± 32	234 ± 23	[a] 4.3 ± 0.9
2011	Sud.	Crooked	46°22'N, 81°02'W	6.5	4.2 ± 0.3	10.7 ± 0.1	< 21	241 ± 8	32 ± 20	284 ± 26	[p] 5.3 ± 0.5
2011	Sud.	Silver	46°24'N, 80°57'W	7.0	3.4 ± 0.3	29.8 ± 0.1	30 ± 14	273 ± 24	27 ± 8	331 ± 29	[p] 5.9 ± 0.3
2010	Sud.	Raft	46°22'N, 81°03'W	7.3	2.2 ± 0.2	7.9 ± 0.1	70 ± 54	171 ± 59	48 ± 16	340 ± 6	[p] 3.4 ± 0.2
2010	RN	Dufault	48°18'N, 79°00'W	7.6	3.8 ± 0.2	28.5 ± 0.5	174 ± 21	234 ± 6	< 21	415 ± 34	[p] 3.8 ± 0.2
2010	Sud.	Crooked	46°22'N, 81°02'W	6.7	5.1 ± 0.1	7.1 ± 0.1	39 ± 0	416 ± 43	< 21	438 ± 14	[p] 6.5 ± 0.1
2011	RN	Dufault	48°18'N, 79°00'W	7.5	5.3 ± 0.2	24.7 ± 0.1	124 ± 7	284 ± 19	41 ± 18	450 ± 7	[p] 5.6 ± 0.1
2011	Sud.	Hannah	46°26'N, 81°02'W	7.9	3.6 ± 0.2	12.2 ± 0.3	141 ± 8	314 ± 13	39 ± 51	486 ± 58	[p] 5.6 ± 1.1
2010	Sud.	Hannah	46°26'N, 81°02'W	7.9	3.5 ± 0.4	12.9 ± 0.2	252 ± 18	279 ± 5	< 21	506 ± 24	[p] 4.9 ± 0.4
2010	RN	Osisko	48°14'N, 79°00'W	8.5	2.3 ± 0.1	36.7 ± 0.1	314 ± 50	146 ± 47	75 ± 86	536 ± 91	[p] 5.2 ± 0.2
2011	RN	Osisko	48°14'N, 79°00'W	7.8	2.7 ± 0.2	34.7 ± 0.3	203 ± 16	209 ± 31	150 ± 53	541 ± 97	[p] 4.1 ± 0.7
2011	RN	Pelletier	48°12'N, 79°03'W	7.8	4.4 ± 0.1	49.0 ± 0.2	246 ± 6	251 ± 1	72 ± 83	569 ± 78	[f] 4.9 ± 0.3
2010	RN	Pelletier	48°12'N, 79°03'W	8.3	3.7 ± 0.9	51.0 ± 4.0	275 ± 67	244 ± 35	83 ± 31	602 ± 123	[f] 5.3 ± 0.3
2011	RN	Rouyn	48°14'N, 78°56'W	7.4	4.4 ± 0.1	186.9 ± 2.3	879 ± 28	244 ± 49	316 ± 52	1440 ± 51	[f] 10.6 ± 0.6
2010	RN	Rouyn	48°14'N, 78°56'W	8.0	3.5 ± 0.2	172.5 ± 10.6	854 ± 82	362 ± 199	407 ± 187	1623 ± 139	[f] 11.5 ± 1.0
2011	Sud.	Kelly	46°27'N, 81°03'W	8.5	5.1 ± 0.4	406.0 ± 3.5	1616 ± 30	528 ± 52	918 ± 209	3063 ± 201	[f] 12.8 ± 0.2

The dominant dissolved Se species varied among our 16 study lakes, that is, concentrations of selenite were highest in 3 lakes (McFarlane, Rouyn, Kelly), those of selenate were highest in 3 other lakes (Arnoux, Dasserat, Pine), and those of organo-Se were highest in 7 lakes (Table 1). Selenite and organo-Se concentrations were approximately equal in Lakes Hannah and Pelletier, whereas concentrations of the three Se species were roughly equivalent in Lake Osisko (Table 1). Lake waters in which selenate dominated ($\geq$ 50%) had pHs $\leq$ 6.6, whereas those in which selenite dominated had pHs $\geq$ 7.4 (Table 1). Three of the four sites with pHs < 6.6 (Arnoux 2 and 4, Dasserat) are situated downstream from abandoned mine tailings (Cyr, 2008), and Se enters this system (at AR-2) as selenate (Table 1). Few comparable data on Se speciation in lakes have been reported.

Selenium concentrations in the insect Chaoborus

To estimate the bioavailability of dissolved Se in our study lakes, we measured Se concentrations in the planktivore *Chaoborus*, which is likely to take up this element via its planktonic food. Selenium concentrations in aquatic organisms are generally recognized as being more useful predictors of Se bioavailability and toxicity than are dissolved Se concentrations (Janz et al., 2010; Luoma and Presser, 2009; Stewart et al., 2010).

In about two-thirds of our study lakes, *Chaoborus* (*Sayomyia*) *punctipennis* was the only *Chaoborus* species present (Table 1). Indeed, it is reported to be common in eastern Canadian lakes in which fish are present (Garcia and Mittelbach, 2008; Hare and Tessier, 1998). In Pine Lake, we collected its sister species *Chaoborus* (*Sayomyia*) *albatus*; the two species are so similar in size and morphology that they are often confounded in ecological studies (Norman Yan, York University, Toronto, personal communication) and their concentrations of Cd (Croteau et al., 2001) and Ni (Ponton and Hare, 2009) are usually the same. In four other study lakes *Chaoborus* (*Chaoborus*) *flavicans* was the only species present. It is larger in size (Croteau et al., 2001) and is reported to have somewhat higher concentrations of Cd (Croteau et al., 2001; Hare and Tessier, 1998) and Ni (Ponton and Hare, 2009) than its two congeners. Likewise, its Se

concentrations were higher than those of *C. punctipennis* by a mean of 0.5 ± 0.2 (SD) μg g^{-1} dry weight in 6 of our 8 study lakes (Ponton and Hare, unpublished data from 2007). Since this difference represents only about 4 to 7% of the Se concentrations of *C. flavicans* in the high-Se lakes (Kelly and Rouyn), we conclude that data for this species can be combined with those of the other *Chaoborus* species for the purposes of estimating Se bioavailability. The small differences in Se concentrations among the study species within a given lake suggest that *Chaoborus* species can be pooled when using them as Se biomonitors thereby avoiding the need to sort them in the field.

Selenium concentrations in *Chaoborus* larvae varied widely among our study lakes (Table 1), which shows that this insect is unable to maintain constant its concentrations of this essential element at the range of Se concentrations measured in these lakes. In contrast, this insect maintains stable its internal Cu and Zn concentrations over wide ranges of dissolved concentrations of these essential metals (Croteau et al., 1998). A lack of Se regulation at the whole animal level has also been reported for freshwater oligochetes and insects (Andrahennadi et al., 2007; Conley et al., 2009; Dubois and Hare, 2009), as well as for many aquatic mollusks, fish and birds (Luoma and Presser, 2009). Wide variations in *Chaoborus* Se concentrations are a prerequisite for using this insect as a biomonitor of Se concentrations in planktonic food webs.

Selenium concentrations in *Chaoborus* larvae varied from 1.0 to 12.8 μg g^{-1} dry weight in our study lakes (Table 1). Since Se concentrations between 3 and 8 μg g^{-1} dry weight in the food of some fish species are reported to cause reproductive effects (Skorupa et al., 1996; Lemly, 2002; May et al., 2008), a diet of *Chaoborus* larvae from some of our study lakes could be potentially toxic to fish. Furthermore, Se concentrations in pelagic copepods from our study lakes were approximately twice those of *Chaoborus* larvae (Ponton and Hare, unpublished data), which implies an even greater potential risk to fish.

Comparison of selenium concentrations in water and in Chaoborus

Selenium concentrations in *Chaoborus* were significantly and strongly (r^2 = 0.88) correlated with those of total dissolved Se (Fig. 1A). The curvilinear form of this

relationship is largely a consequence of the two high-Se lakes (Kelly (KE) and Rouyn (RO)). When these lakes are removed, the relationship is more linear (Fig. 2A; $r^2 = 0.46$). This linear relationship is strengthened by considering organo-Se alone ($r^2 = 0.70$; Fig. 2B), but is no longer significant ($p > 0.05$) if either of the inorganic species is used on their own (Fig. 2C, D). These trends suggest that, with the exception of the high-Se lakes, dissolved organo-Se concentrations best explain Se concentrations in *Chaoborus* larvae. The results of several laboratory studies also suggest that organic forms of Se (e.g., selenomethionine) are more bioavailable than are their inorganic counterparts (Besser et al., 1993; Fournier et al., 2006; Franz et al., 2011; Riedel et al., 1991).

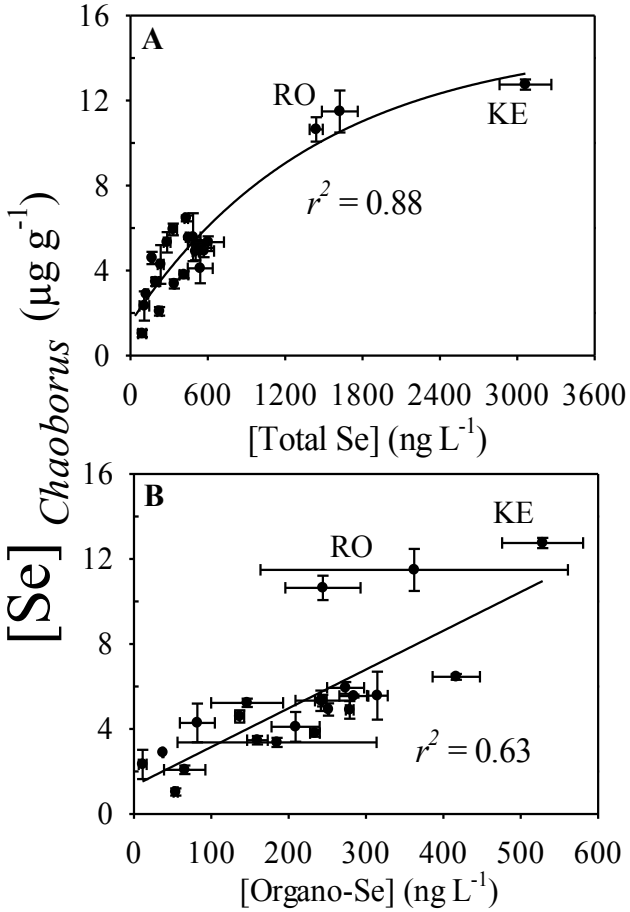

Figure 1. Relationships ($p < 0.05$) between mean (±SD; n = 1-5) Se concentrations in *Chaoborus* larvae ($[Se]_{Chaoborus}$; µg g^{-1} dry weight) and mean (±SD; n = 3) concentrations (ng L^{-1}) of dissolved (A) total selenium or (B) organo-Se in lakewater. KE, Kelly Lake; RO, Lake Rouyn.

When the high-Se lakes are included, dissolved organo-Se concentrations underestimate Se concentrations in *Chaoborus* larvae from these two lakes (Fig. 1B). This result suggests that concentrations of inorganic Se explain in part the Se concentrations in *Chaoborus* from the high-Se lakes, which is consistent with the fact that > 75% of their total dissolved Se is inorganic (Table 1).

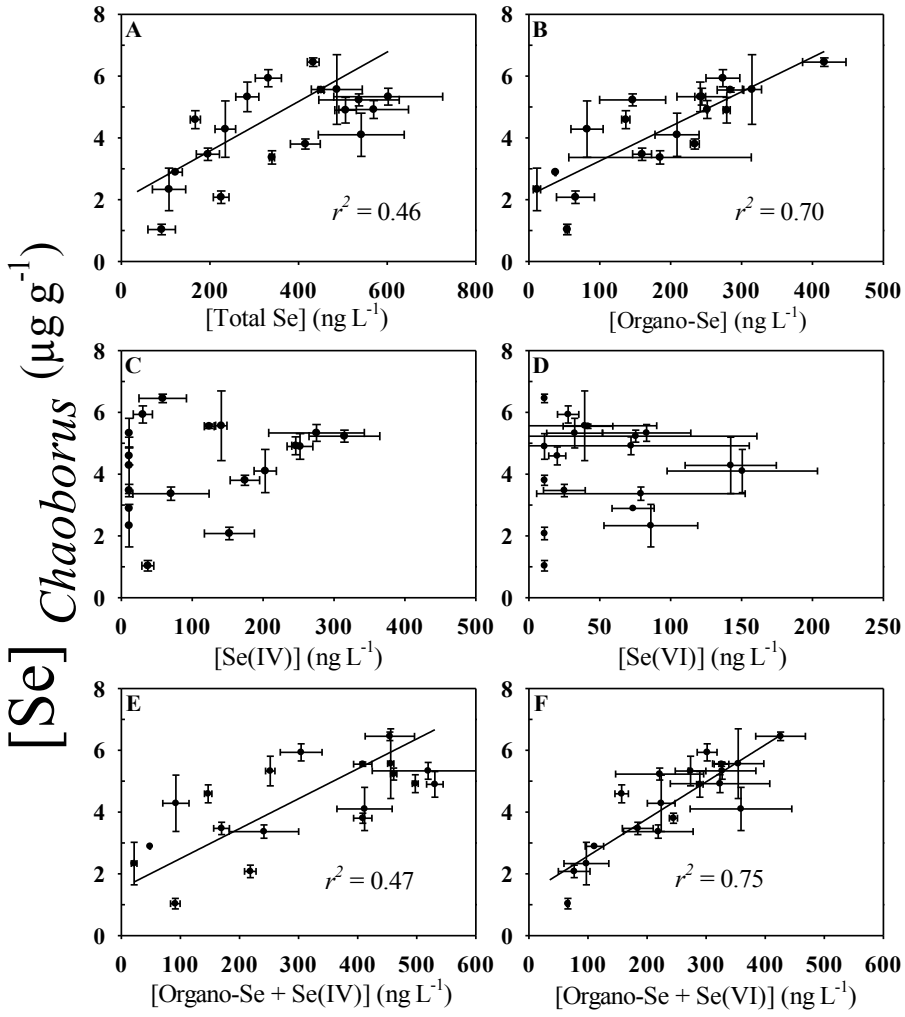

Figure 2. Relationships, without the high-Se lakes (Kelly and Rouyn), between mean (±SD; n = 1-5) Se concentrations in *Chaoborus* larvae ($[Se]_{Chaoborus}$; µg g^{-1} dry weight) and mean (±SD; n = 3) concentrations (ng L^{-1}) of: (A) total dissolved Se ($p < 0.05$), (B) organo-Se ($p < 0.05$), (C) selenite, Se(IV) ($p > 0.05$), (D) selenate, Se(VI) ($p > 0.05$), (E) organo-Se plus selenite ($p < 0.05$), (F) organo-Se plus selenate ($p < 0.05$).

Adding the concentrations of dissolved selenite to those of organo-Se (excluding the two high-Se lakes) weakened the correlation (Fig. 2E; r^2 = 0.47) between Se concentrations in *Chaoborus* and in lakewater. This result belies the fact that algae are reported to accumulate selenite in the laboratory (Besser et al., 1993). This apparent contradiction could be a consequence of the fact that whereas pH does not vary in such laboratory exposures, it did vary widely among our study (3.8-8.5; Table 1). Thermodynamic calculations indicate that although the speciation of selenate (pK_a = 1.7; MINEQL+) would not change over the pH range of our lakes, selenite (pK_a = 8.4; MINEQL+) is likely to be present as two species: $HSeO_3^-$ and SeO_3^{2-} with the former (solid curve in Fig. 3) being dominant at low pHs and the latter (broken curve in Fig. 3) being dominant at high pHs. The proportion of total dissolved selenite (open symbols in Fig. 3) tends to track the rise of SeO_3^{2-} and the percentage of organo-Se tends to follow those of $HSeO_3^-$. If these forms of selenite differ in their availability to algae, this could explain in part why trends in our field data were not explained by considering total dissolved selenite. This hypothesis should be tested in the laboratory.

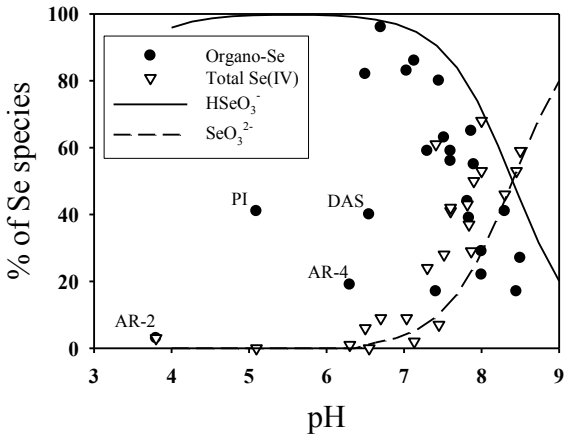

Figure 3. Predicted percentages of selenite as $HSeO_3^-$ (solid curve) and as SeO_3^{2-} (broken curve) compared to those of organo-selenium (closed circles) and total dissolved selenite (Se(IV); open triangles) measured in lakewater at various pHs. Curves for $HSeO_3^-$ and SeO_3^{2-} were obtained by inorganic speciation modeling using MINEQL+ (Environmental Research Software, USA) with total dissolved selenite concentrations and pH as input. AR, Lake Arnoux; DAS, Lake Dasserat; PI, Pine Lake.

In contrast, adding selenate to organo-Se strengthened somewhat the relationship between Se concentrations in *Chaoborus* and in lakewater (r^2 = 0.75; Fig. 2F; excluding the two high-Se lakes). This correlation was strengthened further (r^2 = 0.89) by including high-Se Lake Rouyn (Fig. 4), which suggests that in most Canadian Shield lakes Se concentrations in *Chaoborus* larvae could be used to estimate the sum of dissolved organo-Se plus selenate concentrations in lakewater. The availability of selenate to these planktonic food webs is consistent with results obtained in the laboratory in which the green alga *Chlorella* accumulates selenate at much higher rates than it does selenite (from 10 to 100x, Simmons and Wallschläger (2011) and Neumann et al. (2003), respectively).

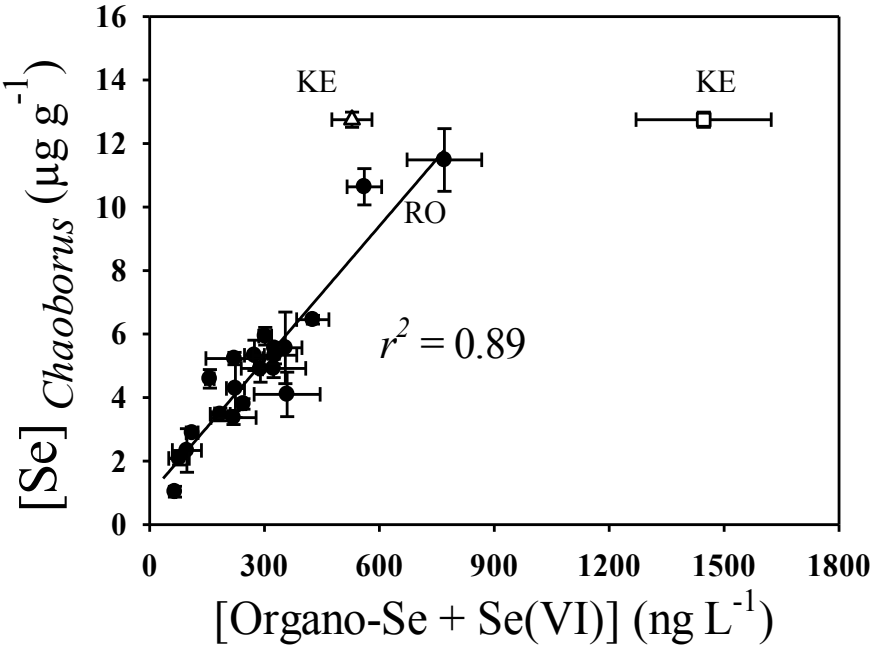

Figure 4. Relationship (p < 0.05) between mean (±SD; n = 1-5) Se concentrations in *Chaoborus* larvae ($[Se]_{Chaoborus}$; µg g^{-1} dry weight) and the sum of the mean (±SD; n = 3) concentrations (ng L^{-1}) of dissolved organo-Se and selenate (Se(VI)). Data for Kelly Lake (not included in regression) are presented in terms of both organo-Se (open triangle) and organo-Se plus selenate (open square). KE, Kelly Lake; RO, Lake Rouyn.

The fact that Kelly Lake does not fit the trend for our other study lakes (Fig. 4) can be explained in two ways that are not mutually exclusive. First, in the laboratory, Se concentrations have been shown to differ widely among algal groups and species (Baines and Fisher, 2001; Riedel et al., 1991). Thus if the phytoplankton community of Kelly

Lake was dominated by algal groups that accumulate less Se than those which dominate the other lakes, this would lead to lower Se concentrations in consumers such as *Chaoborus*. We did not measure Se concentrations in algae to test this hypothesis due to the aforementioned difficulty in isolating living phytoplankton from other similar-sized non-living and living particles in lakewater. The fact that Kelly Lake has been the recipient of municipal sewage has undoubtedly influenced the composition of its phytoplankton community. However it is not unique in this respect, since Lake Rouyn has also been contaminated by municipal sewage.

Second, sulfate concentrations in Kelly Lake were approximately double those of Lake Rouyn and were, at a minimum, 8 times those in the other study lakes (Table 1). Since sulfate can compete with selenate at Se-uptake sites on algae (Fournier et al., 2010; Neumann et al., 2003; Simmons and Emery, 2011), the high sulfate concentrations in Kelly Lake could have reduced Se concentrations in algae and thus those at higher trophic levels. A parallel can be drawn with the competitive influence of H^+ ions on Cd and Ni uptake by organisms in the food chain leading to *Chaoborus* (Orvoine and Hare, 2006; Ponton and Hare, 2009). We note, however, that the competitive influence of higher sulfate concentrations in Kelly Lake may be offset by the higher selenate concentrations in this lake (approximately double those of Lake Rouyn; Table 1), which could explain the similarity in *Chaoborus* Se concentrations between these two lakes. Clearly, laboratory experiments would be needed to elucidate the influence of selenate-sulfate competition on Se bioaccumulation under our field conditions.

Overall, *Chaoborus* larvae are likely to be an effective sentinel for monitoring Se exposure and concentrations in lake planktonic food webs given: the strength of correlations between their Se concentrations and those in lakewater; their wide geographical distribution (we collected them in all of the lakes that we sampled); their ease of collection and identification to species; their large size, compared to other zooplankton, which facilitates Se measurements; their complete dependence on plankton as food (Gosselin and Hare, 2003), in contrast to many field species that change their diet as they grow; and their tolerance of Se (they were present in our high-Se lakes).

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DEUXIÈME ARTICLE

INFLUENCE OF SE SPECIATION, SULFATE AND PH ON SE ACCUMULATION BY PLANKTON AND THE PREDICTION OF SE IN PELAGIC ORGANISMS

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Environmental Context

We show that Se concentrations in planktonic organisms can be predicted by considering the chemical speciation of this element along with sulfate concentrations in lakewater.

Abstract

Selenium concentrations in planktonic food chains (microplankton < 64 μm, copepods and *Chaoborus* larvae) were strongly correlated to the sum of dissolved organic selenide and selenate concentrations in lakewater. To explain these field observations, we exposed the green alga *Chlamydomonas reinhardtii* to selenite, selenate or selenomethionine at various H^+ ion and sulfate concentrations under controlled laboratory conditions. At very low sulfate concentrations, inorganic Se species (selenate >> selenite) were more readily accumulated by this alga than was organic Se. However, at slightly higher sulfate concentrations the uptake of inorganic Se was greatly reduced whereas that of selenomethionine was unaffected. The pH of the exposure water influenced the uptake of selenomethionine and selenite by this alga. Overall, Se concentrations in this alga were 30 times lower than those measured in field-collected microplankton or in field microplankton exposed to Se in the laboratory. This difference is likely explained by the presence of bacteria in the field samples. We used our laboratory measurements of Se uptake to successfully model Se concentrations in pelagic food chains.

Introduction

Aquatic systems can be contaminated by selenium (Se) from agricultural irrigation, metal mining and refining, as well as fossil fuel extraction and combustion (Lemly, 2004). In some regions of California and North Carolina, high Se concentrations have caused deformities in fish and aquatic birds (Lemly, 1993; Presser and Ohlendorf, 1987). Aquatic animals take up Se mainly from their food (Luoma and Rainbow, 2008) and thus their Se concentrations are determined in large part by those of organisms at the base of food webs (e.g., algae and bacteria in planktonic food chains; Stewart et al., 2010), which in turn are controlled by Se speciation in water.

The chemical speciation of Se in freshwater varies depending on the contamination source as well as the retention time and chemistry of the water body (Bowie, 1996; Ponton and Hare, 2013). Selenium in natural waters is present in several oxidation states including selenate (Se(VI)), selenite (Se(IV)) and organic selenide (Se(-II) to Se(+II)). The most common forms of organic selenide in natural waters are selenomethionine (Se-Met) and selenocysteine (Conde and Sanz Alejos, 1997). In the laboratory, investigators have reported either greater bioaccumulation of selenate than selenite (Neumann et al., 2003; Simmons and Wallschläger, 2011), or no difference in bioaccumulation between these inorganic species (Conley et al., 2013; Fournier et al., 2006), or greater selenite accumulation compared to selenate (Luoma and Rainbow, 2008). Although there are exceptions (Baines et al., 2001), organo-Se is generally thought to be the Se species with the highest accumulation potential (Chapman et al., 2010; Besser et al., 1993; Fournier et al., 2006). In the field, Ponton and Hare (2013) reported that the sum of organic selenide and selenate concentrations in lakewater best predicted those in larvae of the phantom midge *Chaoborus*, although high sulfate concentrations reduced the uptake of the inorganic Se species. In the laboratory, sulfate is also reported to reduce the accumulation of selenite (Morlon et al., 2006) and selenate (Fournier et al., 2010) by algae. Likewise, pH could influence selenite bioaccumulation by changing its protonation state (Ponton and Hare, 2013; Riedel and Sanders, 1996; Morlon et al., 2006). Understanding the influence of Se speciation on Se bioaccumulation in the field is

clearly important for the development of a biotic ligand model for Se that could be used in risk assessments for Se in aquatic ecosystems.

To better understand the influence of Se speciation on Se bioaccumulation, we compared the accumulation of selenite, selenate and organic selenide at various H^+ ion and sulfate concentrations by the freshwater green alga *Chlamydomonas reinhardtii*. We used this algal species because it is tolerant of high Se concentrations (Morlon et al., 2005), easy to culture and grows over a wide range in H^+ concentrations (Lustigman and Lee, 1995). To determine if our laboratory results for one algal species are representative of Se accumulation by mixed plankton in the field, we also exposed field-collected microplankton (< 64 μm) to various Se species under conditions that were similar to those used for *C. reinhardtii*. Lastly, we used these measurements to predict Se concentrations in a lake planktonic food chain comprising microplankton, zooplankton (copepods) and their predators, that is, larvae of the insect *Chaoborus* and young of the year yellow perch (*Perca flavescens*). These predators have been used as trace element biomonitors in lake food webs because they are tolerant to trace elements and thus persist in many lakes impacted by emissions from metal smelters (Campbell et al., 2003; Hare et al., 2008; Ponton and Hare, 2009; Ponton and Hare, 2013).

Methods

Study Sites and Field

Collections. We collected microplankton (< 64 μm), crustacean zooplankton (copepods) and larvae of the phantom midge *Chaoborus* in late May and early June in 2010 and 2011 from 12 to 18 lakes located on the Precambrian Shield in the mining areas of Sudbury (Ontario) and Rouyn-Noranda (Quebec), Canada. Lake locations and information on their Se sources and the measurement of dissolved Se speciation and water chemistry are given in Ponton and Hare (2013). Uncontaminated microplankton was collected from Lake Bédard ($47^o16'N,71^o07'W$), which is located 70 kilometers north of Quebec City (Quebec, Canada) on the Precambrian Shield.

Microplankton Collection

We used a 2-meter long, 3-cm diameter, plastic tube to collect integrated samples of epilimnetic water in the center of each lake. These samples were immediately filtered through a 64 μm mesh-aperture plastic sieve, to remove macro-zooplankton, and put in a plastic container. In the laboratory, subsamples (500 mL glass bottles; 1% Lugol's; $n = 3$) were taken for the identification and counting of microplankton using an inverted microscope (Axiovert, 200; Axio Vision 3.0 software) and a flow cytometer (BD FACSCalibur, BD Biosciences). Cell volume and surface area were measured according to the methods of Hillebrand et al. (1999). Filtered lakewater (~40 L) was then centrifuged (Westfalia Separator LWA 205, Centrico Inc.) to concentrate microplankton. The centrifugation process was efficient since algal cell counts in centrifuged supernatant samples were 2 orders of magnitude lower than those prior to centrifugation. Three samples of microplankton were placed on acid-washed Teflon sheeting in an acid-washed 1.5 mL polypropylene centrifuge tube and frozen at -20 ^{o}C until drying, digestion and Se analysis.

Zooplankton Collection

We collected crustacean zooplankton and *Chaoborus* larvae at night by hauling a 64 μm mesh-aperture plankton net horizontally in the water column of each lake. Crustaceans were separated from *Chaoborus* larvae using a 0.5 mm mesh-aperture sieve and both were held in lakewater at field temperatures for transport to the laboratory.

Chaoborus larvae were sorted according to species (Saether, 1972), and 5 samples per lake were prepared by pooling 10-20 similar sized fourth-instars (Carter and Kwik, 1977) per sample. *Chaoborus punctipennis* was the only *Chaoborus* species present in most lakes. Exceptionally, in Lakes Kelly and Rouyn, *Chaoborus flavicans* was the only *Chaoborus* species present. Since Se concentrations in these two species are reported to be similar (Ponton and Hare, 2013), we assumed that Se data for the two could be combined.

Planktonic crustaceans were subject to a short period of anoxia to separate cladocerans from copepods by flotation, decantation and filtration on a 125 µm mesh-aperture plastic sieve. Three samples of copepods (final-instar *Chaoborus* larvae feed readily on copepods; Croteau et al., 2003) were placed on acid-washed, preweighed pieces of Teflon sheeting held in acid-washed, polypropylene, microcentrifuge tubes and frozen at -20 ^{o}C until drying and analysis. The maximum delay between collection and freezing was one day, during which time samples were stored at 4 ^{o}C.

Algal Cultures

All labware was soaked for 1 day in 15% HNO_3 (volume/volume; Omni Trace Grade) and rinsed 5 times with demineralized water and two times with ultrapure water (< 18 MΩ cm) to prevent inadvertent trace element contamination. An agar culture of *C. reinhardtii* (CPCC 11 wild strain) was obtained from the Canadian Phycological Culture Centre at the University of Waterloo, Waterloo, Ontario. Algae were grown in an axenic modified high-salt medium with an ionic strength of 8.4 $mEq \cdot L^{-1}$ (Fortin et al., 2004). One-liter Erlenmeyer flasks were sterilized using the culture medium (without trace elements) at 121 ^{o}C for 15 minutes in an autoclave (Sanyo). After sterilization and cooling, sodium hydroxide, trace elements and algae were added using sterilized pipet tips under a sterile Class 100 laminar flow hood. A sterile piece of cotton (USP Sterile Cotton Roll, U.S. Cotton) was put in the opening of each flask to prevent bacterial contamination. Cultures were held in an environmental growth chamber (Conviron, CMP3023) at 20 ± 0.2 ^{o}C under constant, cool-white, fluorescent lighting (100 $\mu Em^{-2}\ s^{-1}$) and flasks rotated at the rate of 50 rotations per minute. We used ~2 L of algae (separated into 6 1-L sterile Erlenmeyer flasks) growing exponentially for each experiment. One hour before each experiment, the 2 L of culture was centrifuged (Sorval RC50 Plus, Du Pont) twice at 4,000 x *g* for 5 minutes; algae were rinsed between centrifugations with Se-free exposure medium (see next section) and resuspended in about 100 mL of Se-free exposure medium. This rinsing step allowed the removal of algal exudates that can influence Se speciation. The concentrated algal suspension was counted immediately (Counter Multisizer III, Beckman Coulter). Microplankton from Lake Bédard that had been

concentrated by flow-through centrifugation was also resuspended in about 100 mL of Se-free exposure medium prior to inoculation of the exposure medium.

Algae and Microplankton Selenium Exposure

Algae were exposed to Se at a cellular density of about 125,000 cells mL^{-1} under the same light and rotation conditions as the stock culture. To compare these results with those obtained using natural microplankton, Se concentrations are given on a dry weight basis. At the end of the exposure periods, the Se concentration in the exposure medium had changed little (data not shown). Reagent-grade ACS salts were used to create the exposure medium, the final concentrations of which were: NH_4NO_3 (30 μM), KNO_3 (20 μM), $MgCl_2$ (80 μM), $CaCl_2$ (70 μM) and NaCl (100 μM). This exposure medium simulates the average concentrations of major ions in Rouyn-Noranda and Sudbury lakes (unpublished results). Experiments were carried out in 400 mL of exposure medium held in 1 L sterile, polypropylene, Erlenmeyer flasks (in triplicate, except where mentioned).

The nominal Se concentration of all Se species was 63 nM (5 μg L^{-1}), which is the U.S. criterion for the protection of wildlife in softwater (Luoma and Presser, 2009) and approximates Se concentrations in contaminated water bodies (Luoma and Rainbow, 2008). This concentration was not toxic to the algae since very high selenite concentrations (50 μM) are needed to reduce the growth of *C. reinhardtii* (Morlon et al., 2005) and plants are generally insensitive to Se (Chapman et al., 2010). The pH of the exposure medium was maintained constant using 5 mM trishydroxyméthylaminomethane buffer (TRIS, OmniPur Grade, EM Science), and adjustments were made using hydrochloric acid (HCl, AristarUltra Grade, VWR) to maintain the pH at 7.5 for experiments in which pH was not the studied variable. This buffer was used because it's effective pH range is 6.5 to 9.7. Sulfuric acid (ACS grade) was used to obtain the desired sulfate concentrations. The pH, exposure-time and sulfate concentrations used in each experiment are given in the figure titles.

Following Se exposure, algae were centrifuged two times (4,000 x *g* for 5 min) and rinsed between centrifugations with alkaline (pH 8.5), Se-free, exposure

medium to both remove Se adsorbed onto cell membranes and remaining exposure medium. Since inorganic Se is reported to adsorb less on particles at high pH (Lemly, 2004), we rinsed algae with an alkaline solution to remove adsorbed Se. Each algal pellet was removed using a micropipette and placed in an acid-washed 1.5 mL, polypropylene, centrifuge tube and centrifuged (Micromax, Thermo IEC) to remove all remaining water. Pellets were frozen at -20 ^{o}C until drying and analysis.

Analyses

Frozen organisms were freeze-dried (FTS Systems), weighed using a microbalance (Sartorius M2P PRO 11) and placed in acid-washed, high density, polyethylene bottles where they were digested for 2 days in concentrated HNO_3 (Aristar grade; 100 µL per mg sample dry weight) followed by 1 day in concentrated 30% (w/w) hydrogen peroxide (Trace Select Ultra grade; 40 µL per mg sample dry weight); digestate volume was brought up to 1 mL per mg sample dry weight using ultrapure water. Certified reference material (lobster hepatopancreas, TORT-2, National Research Council of Canada) was submitted to the same digestion procedure. Selenium of mass 82 g mol^{-1} (^{82}Se) was measured using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS; Thermo Elemental X Series) and interferences with bromine (^{81}Br + ^{1}H) were corrected using a standard curve of several Br concentrations. Selenium in the reference material (5.7 ± 0.1 (SD) µg g^{-1}; n = 4) was within the certified range (5.6 ± 0.7 (± 95% confidence interval) µg g^{-1}). The detection limit of the ICP-MS for Se was 0.2 µg L^{-1}. Field microplankton samples from some lakes had refractory inorganic matter that was removed by centrifugation before analysis.

Results and Discussion

Selenium Concentrations in a Planktonic Food Chain

Selenium concentrations in the members of a planktonic food chain composed of microplankton (<64 μm), copepods and *Chaoborus* were highly correlated with the sum of the concentrations of dissolved organic selenide and selenate (Figure 1A,B,C). Of these two Se species, organic selenide best predicted Se concentrations in organisms (Ponton and Hare, 2013). Kelly Lake differed somewhat from the other lakes in that adding selenate (solid triangle in Figure 1A,B,C) to organic selenide (solid square in Figure 1A,B,C) over-predicted Se concentrations in organisms. The very high concentrations of sulfate in this lake (4 mmol L^{-1}) likely competed with those of inorganic selenate thereby reducing the accumulation of this inorganic Se species at the base of the food chain (Ponton and Hare, 2013).

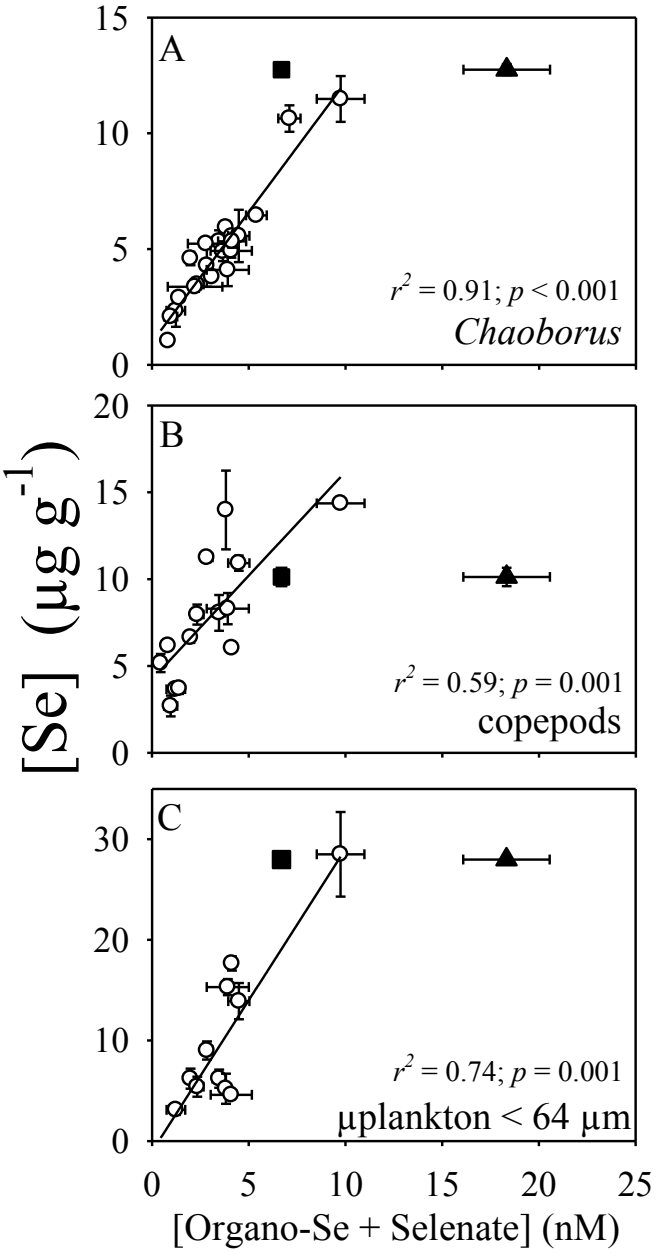

Figure 1: Relationships between Se concentrations (± SD; μg g^{-1} dry weight; n = 3-5) in field-collected A: *Chaoborus* larvae (redrawn from Ponton and Hare (2013)); B: copepods; C: microplankton (< 64 µm) and the concentrations of dissolved organic selenide (organo-Se) plus selenate (nM; n = 3). Closed symbols were not included in the regression line; they refer to data for Kelly Lake (triangles: selenate included; squares: selenate excluded).

Influence of Sulfate on the Uptake of Se Species by *Chlamydomonas reinhardtii*

Selenium concentrations in *Chlamydomonas reinhardtii* exposed to 63 nM (5 μg L^{-1}) selenate were high (225 μg g^{-1} dry weight) without the addition of sulfate (set as 10^{-9} M in Figure 2). At a sulfate concentration of 1 μM, Se concentrations in this alga were reduced by 50% (Figure 2A). This sulfate concentration is 16 times that of selenate, which suggests a much higher affinity of algal membrane transporters for selenate than for sulfate. This result contrasts with those obtained for the bacterium *Escherichia coli* that suggest a higher affinity of sulfate compared to selenate for a shared transporter (Lindblow-Kull et al., 1985). Even at sulfate concentrations as high as 500 μM, this alga accumulated some selenate (inset in Figure 2A). Other studies on green algae (*C. reinhardtii*, *Pseudokirchneriella subcapitata* and *Chlorella* spp.; Williams et al., 1994; Fournier et al., 2010; Neumann et al., 2003, respectively) have also reported competition between sulfate and selenate at shared transporters (Aguilar-Barajas et al., 2011). Ours is the first study to measure the influence of sulfate on selenate uptake by algae over the wide range of sulfate concentrations measured in the field (30 to 4,230 μM in our study lakes).

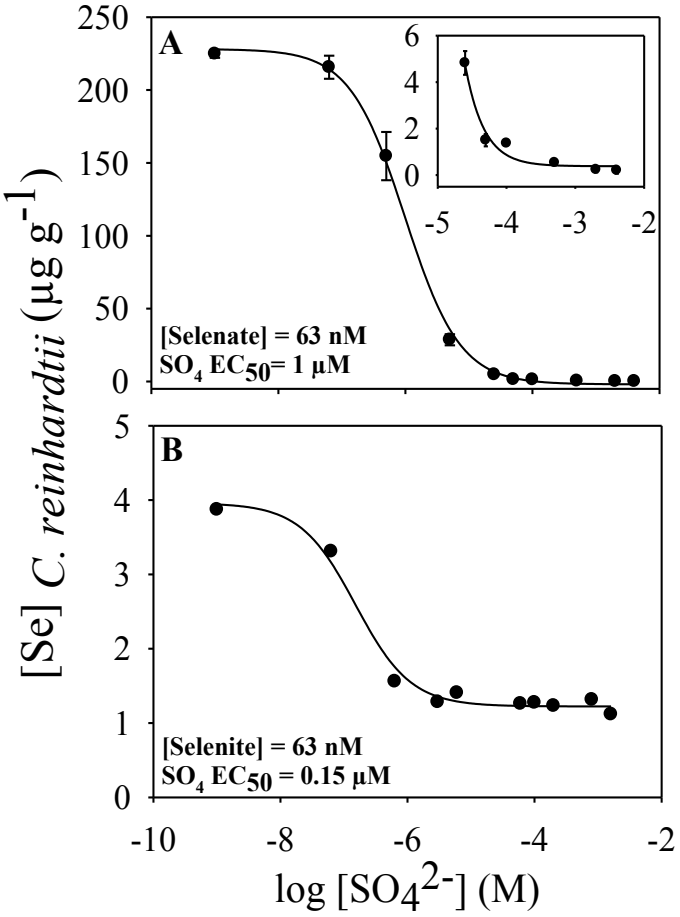

Figure 2: Selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg g^{-1} dry weight) exposed for 12 hours to 63 nM of either A: selenate (means ± SD; n = 3) or B: selenite (n = 1) and at various concentrations of sulphate (log [SO_4]; M). Curves of best fit have $r^2 > 0.99$ and $p < 0.001$. SO_4 EC_{50} are sulfate concentrations that reduced by half the [Se] in *C. reinhardtii*.

The Se concentration in *C. reinhardtii* exposed to selenite without added sulfate in the exposure medium (Figure 2B) was 56 times less than when this algae was exposed to selenate (Figure 2A). To reduce selenite uptake by 50%, it took ~7 times less sulfate (0.15 µM; Figure 2B) than was the case for selenate (1.0 µM; Figure 2A). Other studies report that selenate tends to be less readily accumulated by marine algae than does selenite (Hu et al., 1997; Luoma and Presser, 2009), although neither of these inorganic Se species is taken up readily by marine algae likely because of the high sulfate concentrations in seawater (Hu et al., 1997; 28 mM).

Likewise, in our study, at high SO_4 concentrations (> 10^{-4}) *C. reinhardtii* accumulated more Se when exposed to selenite as opposed to selenate (Figure 2). Sulfate did not reduce the uptake of seleno-methionine by *C. reinhardtii* (data not shown).

Temporal Patterns of Se Uptake by *Chlamydomonas reinhardtii*

When exposed to selenomethionine for 48 hours (Figure 3), *C. reinhardtii* had Se concentrations that were about the same (4 μg g^{-1}) as when it was exposed to selenite (without sulfate at pH 7.5; Figure 2B). It took ~30 hours for the alga to reach a steady state in its Se concentrations when exposed to selenomethionine, even when it had been preexposed to a selenomethionine concentration of 2.5 nM (0.2 μg L^{-1}). In contrast, it took this alga only ~12 hours to reach a steady state in its Se concentrations when exposed to the two inorganic species (Figure 3). We note that the apparent decline in the Se concentrations of algae exposed to selenate (between 12 and 48 hours) was likely caused by dilution due to algal growth. The inorganic and organic Se species also differed because there was an initial lag in the uptake of the organic species whereas this was not the case for the inorganic species (Figure 3). The steady-state concentration of the former was ~3 times those of the latter (Figure 3), which is explained in part by the fact that sulfate (100 μM) in the exposure medium reduced the accumulation of the inorganic Se species (as shown in Figure 2) but not that of the organic species (data not shown). Fournier et al. (2006) reported that for a given sulfate concentration (100 μM), selenomethionine uptake by this same alga was 5 times faster than with either of the inorganic species (we measured a 3x difference in algal [Se]).

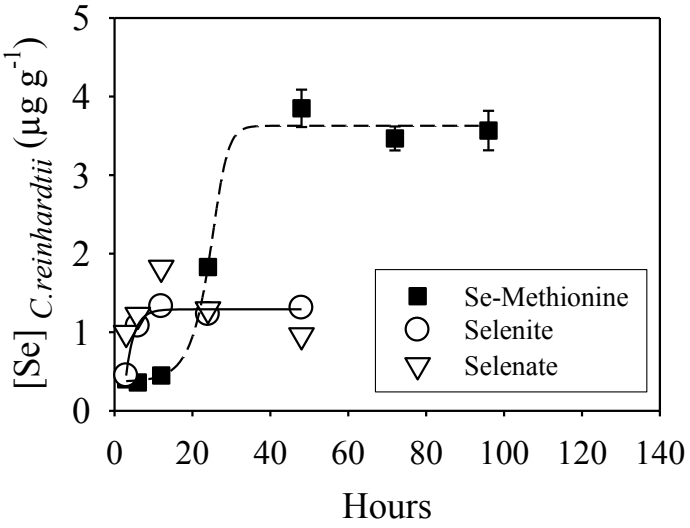

Figure 3: Selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg g^{-1} dry weight) exposed to 63 nM of either selenomethionine (solid squares), selenite (open circles) or selenate (open triangles) for up to 100 hours at a sulfate concentration of 100 µM and a pH of 7.5. Values are means ± SD (n = 3) for selenomethionine (48, 72, 96 h), whereas those for selenate and selenite are single samples. Both curves of best fit have r^2 = 0.99 and p = 0.01.

Influence of pH on the Uptake of Se Species by *Chlamydomonas reinhardtii*. Algae accumulated significantly more Se ($P < 0.05$) when exposed for 12 hours to seleno-methionine at pH 8.5 and 9.0 compared to their Se concentrations when exposed at pH 7.0 and 7.5 (Figure 4A). This difference is explained by slower selenomethionine uptake at the lower pHs since, for example, at pH 7.5, a 30-hour exposure was necessary for algae to achieve a steady state in their Se concentrations (4 µg g^{-1}; Figure 3), whereas at pH 8.5 or 9 (Figure 4A), Se concentrations did not significantly differ ($P < 0.05$) following a 12 hour exposure. Lower pH (7.0 to 5.5) is also reported to reduce by half the uptake of lipophilic cadmium complexes by *C. reinhardtii* and other green algae (Lavoie et al., 2012). The results of our study do not suggest diffusive Se-transport across the cell membrane since Se uptake was slow (Figure 3). Pre-exposing *C. reinhardtii* for three growth cycles to low concentrations of selenomethionine (2.5 nM; 0.2 µg L^{-1}) had no influence on its subsequent uptake of Se, which suggests that this alga does not synthesize additional selenomethionine

transporters when previously exposed to this Se species. Our results suggest that selenomethionine uptake by *C. reinhardtii* is slow because Se is diluted due to algal growth such that it is only when algae stop growing that we measure significant Se accumulation.

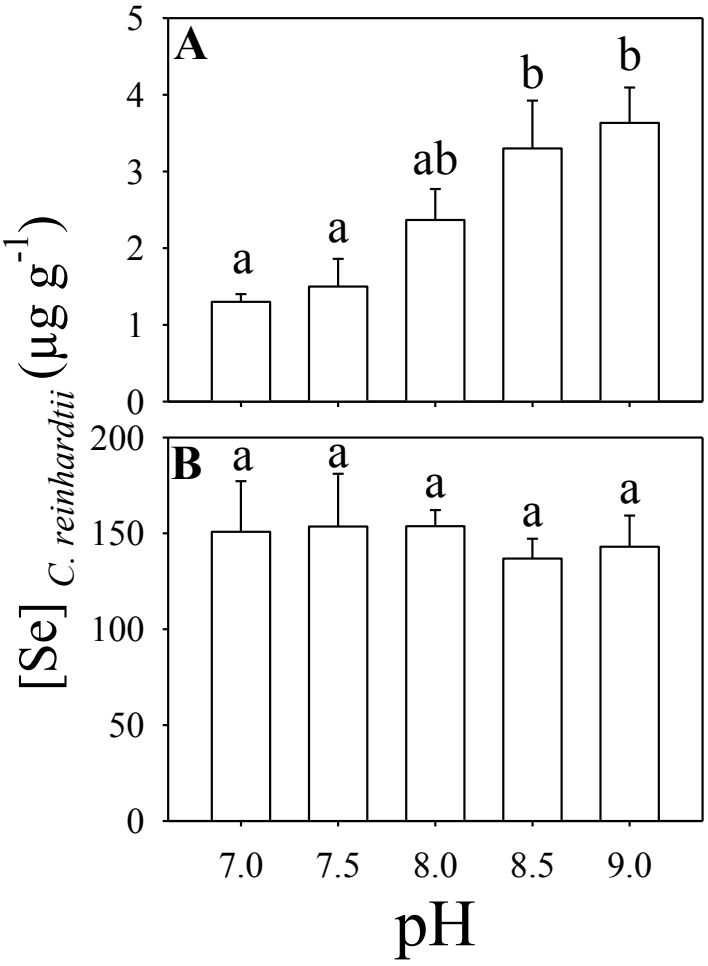

Figure 4: Mean (± SD, *n* = 3) selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg g^{-1} dry weight) exposed for 12 hours to 63 nM of either A: selenomethionine or B: selenate, at various pHs (sulfate absent). Different letters above bars indicate a significant difference ($p < 0.05$).

Selenate accumulation was not influenced over the pH range that we tested (Figure 4B). Although high concentrations of H^+ ions can alter the surface charges of algal cells and influence the uptake of anions (Kinraide, 2003), in the case of selenate, cell surface potential does not appear to influence this anion transporter.

Since the acidity constant of selenate (pK_a = 1.9) falls outside of the pH range that we tested (7-9), pH induced protonation of selenate should not influence uptake of this Se species. In contrast, inorganic speciation of selenite as a function of pH could influence uptake of this Se species.

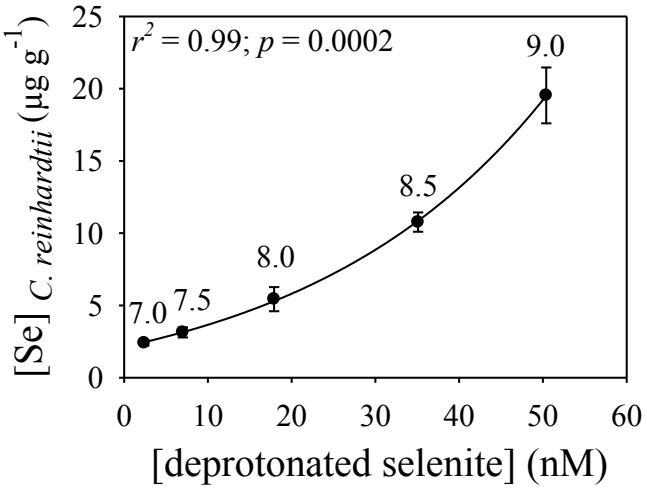

Figure 5: Mean (± SD, n = 3) selenium concentrations in *Chlamydomonas reinhardtii* ($[Se]_{C.\ reinhardtii}$; µg g^{-1} dry weight) as a function of the concentration of the deprotonated selenite species (SeO_3^{2-}, as calculated using MINEQL+). Concentrations of the two dominant selenite species (SeO_3^{2-} and $HSeO_3^-$; pK_a = 8.4) were varied by changing the pH of the exposure solution (given above data points). Algae were exposed to a total selenite concentration of 63 nM for 12 hours.

We measured a significant ($P < 0.05$) pH effect on Se accumulation by *C. reinhardtii* (Figure 5) following 12 hours of exposure to 63 nM selenite. In contrast, two other studies on this algal species reported no clear influence of pH on inorganic Se accumulation (Morlon et al., 2006 (pH 5-8); Riedel and Sanders, 1996 (pH 5-9)). Selenite speciation changes markedly as pH increases from 7 and 9, since the protonated form ($HSeO_3^-$) is progressively replaced by the deprotonated form (SeO_3^{2-}) up to pH 8.4 (pK_a) where ~50% of each Se(IV) species is present (thermodynamic calculation using MINEQL+ Schecher, 1992). Our results suggest that algae assimilate only the deprotonated form of selenite, that is, SeO_3^{2-}, such that concentrations of this species are correlated with those of Se in *C. reinhardtii* (Figure 5). Greater bioavailability of the deprotonated selenite species is consistent with the

fact that it has a higher net charge (-2) and thus is likely to be more reactive with transporters in biological membranes. At pH 9, selenite accumulation was 5 times higher than at pH 7 (Figure 5). This phenomenon can be important in natural systems where Se is present mainly as selenite, as well as where pHs are high, such as in oceans (pH 8.2) or downstream from ponds that have been treated with lime to neutralize acidic effluents (Lemly, 2004; Lakes Kelly and Rouyn; Ponton and Hare, 2013). Although the concentration of selenite was constant in our experiment, in nature, selenite concentrations would increase with increasing pH (Ponton and Hare 2013), which would also favor Se uptake at higher pHs.

Microplankton Selenium Accumulation in the Laboratory. The steady state Se concentrations attained by *C. reinhardtii* in the laboratory were an order of magnitude lower than those measured in field microplankton that were exposed to organo-Se concentrations that were ~10% of those that we used in the laboratory (Figure 1 and Ponton and Hare, 2013). To explain this large difference, we exposed microplankton (< 64 μm) collected from an uncontaminated lake to selenomethionine under the same exposure regime that we used for *C. reinhardtii*. As expected from our field measurements, the steady state Se concentration of microplankton (Figure 6) was ~30 times that attained by *C. reinhardtii* (Figure 3). This result suggests that the difference we measured between Se concentrations in a green alga in the laboratory and those in field microplankton are not explained by a difference in the species of organic Se to which algae are exposed (selenomethionine rather than selenocysteine), but by the types of organisms that make up field microplankton. We conclude that organic selenide accumulation by *C. reinhardtii* is not a good surrogate for processes occurring in the field.

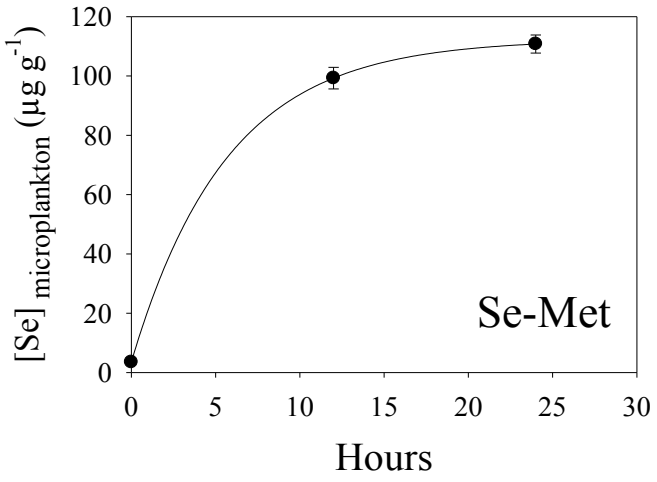

Figure 6: Mean (± SD, *n* = 3) selenium concentrations of field-collected microplankton (< 64 µm; $[Se]_{microplankton}$; µg g^{-1} dry weight) exposed for various lengths of time to 63 nM of seleno-methionine at a sulfate concentration of 100 µM.

To understand this field-laboratory difference, we identified algae in our uncontaminated microplankton samples and found that 70% were Chrysophyceae, Dinophyceae or Euglenophyceae (Figure 7) as opposed to the Chlorophyceae (10%) of which *C. reinhardtii* is a part. Baines and Fisher (2001) reported that some of the algal groups that were important in our microplankton samples (Dinophyceae and Cryptophyceae) accumulate higher concentrations of Se in the laboratory than do many others. Another second major difference between the laboratory algal cultures and field microplankton was that bacteria were absent in the former whereas in our field samples they were more abundant than were algae, that is, 15,500 ± 3,580 cells μL^{-1} compared to 2.5 cells μL^{-1} (*n* = 3), respectively. In fact, bacteria are reported to explain from 34 to 67% of the bioaccumulated Se from selenite uptake in natural samples (Baines et al., 2004). Although when expressed as cell volumes there was no significant difference between the two groups ($1.6\times10^6 \pm 0.4\times10^6$ μm^3 μL^{-1} and 1×10^6 μm^3 μL^{-1}, respectively), in terms of surface area, bacteria in our microplankton samples greatly exceeded algae (30,000 ± 7000 versus 1,150 μm^2 μL^{-1}, respectively), which suggests that the presence of bacteria may be the most important factor explaining the difference in Se accumulation between field microplankton samples and *C. reinhardtii*.

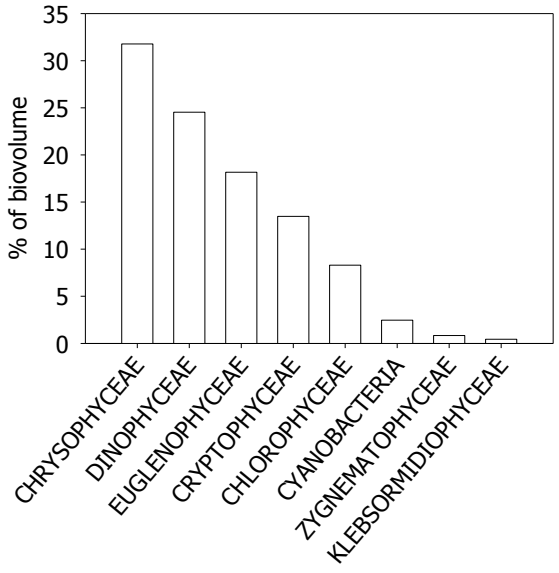

Figure 7: Percentage (%; *n* = 3) of the total biovolume represented by major algal groups in an epilimnetic sample from an uncontaminated lake.

In the laboratory, field-collected microplankton took up less Se (35 μg g^{-1}; Figure 8) than did *C. reinhardtii* (225 μg g^{-1}) when exposed to selenate (without added sulfate) for 12 hours. On the one hand, a sulfate concentration of 0.2 μM ($10^{-6.7}$ M) reduced microplankton selenate accumulation by 50% (Figure 8), whereas the comparable value for *C. reinhardtii* was 1 μM. On the other hand, at the highest sulfate concentration (4 mM), Se concentrations in microplankton (~5 μg g^{-1}) were higher than those in this alga (< 0.2 μg g^{-1}). We measured no significant competitive effect of sulfate on selenite accumulation by microplankton (Figure 8). Furthermore, natural microplankton accumulated more Se when exposed to selenite than to selenate (about 51 μg g^{-1}; Figure 8). Lastly, in the laboratory, organisms in our microplankton samples accumulated inorganic Se species differently than did *C. reinhardtii.*

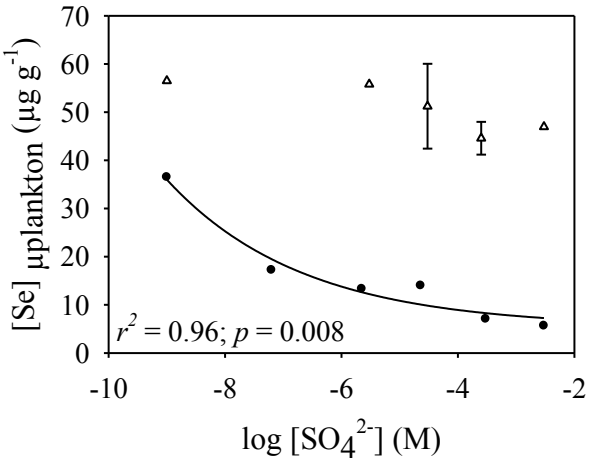

Figure 8: Selenium concentrations in field-collected microplankton (< 64 μm; $[Se]_{microplankton}$; μg g^{-1} dry weight; n = 1) exposed, at pH 7.5, to 63 nM selenate (closed circles) or selenite (open triangles) at various sulfate concentrations.

Estimation of Se Concentrations in Field Plankton from Modeled Parameters. We used our laboratory data to estimate Se concentrations in indigenous planktonic organisms. Because Se accumulation by microplankton better represented field values than did that of *C. reinhardtii*, we used the data from our microplankton Se exposures in the laboratory as input to the model. Steady state Se concentrations in microplankton ($[Se]_{microplankton}$; μg g^{-1}) can be expressed as:

$$[Se]_{microplankton} = k_{d(IV)}[Se(IV)] + \frac{(5.4 + 0.63e^{(-0.43[SO_4])})[Se(VI)]}{63} + k_{d(-II)}[Se(-II)] \qquad (1)$$

where $k_{d(IV)}$ and $k_{d(-II)}$ are partition coefficients (Presser and Luoma, 2010) constants (mL g^{-1}) our uptake study by microplankton of dissolved selenite and organic selenide, respectively. The k_d for these Se species was obtained by dividing the steady state Se concentration in microplankton exposed to selenomethionine (Figures 6; 1,380 nmol g^{-1}) and to selenite (Figure 8; mean = 646 nmol g^{-1}) by the Se concentration in the exposure medium (63 pmol mL^{-1}), which gives a calculated k_d for selenite ($k_{d(IV)}$) of 10,255 mL g^{-1} and a k_d for selenomethionine ($k_{d(-II)}$) of 21,900 mL g^{-1}. In the selenate term of equation 1, the k_d for selenate ($k_{d(VI)}$) is replaced by the equation for the curve presented in Figure 8 (which includes the competitive effect of

sulfate on selenate uptake) divided by the Se exposure concentration (63 pmol mL^{-1}). Note that since we cannot assume that model results are necessarily linear, we do not know if the model can be applied at much higher dissolved Se concentrations.

Using equation 1 along with the estimated values of these constants and the concentrations of the three Se species and sulfate ($[SO_4]$) in lakewater (Ponton and Hare, 2013) we estimated Se concentrations in field microplankton. To estimate Se concentrations in invertebrates at higher trophic levels, we multiplied the values obtained for microplankton by the trophic transfer factors ($TTF = [Se]_{consumer}/[Se]_{food}$) of 1.0 ± 0.7, to obtain values for copepods, and then 0.6 ± 0.2 (n = 13), to obtain those for the predator *Chaoborus*. These consumer-prey relationships had p values < 0.01. For example, modeled values for *Chaoborus* are in good agreement with those measured in the field, as indicated by the similarity of the regression line to the 1 to 1 line in Figure 9. Comparing the contributions of the three Se species to the Se concentrations predicted in microplankton (equation 1), and extrapolating up the food chain to *Chaoborus* larvae, suggests that the majority (74 ± 19 %) of the Se accumulated by this predator is explained by the concentrations of dissolved organic selenide in lakewater. Since microplankton accumulated less selenate than selenite in the laboratory, and because sulfate influenced selenate uptake (Figure 8), the concentrations of selenate do not explain any (0 ± 1 %) of the Se accumulated by *Chaoborus*. In contrast, selenite was not influenced by competition with sulfate (Figure 8), such that it explains 26 ± 19 % of the predicted Se concentrations in *Chaoborus*.

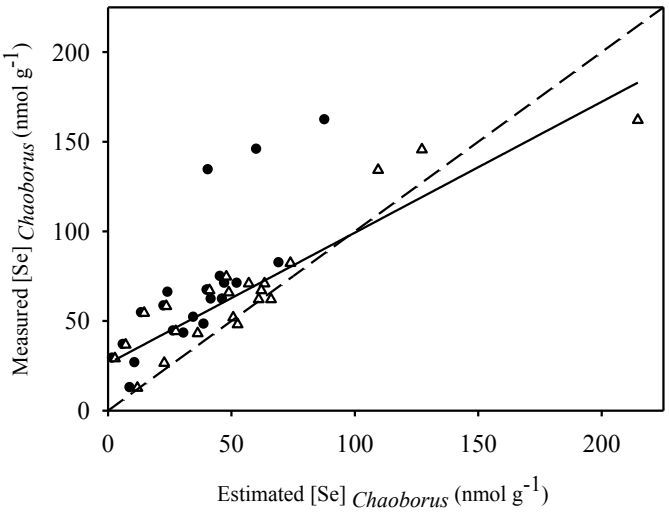

Figure 9: Predicted selenium concentrations in *Chaoborus* larvae (µg g^{-1} dry weight) from 16 lakes as a function of predicted values (from equation 1) based on Se and sulfate concentrations in lakewater and on laboratory measurements of Se uptake by field-collected microplankton. Broken line is 1:1 relationship. Closed symbols are estimations considering dissolved organic selenide concentrations only and open symbols are estimations with both dissolved organic selenide and selenite concentrations.

Overall, our laboratory results suggest that careful attention should be given to the interpretation of laboratory studies of Se uptake by single algal species, as well as the influence of sulfate and pH on Se uptake by microorganisms.

Acknowledgments

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TROISIÈME ARTICLE

FEEDING BEHAVIOR AND SELENIUM CONCENTRATIONS IN YELLOW PERCH (*PERCA FLAVESCENS*)

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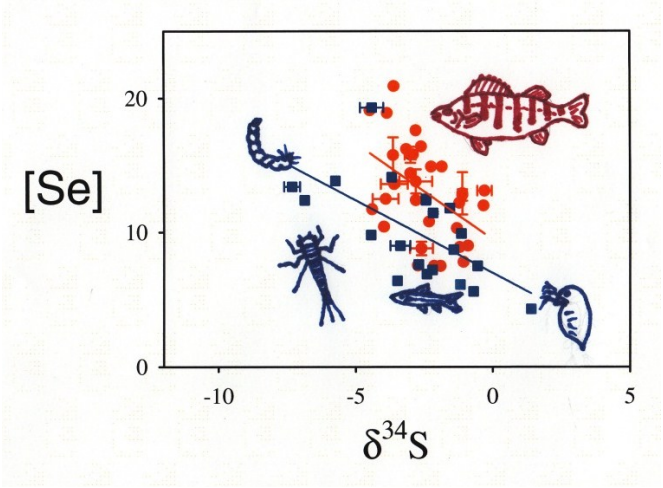
To be submitted at Environmental Science and Technology



Keywords: Selenium, Prey, Sulfur isotopes, Bioaccumulation, Biomagnification, Food web, Fish, Yellow perch, *Perca flavescens*

Abstract

We measured selenium (Se) concentrations in yellow perch (*Perca flavescens)* and their prey in four Se-contaminated lakes located near metal smelters in the Canadian cities of Sudbury and Rouyn-Noranda. In two of these lakes, there was no relationship between yellow perch size and Se concentrations, whereas in the other two, small yellow perch tended to have lower Se concentrations than did larger individuals. Measurements of sulfur stable isotopes ($\delta^{34}S$) in yellow perch muscle and stomach contents showed that larger fish fed less on zooplankton and more on benthic invertebrates than did smaller fish. Because Se concentrations are lower and $\delta^{34}S$ signatures are higher in zooplankton than in sediment-feeding invertebrates, there was an inverse relationship between fish Se concentrations and $\delta^{34}S$ signatures in all of our study lakes. Selenium concentrations in *P. flavescens* were on average 1.6 times those of its prey, which indicates that Se is biomagnified as it moves up the food web in these lakes. Multiplying the Se concentrations of sediment-feeding prey by 1.6 can be used to estimate maximum Se concentrations in yellow perch. Estimated Se concentrations in yellow perch gonads suggest that fish in two of our study lakes are at risk of reproductive toxicity.

Introduction

The essential element selenium (Se) acts as an antioxidant (Rayman, 2000) that reduces the incidence of cancer in animals (Ip, 1998) and counteracts the effects of contaminants such as mercury (Hg; Polak-Juszczak and Robak, 2014). However, the minimum Se concentration required to satisfy physiological needs is only a factor of four below the concentration that is toxic to fish (Lemly, 1993). High concentrations of Se in aquatic systems receiving runoff from dryland irrigation or ash from coal-fired power plants have caused deformities in the embryos of some fish and birds (Presser and Ohlendorf, 1987; Lemly, 2002). Metal smelters can also be an important source of Se to lakes downwind from these sources (Nriagu and Wong, 1983).

In eastern Canada, recent measurements (Ponton and Hare, 2013) have shown that Se concentrations can be high in the plankton of lakes near metal smelters in Rouyn-Noranda (Quebec) and Sudbury (Ontario). Previous studies in this area focused mostly on trace metals such as cadmium (Cd) and nickel (Ni) (Hare and Tessier, 1998; Campbell et al., 2003; Ponton and Hare, 2009) because they were thought to be responsible for toxic effects on aquatic animals (Borgmann et al. 2001, 2004). Selenium was not considered in these latter studies, perhaps because it is more often viewed as a mercury (Hg) antagonist (Pyle et al., 2005; Belzile et al., 2009) than as a potentially toxic element in its own right.

In lakes near Rouyn-Noranda and Sudbury, the yellow perch *Perca flavescens* (Percidae) persists in even highly metal-contaminated lakes and because of this has been the subject of studies on trace metal bioaccumulation, trophic transfer and toxicity (Campbell et al., 2003; Rasmussen et al., 2008). Although the relationship between Se concentrations and toxic effects has not been studied in yellow perch, members of its sister-family (Centrarchidae) are reported to be tolerant to this trace element (Lemly, 1993). Although many fish species (including yellow perch) are reported to take up trace metals such as Cd and Ni mainly from water via their gills (Kraemer et al., 2006; Lapointe and Couture, 2009), Se is reported to be accumulated by fish mainly from their food (Luoma and Rainbow, 2008).

Fish feeding on zooplankton will be exposed to Se in the water column compartment, whereas those feeding on benthos will be exposed to Se in the sediment compartment. Since yellow perch feed on zooplankton and benthos at different times of their life (Rasmussen et al., 2008), they are exposed to Se in both of these compartments. Thus, *P. flavescens* < 10 g are reported to feed only on zooplankton, those between 10 and 100 g feed mainly on benthic invertebrates, and larger individuals consume small fish, although these diet transitions are influence by the densities of the various prey types in a given lake (Rasmussen et al., 2008). If Se concentrations differ in these compartments and in the invertebrates living in them, then yellow perch Se concentrations are also likely to change as they grow. In marine fish, both positive and negative correlations have been reported between fish size and Se concentrations (Burger et al., 2014, Kojadinovic et al., 2007). In San Francisco

Bay, Se concentrations in a given fish species were not related to its size, but fish species differed markedly in their Se concentrations and these differences were related to the types of prey they consumed (Stewart et al., 2004)

Given this lack of knowledge about Se in fish living near metal smelters, and the potential importance of changes in diet on Se exposure, we set out to measure Se in yellow perch and its prey that we collected from lakes located near the metal smelters in Rouyn-Noranda and Sudbury. Emissions reductions from these smelters have led to reduced trace element concentrations in the water and animals from nearby lakes (Belzile and Morris, 1995; Keller et al., 1999; Croteau et al., 2002). However, trace element concentrations in sediments have changed little (Tropea et al., 2010) and thus can still pose a potential hazard to benthic invertebrates and to the fish depending on them for food. Given these facts, we predict that as the diet of yellow perch changes from zooplankton to benthic invertebrates their Se concentrations are likely to increase. Furthermore, in lakes where yellow perch are able to change from eating benthos to small fish, their Se concentrations will either remain constant, if the prey fish are benthivores, or decrease, if their prey fish are planktivores.

The relative importance of zooplankton and benthos in the diet of yellow perch can be determined by examining fish stomach contents. However, this approach ideally requires a large number of samples taken at various times and is subject to error since some prey types are digested more readily than others. An alternative is to measure sulfur (S) stable isotopes in this predator and its prey since $\delta^{34}S$ signatures are reported to be more negative in sediment-feeding than in plankton-feeding invertebrates and these differences are reflected in the $\delta^{34}S$ signatures of their predators (Croisetière et al., 2009). To the best of our knowledge, $\delta^{34}S$ signatures have not been used previously to explain Se concentrations in fish.

We also compared Se concentrations in yellow perch and their prey to determine if this element is biomagnified in the food chains that we studied. Lastly, we evaluated whether the yellow perch populations that we studied are likely to be at risk by comparing Se concentrations in their muscle with those of toxicity guidelines as well as by estimating Se concentrations in their gonads from those in muscle and

comparing these to published values for Se reproductive toxicity. Given the lack of study of this important trace element in mining regions, our study should be useful to those wishing to develop risk assessments for Se in fresh waters.

Methods

Study Sites and Animal Collection and Handling. We collected yellow perch and their prey from mid-May to mid-June for three years (2010 to 2012) in four lakes located on the Precambrian Shield in the mining areas of Sudbury, Ontario (Kelly Lake: 46°27'N, 81°03'W) and Rouyn-Noranda, Quebec (Lakes Dufault: 48°18'N, 79°00'W; Osisko: 48°14'N, 79°00'W; Rouyn: 48°14'N, 78°56'W), Canada. Lakes were chosen because of their relatively high Se concentrations in lakewater and plankton (Ponton and Hare, 2013) due to their proximity to metal smelters. The water columns at our sampling sites were of uniform temperature and saturated in oxygen.

In each year, we collected 5 to 12 yellow perch in the littoral zone of each lake using a 0.5-cm mesh-size seine net (75 m × 1 m), which gave a total of from 12-30 fish from each lake. Live fish were held in a cooler with aerated lakewater until they were measured, weighed and sacrificed on shore by decapitation and decerebration. From each fish, we removed stomach contents and a piece of anterior dorsal muscle. Since we used the other organs of these fish for biochemical measurements, they were not available for Se measurements. For this reason, in 2009, we also collected yellow perch from another Sudbury area lake (Lohi Lake; 46°23'N, 81°02'W) so that we could determine if Se concentrations in fish muscle differed from those of other organs (Supporting Information, Figure S1), as well as to estimate the likelihood that Se concentrations in the reproductive organs of yellow perch were potentially toxic. Fish parts and stomach contents were placed individually into acid-washed, 1.5-mL, polypropylene, centrifuge tubes and stored at -20 °C. In the laboratory, fish stomach contents were thawed, identified and counted in ultrapure water under a dissecting microscope then refrozen at -20 °C for later analysis.

Benthic prey were collected using an Ekman grab at the same site in each lake at which we collected yellow perch. Mud samples were sieved in lakewater using a 0.5-mm mesh-size net and the invertebrates retained were held in a plastic bag in

lakewater until their return to the laboratory where they were sorted according to lowest taxonomic level possible (SI, Table S1) and held at 4 ^{o}C for 3 days to depurate their gut contents. We retained only prey types that we had observed in yellow perch stomach contents for Se analyses. Three individuals of each taxon were placed on a pre-weighed piece of acid-washed Teflon sheeting in an acid-washed micro-centrifuge tube that was frozen at -20 °C until analysis.

Pelagic prey were collected at night by hauling a 64 μm mesh-size plankton net horizontally in the water-column. We sieved bulk zooplankton to separate larvae of the phantom midge *Chaoborus* from micro-crustaceans (cladocerans and copepods). We verified under a microscope that plankton fractions were composed of at least 90% micro-crustaceans by volume. Samples for chemical analysis were prepared by placing either 10-20 similar-sized fourth-instar *Chaoborus* larvae or ~10 mg wet weight of crustacean zooplankton on acid-washed, pre-weighed pieces of Teflon sheeting in acid-washed micro-centrifuge tubes and frozen at -20 °C until analysis.

Analyses. Frozen samples were crushed into a homogeneous powder in polypropylene centrifuge tubes using a plastic pestle and subsamples were taken for the measurement of Se and sulfur (S) stable isotopes.

Subsamples (3-4 mg) for S stable-isotope analysis were put into tin capsules, along with ~8 mg of vanadium pentoxide powder as a catalyst, that were placed in a 96-well microplate. Blind duplicates were made for quality assurance. Sulfur isotopic signatures are reported as $\delta^{34}S$ (‰) = $[(^{34}S/^{32}S_{\text{sample}} / {}^{34}S/^{32}S_{\text{standard}}) - 1] \times 10^{3}$, where the S standard is from the Canyon Diablo Troilite (CDT). Sulfur stable-isotopes were measured by elemental analyser – isotope ratio mass spectrometry at Iso-Analytical Limited (Crewe, UK) using a Sercon elemental analyser and 20-20 mass spectrometer (Sercon Ltd, Crewe, UK). The certified reference materials used during S isotopic-analyses were IAEA-SO-5 (barium sulphate, $\delta^{34}S$ = + 0.5‰; IAEA) and IA-R027 (whale baleen, $\delta^{34}S$ = + 16.30‰; Iso-Analytical working standard). Values for both were within the certified ranges (+0.34 ± 0.2 ‰ and +16.24 ± 0.28 ‰, respectively). We also measured both C and N stable isotopes, however these data are not shown because they varied little among prey types and yellow perch from a given lake.

Subsamples (1 to 15 mg dry weight) for Se analysis were weighed and placed in acid-washed, 15 mL, high-density polyethylene bottles where they were digested at room temperature for 2 days in concentrated nitric acid (Aristar grade; 100 μL per mg sample dry weight) followed by 1 day in concentrated hydrogen peroxide (Trace Select Ultra grade; 40 µL per mg sample dry weight); digestate volume was completed to 1 mL per mg sample dry weight using ultrapure water. Certified reference material (lobster hepatopancreas, TORT-2, National Research Council of Canada) was submitted to the same digestion procedure. Selenium of mass 82 g mol^{-1} was measured using Inductively Coupled Plasma - Mass Spectrometry (ICP-MS; Thermo Elemental X Series) and interferences with bromine (Br) and hydrogen ($^{81}Br + {}^{1}H$) were corrected using a standard curve of several Br concentrations. Selenium in the reference material (5.7 ± 0.1 (SD) μg g^{-1}; n = 4) was within the certified range (5.6 ± 0.7 (95% CI) μg g^{-1}) and the detection limit of the ICP-MS for Se was 0.2 ppb. Standard deviations reported for individual values are for three analyses of the same sample.

Statistical Tests. We compared our data for yellow perch weight, Se concentrations and $\delta^{34}S$ signatures in a given lake using bivariate scatterplots, then tested the adequacy of fit of linear and exponential regression models when assumptions of normality (Shapiro-Wilk test) and equality of variances (Levene's test) were satisfied. To determine if we could combine our data for a given prey type from various lakes, we used the equation:

$$[Se] = a + b(\text{isotope}) + (\text{isotope}) * \text{Lake}$$

which allowed us to compare the slopes (the "(isotope) ∗ Lake" term) and y-intercepts (the "isotope" term) among lakes. The lack of significant differences between the slopes and y-intercepts for Lakes Dufault and Osisko as well as between Lakes Kelly and Rouyn (*post hoc* Tukey multiple comparisons) indicated that our prey data for these lakes could be combined. Differences among prey Se concentrations and $\delta^{34}S$ signatures were assessed using ANOVA followed by a non-parametrical Dunn's test on ranks. Statistical analyses were carried out using Systat version 11, SigmaStat version 3.5 and Jump 9.0 (SAS Institute Inc.). A p value of 0.05 was used as the threshold for significance.

Results and Discussion

Since there was no significant difference ($P > 0.05$) among years in mean Se concentrations in yellow perch from most lakes (ANOVA, T test and Mann-Whitney), we combined data for all years for each lake. Exceptionally, Se concentrations in yellow perch from Kelly Lake differed between 2011 and 2012 likely due to differences in diet among the fish captured (as discussed below).

In a given lake, Se concentrations varied among individual yellow perch by a factor of two to five (Figure 1, left panels; SI, Table S1). In Lakes Dufault and Kelly, these individual differences were not random since Se concentrations increased significantly ($P < 0.05$) with increasing fish weight up to ~25 g (Figure 1, left panels). Above this size, Se concentrations in yellow perch (Figure 1, left panels) either declined somewhat (Lake Dufault) or remained stable (Kelly Lake). No clear trends in Se concentrations with fish weight were observed in Lakes Osisko and Rouyn (Figure 1, left panels). In other fish species, fish size and Se concentrations are reported to show either a positive relationship (several marine predators (Kojadinovic et al., 2007) and trout (Burger et al., 2014)), a negative relationship (halibut; Burger et al., 2014), or no relationship (eels (Polak-Juszczak and Robak, 2014), carp (Levengood et al. 2014) and Arctic char (Gantner et al. 2009)). The variety of relationships that we observed between yellow perch weight and Se concentrations is likely a consequence of lake to lake differences in feeding habits since yellow perch are reported to change their feeding habits as they grow (Rasmussen et al., 2008) and the availability of various prey types (zooplankton, benthos, small fish) is likely to differ among lakes.

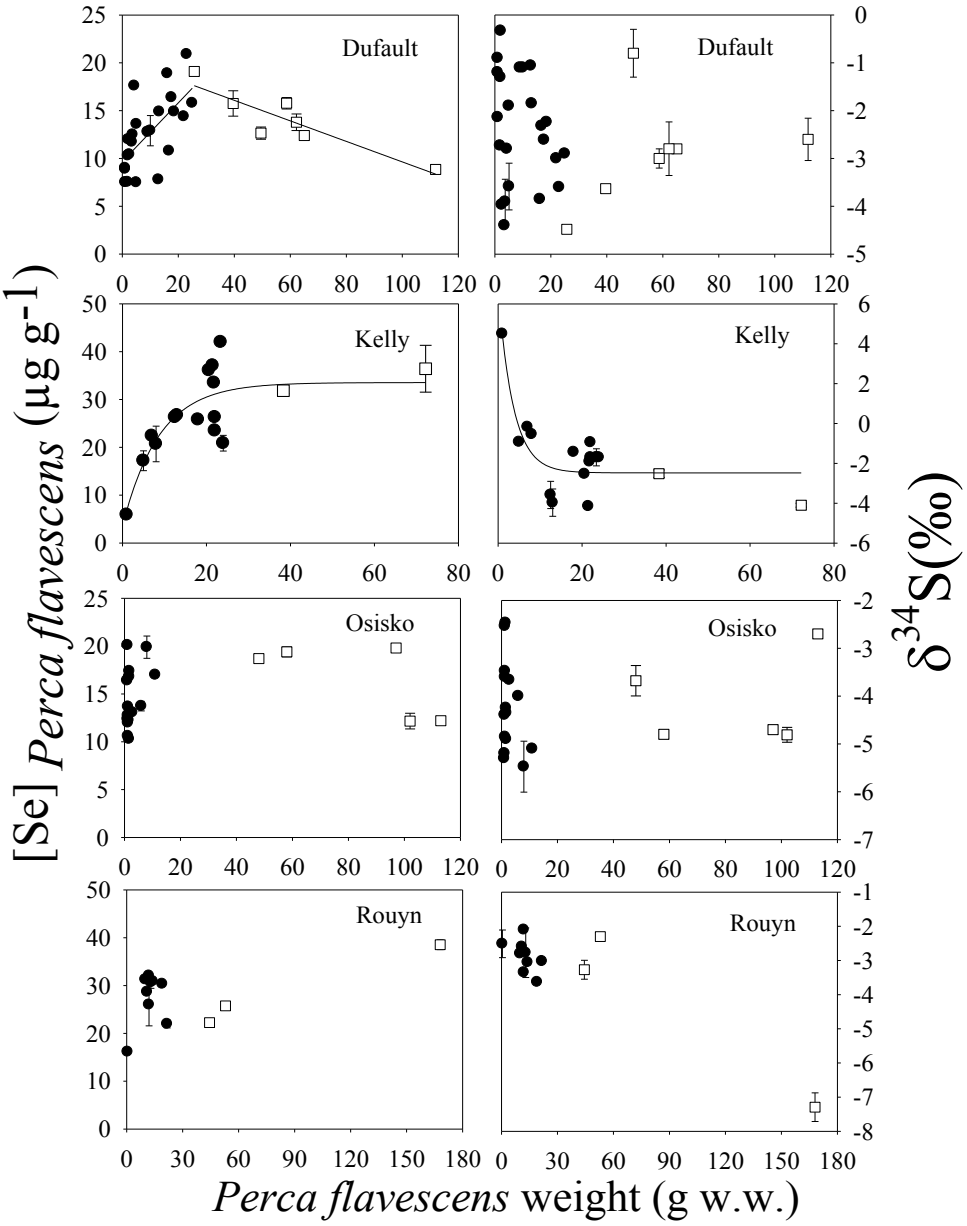


Figure 1. Mean (±SD) *Perca flavescens* selenium concentrations ([Se], μg g^{-1} dry weight; left panels) and sulfur stable-isotope signatures (δ^{34}S, ‰; right panels) as compared to the individual wet weights (grams) of individual yellow perch from our study lakes. Fish having wet weights < 25 g are represented by solid symbols, whereas heavier fish are represented by open squares. Linear or curvilinear regression lines of best fit are shown for significant relationships ($P < 0.05$).

To determine the type of prey on which yellow perch feed, we measured S stable isotopes in this fish and their prey since $\delta^{34}S$ signatures are reported to differ between invertebrates feeding on plankton and those feeding on anoxic sediments (Croisetière et al., 2009). This difference is a result of the fact that $\delta^{34}S$ signatures tend to be more negative in anoxic sediments than in the sulfate dissolved in the oxic zone of lakes such that animals feeding in these two zones show a corresponding difference in their $\delta^{34}S$ signatures (Croisetière et al., 2009). In our study lakes, this difference is not likely to be due to sulfur isotope fractionation due to bacterial dissimilatory sulfate reduction (Brunner and Bernasconi, 2005) because sulfate is completely consumed in sediments (Croisetière et al., 2009). Other mechanisms that could explain such a difference include fractionation during sulfate diffusion across the sediment-water interface (Peterson and Fry, 1987), S fractionation in the drainage basins of these lakes (Cole et al., 2006), and historical changes in the isotopic composition of sulfate due to variations in atmospheric SO_2 sources (Nriagu and Soon, 1985).

There was no significant ($P > 0.05$) difference between the mean (± standard deviation (SD)) $\delta^{34}S$ signatures of yellow perch (-3.0 ± 1.3 ‰) and those of the prey in their guts (-2.8 ± 2.9 ‰), which is consistent with that fact that there is little fractionation of sulfur isotopes between consumers and their food (Peterson and Fry, 1987; McCutchan et al., 2003).

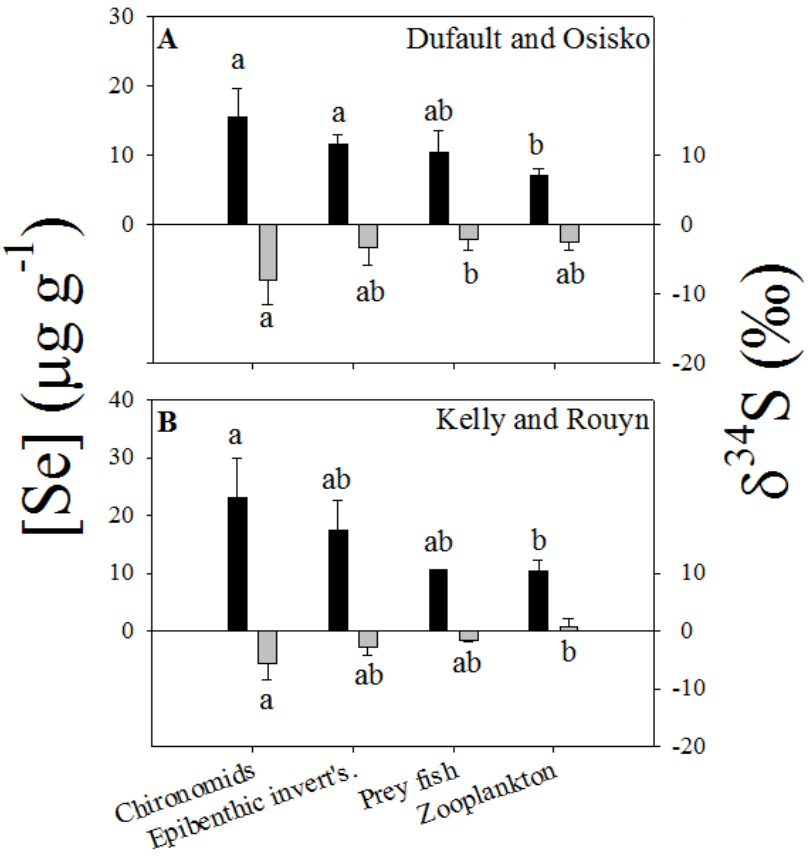


Figure 2. Selenium concentrations ([Se], μg g^{-1} dry weight; black bars) and sulfur stable isotope signatures ($\delta^{34}S$, ‰; gray bars) of prey consumed by *Perca flavescens* collected in either Lakes Osisko and Dufault (upper panel) or Lakes Rouyn and Kelly (lower panel). Different letters represent a significant difference between prey types.

The $\delta^{34}S$ signatures of zooplankton were significantly more positive than those of sediment-feeding chironomid larvae (Figure 2), which suggests that yellow perch feeding on zooplankton should have more positive $\delta^{34}S$ signatures than those feeding on benthos. In lakes where individual small yellow perch feed on either zooplankton or benthos, their $\delta^{34}S$ signatures should cover a wide range. Although previous reports suggest that small (< 10 g) yellow perch feed almost exclusively on zooplankton (Rasmussen et al., 2008), in our study lakes some fish of this size class fed on zooplankton (6 fish from 3 lakes) whereas others fed on benthos (9 fish from 4 lakes; SI, Table S1). For example, in Kelly Lake, we collected a 1 g fish that had

consumed 22 chironomids whereas an 8 g fish had only zooplankton in its stomach. This wide range of feeding habits among small yellow perch (< 10 g) explains the correspondingly wide range in their $\delta^{34}S$ signatures, especially in Lakes Dufault and Osisko (Figure 1, right panels). In contrast, larger fish (10 to 25 g; Figure 1, right panels) showed a more narrow range in the $\delta^{34}S$ signatures which suggests that as fish age their diet becomes less diverse, likely because they eat less zooplankton, which provides less food for the same effort than benthic invertebrates (Rasmussen et al., 2008).

Above a mass of ~25 g, the small numbers of yellow perch that we collected had Se concentrations (Figure 1, left panels) that tended to either decline (Lake Dufault) or remain stable (Kelly Lake). This difference is likely explained by the types of prey available to the yellow perch in a given lake. On the one hand, where small fish such as minnows are not abundant, yellow perch are likely to remain dependent on benthos and thus their Se exposure should remain constant as their weight increases above 25 g. On the other hand, in lakes where small fish are available as prey, it would be energetically advantageous for yellow perch to shift from feeding on benthic invertebrates to fish (Rasmussen et al., 2008). In this case, the trend in perch Se concentrations will depend on the diet of the prey fish. Where prey fish are planktivores, then the Se concentrations of yellow perch are likely to decline since Se concentrations in zooplankton tend to be lower than those in benthos (Figure 2). This was the case in Lake Dufault (Fig. 1; left panel), where Se concentrations in prey fish were not significantly different from those of zooplankton but were significantly lower than those in chironomids (Figure 2, lower panel). In contrast, if prey fish are benthivores, then the Se concentrations of large yellow perch are likely to remain stable. This was the case in Lake Rouyn where the largest yellow perch (168 g) that we collected did not have lower Se concentrations and ate both benthos (damselfly larvae) and small fish (Se concentration of 25.5 μg g^{-1} and $\delta^{34}S$ signature of -2.4; SI, Table S1). In Kelly Lake, where yellow perch Se concentrations also remained high, one of the two large yellow perch (> 25 g) that we collected had chironomids in its stomach (SI, Table S1), which suggests that large yellow perch in this lake remain benthivorous likely due to the small densities of suitable prey fish.

Overall, our results suggest that although yellow perch Se concentrations vary with fish size, the relationship between these two variables depends on the availability of prey, and thus perch feeding habits, in a given lake. Furthermore, these results for yellow perch suggest that considering the diet of other fish species could help to explain variations in their Se concentrations with body size.

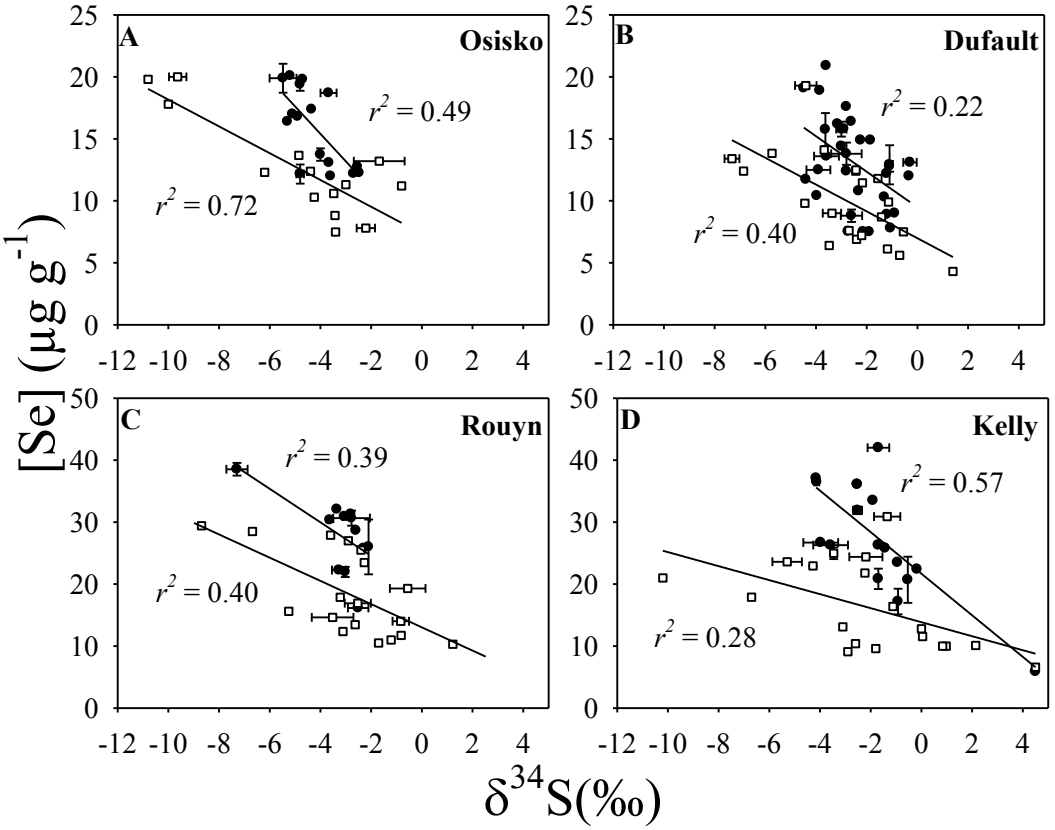


Figure 3. Selenium concentrations ([Se]; μg g^{-1} dry weight; right panels) as a function of sulfur stable-isotope signatures (δ^{34}S; ‰) in yellow perch (closed circles) or in their prey (open squares) from our study lakes. All linear regressions are significant at $P <$ 0.03.

Ignoring differences in yellow perch weight, there was a clear relationship between fish Se concentrations and δ^{34}S signatures in our four study lakes (Figure 3). On the one hand, lower Se concentrations and more positive δ^{34}S signatures were associated with yellow perch that depended on zooplankton, either directly as prey, or indirectly when feeding on planktivorous minnows. On the other hand, higher Se

concentrations and more negative $\delta^{34}S$ signatures were associated with yellow perch feeding on benthic chironomids. These trends were mirrored in the Se concentrations and $\delta^{34}S$ signatures of planktonic and benthic prey (Figures 2 and 3). Consistent with these trends is the fact that trout tend to accumulate more Se when feeding on sediment-based food webs than when feeding on periphyton-based food webs (Orr et al., 2006). Furthermore, lake chub are reported to have higher Se concentrations when they feed on benthic insects than when they do not have access to benthic prey (Phibbs et al., 2011), which results in an inverse relationship between their Se concentrations and $\delta^{34}S$ signatures.

These trends suggest that Se is more bioavailable in anoxic sediment than in surface oxic sediments or the water column. Such a trend has been reported for two groups of *Chironomus* species that feed either on anoxic sediment particles or on superficial oxic sediment particles. These species groups differed in both their $\delta^{34}S$ signatures and their concentrations of cadmium, zinc (Martin et al., 2008; Proulx and Hare, 2014) and Se (Proulx, 2014), with those species feeding on anoxic particles having more negative $\delta^{34}S$ signatures and correspondingly higher Se concentrations (as in our study) but lower concentrations of cadmium and zinc than species feeding on oxic particles. These differences in cadmium and zinc bioaccumulation were not related to the concentrations of cadmium and zinc in bulk sediment and are thus likely explained by differences in the bioavailability of these metals in anoxic and oxic sediments (Martin et al., 2008).

There are several hypotheses that might explain why Se bioavailability is higher in anoxic sediments, all of which depend on the fact that organic forms of Se tend to be more bioavailable than inorganic Se (oxyanions and selenide; Schlekat et al., 2000). First, dissimilatory sulfur bacteria living in anoxic sediments reduce Se oxyanions to more bioavailable forms such as organic selenide (Schlekat et al., 2000). Second, dead planktonic organisms that are deposited as sediment contain mostly organic selenides as opposed to inorganic Se (Doblin et al., 2006). Third, selenite can be reduced to elemental Se (Se^0) by iron oxides at the surface of sediments through abiotic reactions (Chen et al., 2009). These processes can account for over 70% of the reduced organic Se pool in sediments, of which 30 to 40% is generally organic

selenide (Schlekat et al., 2000; Velinsky and Cutter, 1991; Martin et al., 2011). In the oxic water column, Se oxyanions are less readily taken up by primary producers than is organic selenide and the uptake of these inorganic Se species is inhibited by competition with sulfate ions (Ponton and Hare, 2013; Morlon et al., 2006). A recent study by Franz et al. (2013) demonstrated that sedimentary organic selenide, and to a lesser extent elemental Se, are taken up by *Chironomus dilutus*. Furthermore, this insect persisted even when it had accumulated Se concentrations as high as 100 μg g^{-1} d.w. (Gallego-Gallegos et al., 2013), which made it an important source of Se for its predators. Lastly, Schlekat et al. (2000) showed that organic selenide produced by diatom cells and sedimentary bacteria was more bioavailable to the bivalve *Potamocorbula amurensis* than was abiotically formed elemental Se.

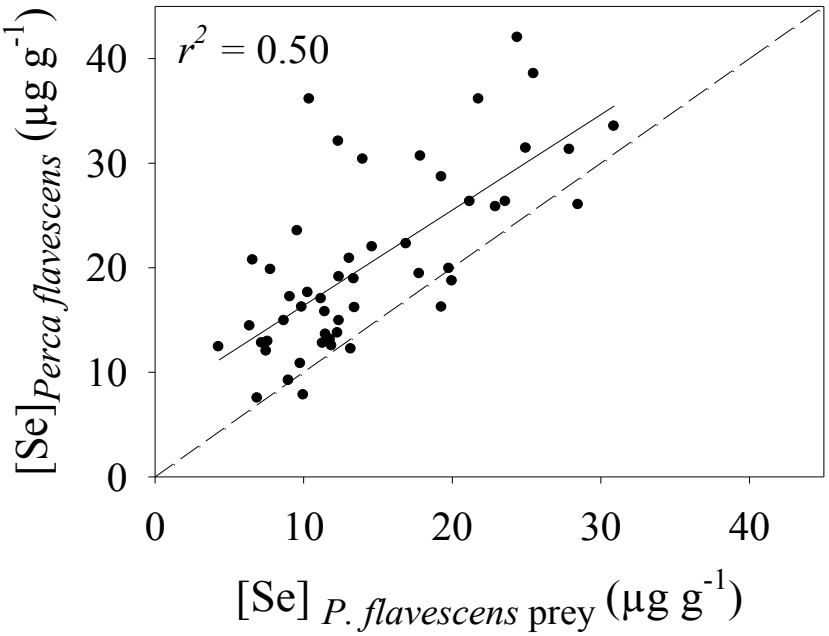


Figure 4. Selenium concentrations ([Se]; μg g^{-1} dry weight) in individual yellow perch from our four study lakes as a function of Se concentrations of prey in their stomachs ($P < 0.001$). The dotted line represents a 1:1 relationship.

Selenium concentrations in yellow perch muscle were generally higher than those in their gut contents (Figure 4; note 1 to 1 line) such that the mean trophic transfer ratio ([Se] in consumer / [Se] in food) in our study lakes was 1.6 ± 0.7 (SD, *n* = 51). This value is at the high end of those reported for various fish species (0.5 to 1.6; Presser and Luoma, 2010). This trophic transfer factor can be used to estimate the maximum Se exposure to yellow perch. In our study lakes, the sediment-feeding insect *Chironomus* had the highest Se concentrations (31 μg g^{-1}) of any yellow perch prey. This high concentration is likely a result of exposure to higher concentrations of bioavailable Se in sediments. An alternative hypothesis is that *Chironomus* larvae have higher Se concentrations than did the other invertebrates because they either take up Se more efficiently from their food (Schlekat et al., 2002) or lose Se less readily (Stewart et al., 2004). The fact that *Chironomus* larvae feeding on deep anoxic sediments tend to differ in their trace elements concentrations from those feeding on surface oxic sediments (Proulx and Hare, 2014 and unpublished data) argues against a general physiological propensity to accumulate higher concentrations of trace elements than the other invertebrates that we studied. Multiplying *Chironomus* Se concentrations by the trophic transfer factor of 1.6 can be used to estimate maximum Se exposure to yellow perch. In the absence of *Chironomus* larvae, other sediment-feeding invertebrates such as tubificid oligochaetes might also be suitable for estimating maximum Se exposure to yellow perch and other fish species that feed on benthos.

To assess the likelihood that yellow perch in our study lakes are suffering toxic effects, we compared their Se concentrations to those reported to induce deformities in the taxonomically-related Centrarchidae (Lemly, 1993). Since the Se concentrations reported for this family are in terms of whole fish, we multiplied our values for Se in muscle by 0.75 (as reported for *Esox lucius*; Muscatello et al., 2006). The highest Se concentration that we measured in yellow perch is at the inflexion point of the exponential curve at which deformities occurred in 15% of individuals, whereas the mean concentrations in yellow perch from Lakes Kelly and Rouyn would correspond to 10% deformed fish and those for Lakes Dufault and Osisko would likely be undetectable (Lemly, 1993).

We also compared our yellow perch Se measurements to the ovarian Se concentration that is reported to produce toxicity in many fish species (20 μg g^{-1} dry weight; DeForest et al., 2011). Using our data for Lohi Lake yellow perch, we estimated that Se concentrations in muscle are 155% of those in gonads (SI, Figure S1; values for Se in ovaries fell within those for testes) such that yellow perch having a muscle Se concentration > 31 μg g^{-1} would be at risk of Se reproductive toxicity. Using this threshold, we estimate that 38% and 33% of the yellow perch from Lakes Kelly and Rouyn, respectively, are at risk of Se toxicity, whereas in our other two study lakes no Se concentrations exceeded this threshold value. The fact that Se concentrations in the biota, sediments and water of most lakes in the regions of Rouyn-Noranda and Sudbury are reported to be lower than those in Lakes Kelly and Rouyn (Ponton and Hare, 2013), suggests that Se is not a major risk factor for fish in the majority of these lakes. Although we did not measure yellow perch population sizes in our four study lakes, we note that the lowest catch per unit effort (data not shown) was in the lakes in which Se toxicity is likely to occur (Lakes Kelly and Rouyn).

A healthy Se intake for humans is reported to be ~140 µg/day (Gartrell et al., 1986), whereas consumption of >400 µg of Se per day is considered to be dangerous (FAO 2001). Given the mean Se values in yellow perch from Lakes Dufault and Osisko (13.9 ± 3.6 µg g^{-1} (dry weight) or 2.8 µg g^{-1} (wet weight; dry to wet weight factor of 5.0; CRESP, 2006)), humans should avoid consuming more than 12 filets per month (filet size of 0.23 kg fresh weight) from these lakes. Since yellow perch Se concentrations in Lakes Kelly and Rouyn are about double those in the other two study lakes, a maximum of six filets per month in these lakes is advisable.

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Supporting Information Available

Table S1 gives the length, wet weight and stomach contents (when present) of all yellow perch (*P. flavescens*) that we collected. Also given are the Se concentrations and S stable isotopic signatures in prey and in yellow perch dorsal muscle. Figure S1 compares Se concentrations in the stomach contents and various organs of *P. flavescens* from Lohi Lake, Ontario. This material is available free of charge via the internet at http://pubs.acs.org.

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Supplementary Information

Table S1: Total length (cm) and wet weight (g; w.w.) of *Perca flavescens*, as well as the selenium concentrations ([Se] µg g^{-1} dry weight) and sulfur isotopic signatures of *P. flavescens* muscle and prey (*P. flavescens* gut contents or wild animals) from the four study lakes. Error terms are analytical standard deviations. Prey items include the insects Chironomidae (Diptera), *Chaoborus* (Diptera), Corixidae (Hemiptera), dragonflies (Anisoptera, Odonata), and damselflies (Zygoptera, Odonata), as well as the amphipod crustacean *Hyalella*, molluscs (Gastropoda, snails; Bivalvia, clams), and water mites (Hydracarina). Selenium concentrations in bold font (>31 µg g^{-1}) represent a risk of Se toxicity (DeForest et al. 2011)

Lake	Length	Weight	[Se] *P. flavescens*	δ^{34}S *P. flavescens*	[Se] prey	δ^{34}S prey	Prey type
	cm	g w.w.	µg g^{-1} d.w.	(‰)	µg g^{-1} d.w.	(‰)	(% of total mass)
	5.5	1	16.1	-2.5 ± 0.4	13.5	-2.6	75% Copepoda, 20% Chironomidae, 5% *Chaoborus*
	9.9	10	**31.3**	-2.8	27.9	-3.6	20% Chironomidae, 80% Cladocera
	10	11	28.7	-2.6	19.3	-0.6 ± 0.7	Zygoptera
	10.2	12	26.0 ± 4.4	-2.1	28.5	-6.7	Chironomidae
	10	12	**32.1**	-3.4	12.4	-3.1	Zygoptera
	10	13	30.6 ± 1.2	-2.8 ± 0.7	17.9	-3.2	40% Trichoptera, 60% Zygoptera
	10.6	14	**30.9**	-3.1 ± 0.1			
	11.3	19	30.3 ± 0.4	-3.6	14.0	-0.8 ± 0.3	90% Zygoptera, 10% *Hyalella*
	12.5	22	22.0 ± 0.8	-3.0	14.6	-3.5 ± 0.8	60% Zygoptera, 10% *Hyalella*, 30% Corixidae
Rouyn	15	44	22.3	-3.3 ± 0.3	16.9	-2.5 ± 0.5	50% Zygoptera, 10% *Hyalella*, 35% Gastropoda
	16.4	53	25.8	-2.3			
	24.7	168	**38.5 ± 1.0**	-7.3 ± 0.4	25.5	-2.4	70% minnows, 30% Zygoptera
					10.3	1.2 ± 1.8	Copepoda
	3.2	1			10.5	-1.7 ± 0.2	minnows (Cyprinidae)
					10.6	-1.2	*Chaoborus*
					11.7	-0.8 ± 0.5	Copepoda
					15.6	-5.2 ± 0.2	Anisoptera
					23.5	-2.3	Trichoptera
					27.0	-2.9	Trichoptera

					29.4	-8.7 ± 0.4	Chironomidae
	6.3	1	5.9	4.5		-3.2	Chironomidae (11 pupae, 11 larvae)
	9.3	5	17.2 ± 2.1	-0.9	9.1	-2.9	Chironomidae (10 pupae)
	9.4	7	22.4	-0.2			
	9.5	8	20.7 ± 3.7	-0.5	6.6	4.5	Cladocera
	10	13	26.7	-4.0 ± 0.7			
	11	13	26.3	-3.6 ± 0.7	21.2		50% Chironomidae, 50% Cladocera
	11	18	25.8	-1.4	22.9 ± 0.1	-4.3	90% Chironomidae (pupae), 10% Zygoptera
	12	21	**36.1**	-2.5	21.8	-2.2	80% Zygoptera, 20% adult Diptera
	12	22	**33.5**	-1.9	30.9	-1.4 ± 0.5	Chironomidae (pupae)
	11.7	22	**37.1**	-4.2			
	12	22	26.3	-1.7	23.6	-5.3 ± 0.6	Chironomidae (pupae)
Kelly	12.7	22	23.5	-0.9	9.6	-1.8	60% Chironomidae (pupae), 40% Zygoptera
	12.4	23	**42**	-1.7 ± 0.4	24.4	-2.2 ± 0.7	33% Cladocera, 66% Zygoptera
	13	24	20.9 ± 1.6	-1.7	13.1	-3.1	60% Chironomidae (pupae), 40% Zygoptera
	15	38	**31.9 ± 0.6**	-2.6 ± 0.2	25.0 ± 0.9	-3.46	Chironomidae (pupae)
	18	72	**36.5 ± 4.9**	-4.2 ± 0.1			
					10.0	1.0 ± 0.2	Copepoda
					10.1	2.1	Copepoda
					11.5	0.1 ± 0.6	*Chaoborus*
					12.8	0	*Chaoborus*
					16.4	-1.1	Zygoptera
					17.9	-6.7	*Chironomus dilutus* larvae
					21	-10.2	*Chironomus plumosus* larvae
	5	1	7.5	-2.1			
	5.2	1	8.9	-1.2			
	5.1	1	9	-0.9			
Dufault	5.2	2	7.5	-2.7			
	5.4	2	10.3	-1.3			
	5.5	2	12	-0.3	7.5	-0.6	Cladocera
	7	3	11.7	-4.4			

	6.3	3	10.4	-4.0		
	6.9	4	12.5	-3.9 ± 0.5	11.9	Ephemeroptera
	7.5	4	17.6	-2.8	10.3	50% Hydracarina, 25% Bivalvia, 25% Chironomidae
	8.5	5	7.5	-1.9	6.9	-2.4 Cladocera
	7.5	5	13.6	-3.6 ± 0.5	11.5	50% Ephemeroptera, 50% Chironomidae (pupae)
	9.9	9	12.8 ± 0.4	-1.1	7.2	-2.2 Cladocera
	10.3	10	12.9 ± 1.6	-1.1	7.6	-2.7 Cladocera
	10	13	14.9	-1.9	8.7	-1.4 Zygoptera
	10.6	13	7.8	-1.1	10	Chironomidae (pupae)
	10	16	18.9	-3.9	13.4	-7.3 ± 0.3 Chironomidae (adults and larvae)
	11.5	17	10.8	-2.3	9.8	-4.4 90% minnows (Cyprinidae), 10% Trichoptera
	11.7	18	16.4	-2.6		
	11	19	14.9	-2.2	12.4	-2.4 70% Chironomidae; 30% Hirudinea
	12.7	22	14.4	-3.0		
	13.5	23	20.9	-3.6		
	13	25	15.8	-2.9		
	13	26	19.1	-4.5	12.4	-6.86 not identifiable
	14.5	40	15.8 ± 1.3	-3.6	11.5 ± 0.1	-2.2 Trichoptera
	17	50	12,6 ± 0.6	-0.8 ± 0.5	11.8	-1.6 Minnow (Cyprinidae)
	16.5	59	15.8 ± 0.6	-3.0 ± 0.2	9.9	-1.1 75% minnows (2 Cyprinidae), 25% Zygoptera
	17.5	62	13.8 ± 0.8	-2.8 ± 0.6	6.4	-3.5 crayfish
	18	65	12.4	-2.8	4.3	1.4 minnows (2 Cyprinidae)
	21.5	112	8.9 ± 0.5	-2.6 ± 0.4	9	-3.4 ± 0.4 crayfish
					5.6	-0.7 *Chaoborus*
					6.1	-1.2 zooplankton
					12.5	-2.4 minnows (Gobiidae)
					13.8	-5.7 Ephemeroptera
					14.1	-3.7 minnows (Gobiidae)
	5.3	1	12	-3.6		
Osisko	5.2	1	16.4	-5.3		
	4.6	1	12.8	-2.5		

4.6	1	20.1	-5.2			
4.9	1	12.3	-2.5			
5.4	2	16.8	-4.9			
5.3	2	17.4	-4.3			
6.3	3	13.1	-3.7			
9.5	6	13.7 ± 0.5	-4.0	12.3	-6.2	90% Chironomidae, 10% Trichoptera
9.4	8	19.9 ± 1.2	-5.5 ± 0.5	19.8	-10.8	Chironomidae
10	11	17	-5.1	11.2	-0.8	90% Zygoptera, 10% *Hyalella*
16	48	18.7	-3.7 ± 0.3	20	-9.6	Chironomidae (adults)
17.8	58	19.4 ± 0.5	-4.8	17.8	-10	Chironomidae (adults)
20.5	97	19.8	-4.7	7.8	-2.2	80% *Perca flavescens*, 20% Bivalvia
21.6	102	12.2 ± 0.8	-4.8 ± 0.2	11.3	-3	minnow (1 Cyprinidae)
21	113	12.2	-2.7	13.2	-1.7	minnows (4 Cyprinidae)
				7.5	-3.4 ± 0.2	*Chaoborus*
				8.8	-3.4 ± 0.2	Copepoda

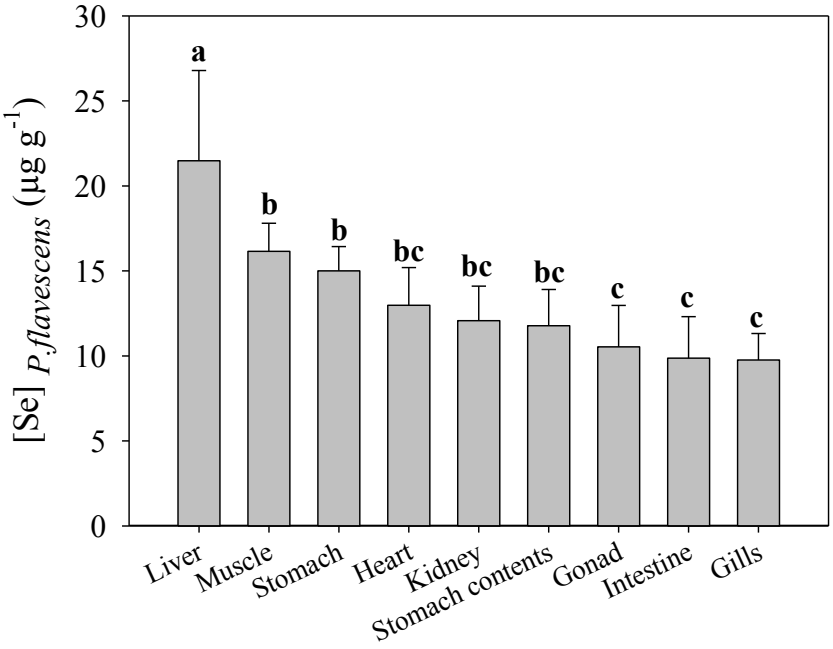

Figure S1: Mean Se concentrations (μg g^{-1} dry weight; + standard deviation; n = 6-8) in the various organs and stomach contents of *P. flavescens* (yellow perch) collected from Lohi Lake, Sudbury, Ontario. Different letters represent significant differences ($P < 0.05$).

QUATRIÈME ARTICLE

WILD YELLOW PERCH (*PERCA FLAVESCENS*) OXIDATIVE STRESS INDUCED BY CADMIUM AND LOW SELENIUM EXPOSURE

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Keywords: Yellow perch, *Perca flavescens*, Glutathione, Malondialdehyde, Selenium, Cadmium, Zinc, Subcellular fractionation, Oxidative Stress.

Abstract

Yellow perch (*Perca flavescens*) collected from 11 lakes in the mining regions of Sudbury (ON) and Rouyn-Noranda (QC) display wide ranges in the concentrations of several potentially-toxic trace elements (cadmium (Cd), nickel (Ni), selenium (Se), and thallium (Tl)) in their livers. To determine if these elements are causing oxidative stress in these fish, we measured several biochemical indicators (glutathione, glutathione disulfide and malondialdehyde) in yellow perch livers. We conclude that 44% of the yellow perch that we collected were at risk of cellular oxidative stress and lipid peroxidation. The essential trace-element selenium (Se) appears to act as an antioxidant because higher liver Se concentrations were coincident with lower proportions of glutathione disulfide compared to glutathione and lower concentrations of malondialdehyde. Furthermore, fish suffering oxidative stress had higher proportions of Cd, Cu and Zn in sensitive subcellular fractions (organelles and heat-denatured proteins) than did fish not suffering from stress. This result suggests that reactive oxygen species oxidize metal-binding proteins and thereby reduce the capacity of fish to bind trace metals. High Cd concentrations in metal-sensitive subcellular fractions likely further exacerbate the negative effects of the low Se exposure.

Introduction

In mining regions, the combined and individual effects of trace metals such as Cd, Cu, Ni and Zn have been the object of many environmental studies (Luoma and Rainbow, 2008; Campbell et al. 2003; Paquin et al., 2002; Balistrieri and Medane, 2014). Although metal smelters emit a large number of trace metals, not all are responsible for toxic effects on aquatic organisms. For example, in Rouyn-Noranda, Quebec, Cd is reported to be the metal that is most likely to cause toxic effects to invertebrates and fish (Borgmann et al., 2004; Campbell et al., 2008), whereas in the vicinity of Sudbury, Ontario, Ni is thought to be the metal that is responsible for toxic effects on aquatic animals (Borgmann et al., 2001; Campbell et al., 2003). To assess

the physiological consequences of exposure to metals in these regions, Giguère et al. (2005) measured oxidative stress in young of the year yellow perch (*Perca flavescens*) and reported that cellular stress was lower at higher metal concentrations in this fish. They suggested that the explanation for this counter-intuitive result was that metal-exposure induced production of the metal-binding protein metallothionein (MT), which acted as an anti-oxidant (Variengo et al., 2000) in these fish.

We test an alternative hypothesis to explain the counter-intuitive results of Giguère et al. (2005). These researchers did not consider the influence of Se, which is also in the emissions of smelters located near their study lakes (Ponton and Hare, 2013). This essential element (Young et al., 2008; Janz, 2011) is a strong anti-oxidant (Rayman, 2000) that protects animals from cancer (Ganther, 1999), as well as the toxic effects induced by mercury (Hg) (Whanger, 1992) and Cd (Wang et al., 2013; Trabelsi et al., 2013). This protective effect occurs over a narrow concentration range in that Se concentrations only 4 times higher than the minimum necessary for cell functioning can be toxic and cause deformities in fish embryos (Presser and Ohlendorf, 1987; Lemly, 1993). We suggest that the anti-oxidant properties of Se could explain the reduction in fish oxidative stress reported by Giguère et al. (2005).

To test this hypothesis, we collected yellow perch of various sizes from 11 lakes in two mining regions and measured the trace metals studied by Giguère et al. (2005; Cd, Cu, Ni and Zn) as well as Se and Tl in these fish. We also fractionated yellow-perch liver tissues to measure trace element concentrations in subcellular fractions that are sensitive to metals (organelles and enzymes) compared to those in which metals are detoxified (bound to metallothionein-like proteins). Lastly, we measured three indicators that have been used to evaluate oxidative cellular stress, that is, malondialdehyde (MDA), glutathione (GSH) and glutathione disulfide (GSSG). Our results should help risk assessors to better understand the interactions among trace elements in aquatic systems.

Methods

Collection of Yellow Perch. We collected yellow perch (*Perca flavescens*) from mid-May to mid-June in 2010 and 2011 from 11 lakes located near metal smelters located in the cities of Sudbury, Ontario (Lakes Hannah, Kelly, Lohi, McFarlane, Tilton) and Rouyn-Noranda, Quebec (Lakes Dufault, Duprat, Kinojevis, Osisko, Pelletier, Rouyn). Selenium (Ponton and Hare, 2013) and trace-metal (Hare et al., 2008; Proulx and Hare, 2014) concentrations are reported to vary widely in animals from these lakes.

In each year, we collected 5-12 yellow perch in the littoral zone of each lake using a 0.5-cm mesh-size seine net (75 m × 1 m). Fish collected encompassed a wide range in wet weights (7-168 g) and feeding habits (Ponton and Hare, 2015). Live yellow perch were held in a cooler with aerated lakewater until they were weighed, measured and sacrificed by decapitation and decerebration in a mobile laboratory. The liver was removed from each fish and placed in a 1.5 mL, acid-washed (15% v/v; Omnitrace grade HNO_3), polypropylene, centrifuge-tube that was flash frozen in liquid nitrogen for transport to the laboratory where it was held at -80 ^{o}C until analysis.

Sample Preparation. Eeach liver was thawed on ice and cut into five parts, ranging in weight from 20 to 150 mg wet weight, for the measurement of: (1) MDA, (2) total glutathione, (3) GSSG, (4) trace elements, (5) trace-element subcellular partitioning and MT. Appropriate buffers were added at a ratio of 3:1 (buffer (mL): tissue (mg)) and each liver subsample was homogenized, on ice, using a plastic pestle fixed to a bench drill, for 3 seconds at 30 second intervals for a total of 5 minutes. Phosphate buffer (NaCl: 8 g L^{-1}; KCl: 0.2 g L^{-1}; Na_2HPO_4: 1.44 g L^{-1}; KH_2PO_4; 0.24 g L^{-1}; all ACS reagents) was used in all cases, with the addition of a thiol scavenger for GSSG measurements (to remove GSH; 3 µL of scavenger per 10 µL buffer) or butylated hydroxytoluene (BHT; 5 mM) to prevent oxidation during MDA measurements. Following homogenization, 200 µL was removed for later use in calculating mass balances to verify the centrifugation protocol. Homogenates were centrifuged (Micromax, Thermo IEC) at 800 x *g* for 10 minutes to remove intact cells, cellular

membranes, and nuclei, then 10 µL of supernatant was collected for measuring protein concentrations prior to freezing at -80 ^{o}C until analysis. Protein concentrations were measured using the Bradford method (Bradford, 1976; Bio-Rad Protein Assay Kit).

Malondialdehyde (MDA) Analysis. We used a lipid-peroxidation microplate assay kit (model FR22, Oxford Biochemical Research Inc.) to measure MDA concentrations in yellow perch livers. This assay is based on the reaction of one molecule of MDA with two molecules of N-methyl-2-phenylindole to yield a stable chromophore. We began the assay by digesting 140 µL of liver supernatant in 455 µL of N-methyl-2-phenylindole and 105 µL of hydrochloric acid in a 45 ^{o}C bath for one hour. The digested samples were centrifuged (Micromax, Thermo IEC) at 15,000 x *g* for 15 minutes and their absorbance read in a 96-well microplate at 586 nm (Bio UV-visible spectrophotometer coupled with a 50 MPR microplate reader, Cary, Varian). A standard curve, using MDA provided in the kit, was used to verify the method's accuracy ($r^2 = 0.99$; $p < 0.0001$).

Reduced Glutathione (GSH) and Glutathione Disulfide (GSSG) Analysis. We used a microplate assay kit (model GT40, Oxford Biochemical Research Inc.) to measure the concentrations of total glutathione (GSH+GSSG), GSSG and, by difference, GSH (Tietze, 1969). When samples are collected, glutathione peroxidase can continue to function and GSH can be oxidized by atmospheric oxygen. This can lead to increased levels of GSSG. To counter act this process and "lock-in" the GSSG level, we add the thiol scavenger when we took out the samples from the freezer (-80 ^{o}C). It is important to split the samples; one for total glutathione (no scavenger) and one for GSSG (with scavenger). The scavenger quickly binds to thiol groups taking GSH out of the measurement. The GSSG sample is then exposed to glutathione reductase in the presence of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid ; DTNB). The glutathione reductase converts the GSSG to two GSH. The GSH reacts with the DNTB very quickly before it can react with the scavenger.

We first deproteinated samples with ice-cold metaphosphoric acid (MPA; 5%) and then centrifuged them at 1,000 x *g* (Micromax, Thermo IEC) for 10 minutes at 4 ^{o}C. We combined 50 µL each of deproteinated liver supernatant (with or without scavenger), Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid; DTNB) and recombinant glutathione reductase in a 96-well microplate that was shaken for 5 minutes before 50 µL of NADPH was added to each well prior to reading the samples at 412 nm for 10 minutes. The rate of reduction over ten minutes is proportional to the GSSG (with scavenger) or total glutathione (without scavenger) concentrations. GSH and GSSG standards provided with the kit were used to obtain calibration curves from 0.05 to 1.5 µM for GSSG and from 0.1 to 3 µM for GSH (both $r^2 > 0.99$ and $P < 0.0001$).

Subcellular Trace-Element Partitioning. We fractionated yellow-perch livers to measure trace-element concentrations in three subcellular fractions, that is, organelles (mitochondria, Golgi apparatus, lysosomes), heat-denatured proteins (HDP; enzymes, amino acids, nucleic acids) and heat-stable proteins (HSP; cysteine, GSH and metallothionein-like proteins). Samples of liver homogenate were first centrifuged (Micromax, Thermo IEC) at 800 x *g* for 10 minutes to separate granules and cell debris (pellet) from the remaining fractions (supernatant). The supernatant was then centrifuged at 100,000 x *g* for 60 minutes to obtain the organelles fraction as a pellet. The resulting supernatant was heated at 80 ^{o}C for 10 minutes and held on ice for 1 hour prior to centrifugation at 50,000 x g for 10 minutes to obtain heat-denatured proteins (pellet) and heat-stable proteins (supernatant). We used a Sorvall WX Ultra Series 100 centrifuge for these high-speed centrifugations (Thermo Scientific with a F50L-24×1.5 rotor). We measured the total protein concentration in this supernatant so that we could express trace-element concentrations in terms of total protein concentrations in the HSP fraction. Trace element concentrations in organelles and HDP are on a dry weight basis.

Trace-element Analysis. Frozen liver subsamples and pellets (debris and granules; organelles; HDP) from the subcellular fractionation procedure were freeze-dried, weighed and placed in acid-washed 12-mL high density polyethylene tubes where

they were digested for 2 days in concentrated HNO_3 (Aristar grade; 100 µL per mg tissue dry weight), heated at 60 ^{o}C for 12 hours, then held for 1 day in concentrated hydrogen peroxide (40 µL per mg tissue dry weight; Trace Select Ultra grade); digestate volume was completed to 1 mL per mg tissue dry weight using ultrapure water. Heat stable protein samples were diluted and acidified to 10% HNO_3 (v/v). Certified reference material (lobster hepatopancreas, TORT-2, National Research Council of Canada) was submitted to the same digestion procedure. Selenium of mass 82 g mol^{-1} was measured by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS; Thermo Elemental X Series) and interferences with bromine (Br) and hydrogen ($^{81}Br + {}^{1}H$) were corrected using a standard curve of several Br concentrations. All the trace elements (except Tl; not present in TORT-2) were within the certified ranges for this standard.

Calculations and Statistical Analyses. Differences in mean liver trace element concentrations among lakes were assessed by an ANOVA followed by a non-parametrical Dunn's test on ranks. A toxicity threshold for MDA was determined by piece-wise iterative regression as follows. Starting with 10 values having GSH/GSSG ratios >> 0 (Figure 1A), we regressed these values against MDA concentrations, then added one additional value at a time and repeated the regression until as many observations as possible were included. The goal of these iterations was to minimize the error at the ordinate (MDA concentration at $X = 0$) while maintaining a slope that was not significantly different from 0. The slope became significantly different from 0 when n reached 28 values, which corresponded to a threshold value of 0.45 ± 0.06 µmol MDA g^{-1} protein (the y intercept ± standard error of the intercept). We conclude that individuals exceeding this value, that is, those having an MDA concentration ≥ 0.51 µmol g^{-1} protein, are at risk of oxidative stress; these yellow perch are represented by open symbols in the following figures. Relationships between all variables studied (e.g. MDA, [TE], % GSSG) were examined by bivariate scatter plot. When those plots indicated a relationship, regression models (e.g. exponential rise or decay, linear) were tested after necessary assumptions (normality and homoscedasticity) were satisfied. Statistical analyses were performed using JUMP

9.0.0 (SAS Institute Inc.) and Sigmaplot (version 10.0; Systat Software). A p value of 0.05 was use as a threshold for statistical test.

Results and Discussion

Trace Element Concentrations in Yellow Perch Liver. Concentrations of the essential element Cu showed no significant differences among lakes or between regions (Figure 1). In contrast, previous studies in the same regions have reported lake-to-lake differences in yellow perch liver Cu concentrations, which may be explained by the fact that these studies included lakes that were not contaminated by trace elements (Giguère et al., 2004; Pierron et al., 2009). Likewise, concentrations of the essential element Zn differed little among lakes, with only yellow perch from Kelly Lake having significantly lower Zn concentrations that those of fish from the other study lakes (Figure 1). This limited variability in yellow perch Zn concentrations is consistent with the results of a previous study in these regions (Niyogi et al., 2007).

In contrast to Cu and Zn, concentrations of the essential elements Ni and Se varied more widely among fish from our study lakes. Nickel concentrations were notably higher in the livers of yellow perch from Sudbury-area lakes than in those collected from lakes near Rouyn-Noranda (Figure 1). This difference is consistent with the fact that there has been a long history of Ni mining and smelting in the Sudbury area (Gunn, 1995), whereas, in the Rouyn-Noranda area, Cu is the main metal of interest (Couillard et al., 2004). Similar differences in Ni concentrations between yellow perch and planktonic insect larvae from the two regions have been reported previously (Pierron et al., 2009; Ponton and Hare, 2009, respectively). The measured differences in Ni concentrations among Sudbury-area fish cannot be explained simply by proximity to the smelters, since Kelly Lake is closer to the smelters than are Hannah and McFarlane Lakes yet Ni concentrations in fish from the former lake were much lower than those in the latter two lakes (as were those of Cd, Cu and Zn). It is likely that the very high productivity of Kelly Lake, due to the influence of sewage effluents, reduces the availability of these trace elements to

organisms at the base of the food web in this lake. Selenium concentrations in yellow perch liver also varied among lakes but, unlike Ni, its concentrations did not differ markedly between the two regions (Figure 1). Variations in fish Se concentrations are likely explained by both physico-chemical (pH, water residence time) and biological (food web structure) differences among the study lakes (Ponton and Hare, 2013).

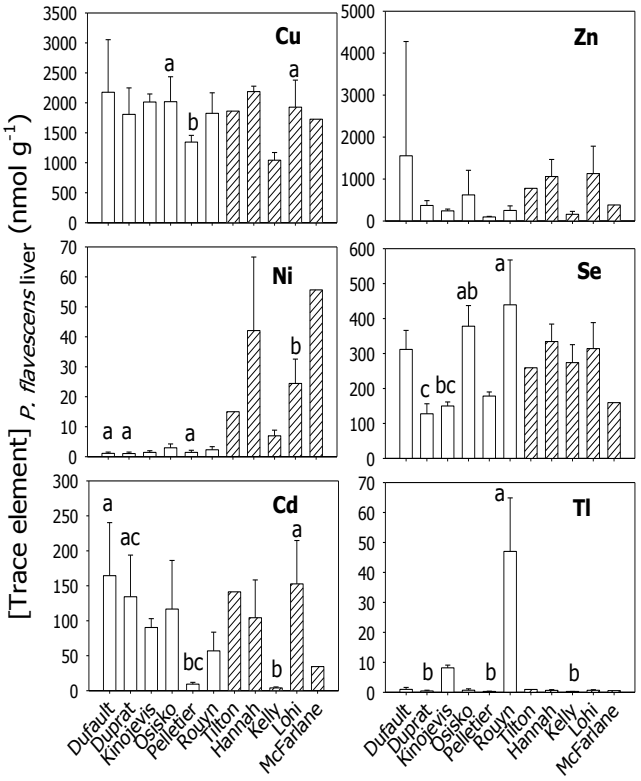

Figure 1: Mean (± standard deviation; nmol g^{-1} dry weight) copper (Cu), zinc (Zn), selenium (Se), thallium (Tl), nickel (Ni) and cadmium (Cd) concentrations in the livers of *Perca flavescens* from lakes near Rouyn-Noranda (open bars) and Sudbury (striped bars). For a given trace element, significant differences among lakes are represented by different letters.

Liver concentrations of the non-essential elements Cd and Tl differed widely among fish from the various lakes (Figure 1). Cadmium concentrations in animals from lakes in this study area are known to be determined by a variety of physico-chemical (pH, dissolved organic matter) and biological (food web structure) factors (Giguère et al., 2004; Hare et al., 2008). Thallium concentrations were very low in

the livers of yellow perch from our study lakes, with the exception of Lake Rouyn, which is located directly downstream from the settling pond of the Rouyn-Noranda smelter (MDDEP, 2011), and Lake Kinojevis, which is a few kilometers downstream from Lake Rouyn (Figure 1). In contrast, Tl concentrations in a Sudbury lake located downstream from a smelter settling-pond (Kelly Lake; Sadiq et al., 2002) were quite low. The difference may be explained by the fact that in the 1990's the feed to the Rouyn-Noranda smelter changed from local ores to a mix of ore and recycled electronics which contain Tl semiconductors (Nriagu, 2003) and may be the source of the Tl in the lakes downstream from the smelter. Alternatively, this difference between Lakes Rouyn and Kelly could be related to the fossil fuel used in smelter processes (e.g., coal versus natural gas) since Tl is known to be released during the burning of coal (Nriagu, 2003).

Oxidative Stress. A lower ratio of glutathione relative to its disulfide (GSH/GSSG) is thought to favor lipid peroxidation, which can be assessed by measuring MDA (McCay and Powell, 1976). As expected, low GSH/GSSG ratios were correlated with high MDA concentrations in yellow perch livers (Figure 2A), which suggests that our two independent measurements of oxidative stress risk are in good agreement.

Using a piece-wise iterative regression technique (see Statistical Analysis), we estimated that at MDA concentrations above a threshold value of 0.45 ± 0.06 μmol MDA g^{-1} protein (± standard error (SE); open symbols in Figures 2-6), lipid peroxidation is likely to have a negative effect on fish in these populations. This result suggests that 44% of the 50 yellow perch that we collected were at risk of cellular damage through lipid peroxidation because of a low GSH/GSSG ratio in their liver cells. Individual yellow perch from a given population showed wide differences in their GSH/GSSG ratio and MDA concentrations (Figure 2) that were not related to their size (data not shown), which suggests that large numbers of fish are needed to accurately assess population risk. Note that young (< 1 year) yellow perch from 3 (Dufault, Hannah, Osisko) of our study lakes are reported to have MDA concentrations (< 0.3 μmol g^{-1} of hepatic protein; Giguère et al., 2005) below our calculated threshold for toxic effects, which suggests that very young individuals were not suffering from lipid peroxidation in these lakes. MDA concentrations in the

livers of rainbow trout exposed to Cd and/or chromium in the laboratory (0.5 to 5.4 μmol g^{-1}; Talas et al., 2008) fall within the range of the concentrations that we measured in wild yellow perch (0.1 to 6.7 μmol g^{-1}).

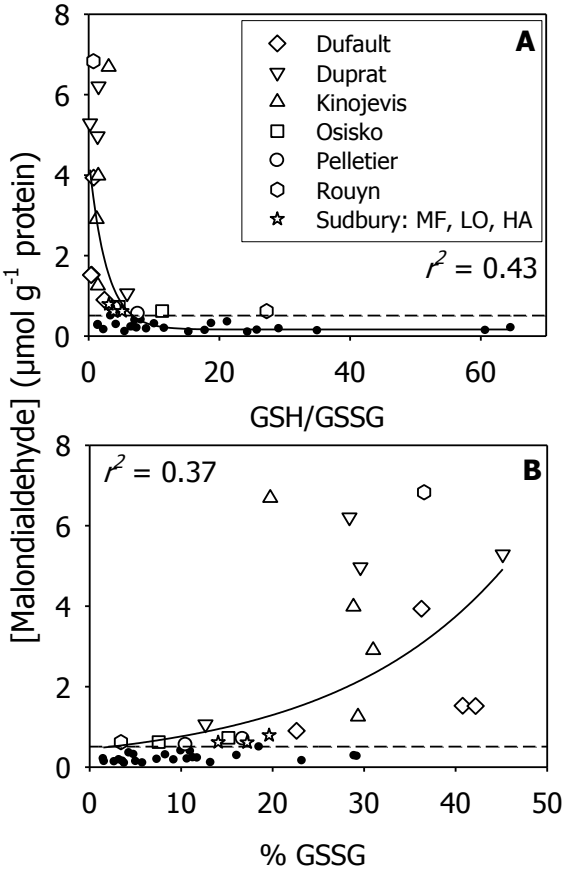

Figure 2: Concentrations of malondialdehyde (MDA; μmol g^{-1} protein) as a function of (A) the ratio of reduced glutathione (GSH) to glutathione disulfide (GSSG) and (B) the percentage of GSSG (of the total GSH+GSSG) in the liver of yellow perch. Closed symbols represent livers with MDA concentrations < 0.51 μmol g^{-1}, as indicated by the broken line. Sudbury lakes (stars) include Hannah (HA), Lohi (LO) and McFarlane (MF). *P* values < 0.0001.

An alternative means of visualising the data shown in Figure 2A is to relate the concentration of MDA to the percentage of GSSG (Fig. 2B) or GSH (regression not shown) relative to total glutathione (GSH+GSSG). Figure 2B suggests that at GSSG values above 30% there is a high risk of lipid peroxidation, as reflected in the higher concentrations of MDA. This percentage is consistent with the fact that

healthy cells and tissues should have < 15% of their glutathione in its oxidized form (GSSG) if they are to effectively cope with reactive oxygen species such as hydrogen peroxide (Ishikawa and Sies, 1976; Mannervick et al., 1976).

Giguère et al. (2005) reported that high concentrations of several trace metals (Cd, Cu, Ni, Zn) were correlated with lower MDA concentrations in young of the year yellow perch from our study areas. Noting that fish exposed to these high metal concentrations had increased concentrations of the metal-binding protein MT, they suggested that these increased MT concentrations allowed yellow perch to maintain the concentrations of metabolically active metals at safe levels. However, MT also acts as an antioxidant that could counteract lipid peroxidation (Viarengo et al., 2000). We suggest that the results of Giguère et al. (2005) could also be explained by the fact that the concentrations of the metals that they measured were likely correlated with those of Se (they did not measure this element), since all of these trace elements have common sources (the metal smelters), and it is Se that is acting to protect fish from lipid peroxidation. Selenium is known to favour the reduction of reactive oxygen species due to its presence in glutathione peroxidase, which catalyses the reduction of hydrogen peroxide (H_2O_2) to water (Flohé et al., 1973).

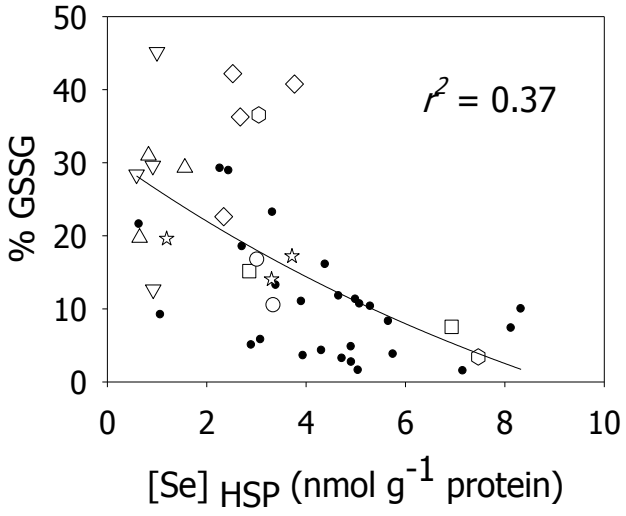

Figure 3: Percentage of glutathione disulfide (% GSSG) as a function of Se concentrations in the heat-stable protein fraction (HSP; nmol g^{-1} protein) in the liver of yellow perch. Symbols are explained in Figure 2 legend. *P* value < 0.0001.

The percentage of GSSG in yellow-perch liver showed a significant decline as Se concentrations in the heat-stable proteins fraction (HSP) increased (Figure 3). Note that in the whole liver, the relationship between Se and GSSG was not significant. Selenocysteine is likely found in the HSP fraction and is the most active form of the selenium amino acids in cells (Janz, 2011; Mannervick et al., 1976; Wang and Rainbow, 2010). The relationship shown in Figure 3 suggests that elevated Se concentrations reduce the concentrations of reactive oxygen species and consequently the proportion of GSSG (Chaudière and Ferrari-Iliou, 1999; Rayman, 2000) in yellow-perch livers.

Subcellular Partitioning of Trace Elements. The majority of the yellow perch that were undergoing oxidative stress (open symbols in Figure 4) had higher proportions of Cd, Cu and Zn in cell fractions that are potentially sensitive to metals (organelles and HDP) than did fish that were not undergoing oxidative stress (closed symbols and associated regression line in Figure 4). This apparent spillover into sensitive fractions in stressed individuals occurred along the entire range in metal concentrations rather than in only the most contaminated fish. Jacob et al. (1998) suggested that, in mammals, reactive oxygen species (ROS) and GSSG oxidize metal-containing clusters of MT, which reduces its affinity for essential metals that are then transported to the enzymes that require them. In contrast, GSH keeps metals bound to MT through its reducing potential. Our results for yellow perch are in agreement with the observations of Jacob et al. (1998) since they suggest that low Se concentrations induce oxidative stress (Figure 3) and that this stress induces a spillover of metals into putative metal-sensitive cell fractions in the liver of *P. flavescens* (Figure 4).

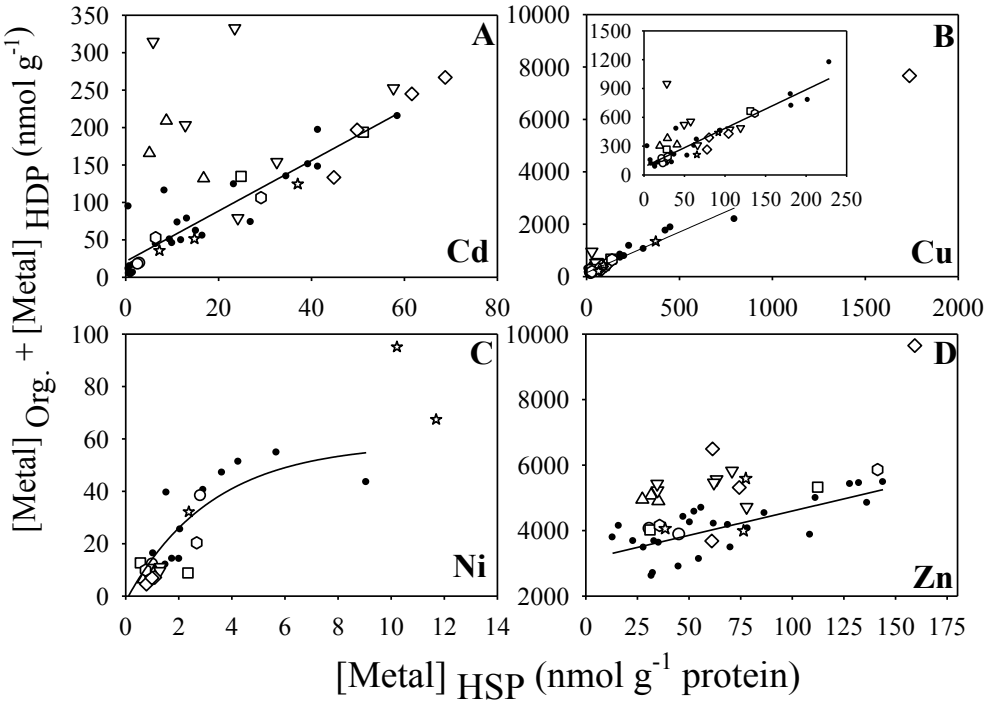

Figure 4: Cadmium, copper, nickel and zinc concentrations in the sensitive subcellular compartment (sum of metal concentrations in the heat denatured protein and organelle fractions; $[M]_{HDP}+[M]_{Org.}$; nmol g^{-1}) as a function of their concentrations in the heat stable protein fraction (HSP; nmol g^{-1} protein) of yellow perch livers. Symbols are explained in the Figure 2 legend. Metal concentrations in the sensitive fractions are on a dry weight basis but those in the HSP fraction are on the basis of the protein content of the final supernatant (see *Methods*). Regressions are based on solid symbols only, that is, non-stressed fish.

To better compare trends in the concentrations of Cd and Zn that spillover into sensitive cell fractions, we used the following equation:

$$\Delta[\mathrm{M}] = [\mathrm{M}]_{\mathrm{HDP}} + [\mathrm{M}]_{\mathrm{org.}} - (a_{\mathrm{M}}[\mathrm{M}]_{HSP} + b_{\mathrm{M}}) \qquad (1)$$

where $\Delta[M]$ (nmol g^{-1}) is the difference between the concentration of a given metal in the sensitive fractions of individual fish (sum of the HDP and organelles (org.) fractions) and the concentration of that metal represented by the regression lines in Figure 4A and D. Note that Cd and Zn are often bound to the same molecules in

animal cells (Klaassen et al., 1999; Rabenstein, 1976). Figure 5 shows the curvilinear regression between Δ[Cd] and Δ[Zn]. We interpret the exponential form of this curve to mean that when MT is oxidized (either by ROS or GSSG; Jacob et al., 1998), Zn is released initially, because it has a lower affinity for MT, whereas Cd is released at higher ROS concentrations. We did not calculate Δ[Cu] and Δ[Ni] because these metals appear to be less impacted by oxidative stress, although spillover was apparent in fish from Lakes Duprat, Dufault and Kinojevis for Cu (small panel in Figure 4B) and in one fish from Hannah Lake in the case of Ni (Figure 4C).

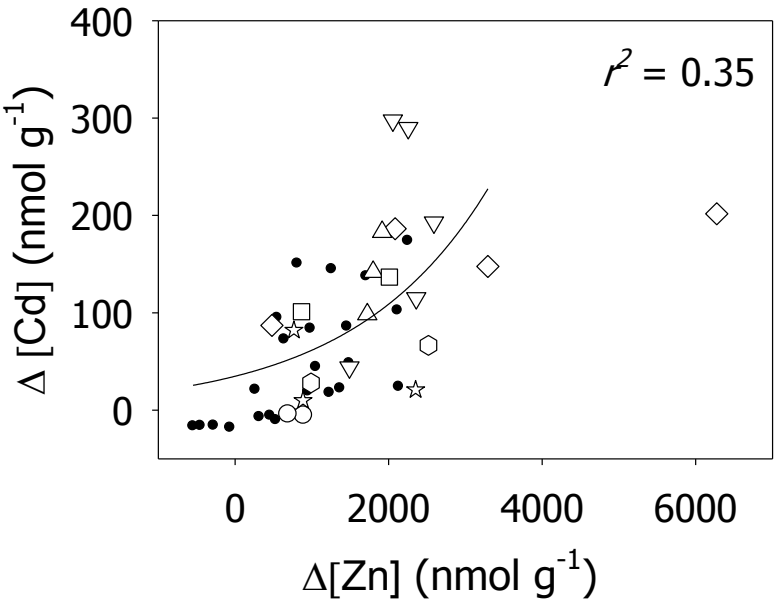

Figure 5: Difference in the delta cadmium concentrations (Δ[Cd]; nmol g^{-1}) as a function of the difference in the delta zinc concentrations (Δ[Zn]; nmol g^{-1} both calculated from equation 1) in yellow perch livers (one extreme Δ[Zn] value excluded from regression). Symbols explained in Figure 2 legend. *P* value < 0.0001.

Cadmium and Selenium Antagonistic Effects. We observed a correlation between MDA concentrations and those of ΔCd (Figure 6A), but not with those of ΔZn (regression not shown). This suggests that the negative physiological effects in fish (mainly those from Rouyn-Noranda suffering from cellular stress) are likely due to Cd exposure, as has been reported by others (Levesque et al., 2003; Borgmann et al., 2004; Campbell et al., 2008). In contrast, we observed that Se concentrations in yellow-perch liver are negatively correlated with those of MDA (Figure 6B), which suggests that Se protects fish from lipid peroxidation (as mentioned above; Figure 3). This is the first reported evidence for an antagonistic effect between Se and Cd in nature.

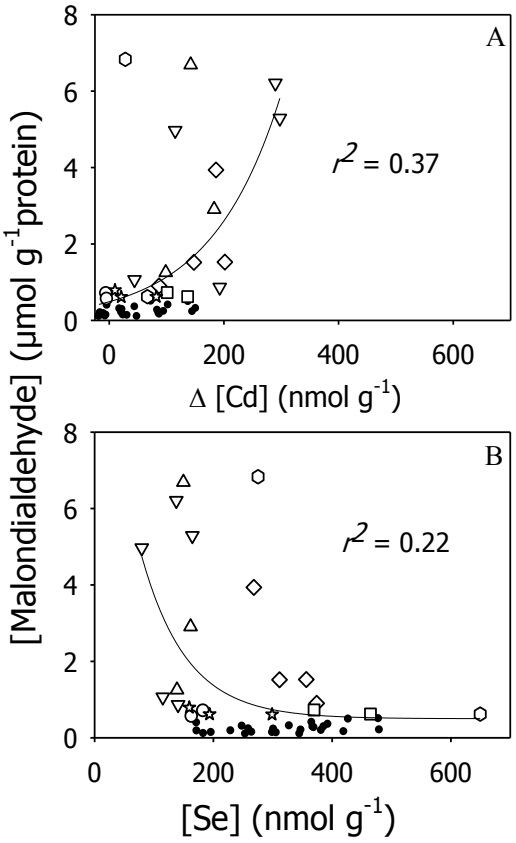

Figure 6: Concentrations of malondialdehyde (MDA; µmol g^{-1} protein) as a function of (A) Δ[Cd] (nmol g^{-1}; calculated from equation 1; $P < 0.0001$) and (B) total Se concentrations ([Se]; nmol g^{-1} d.w.; $P = 0.005$) in *P. flavescens* livers. Symbols explained in Figure 2 legend.

In the laboratory, rats and mice co-exposed to Cd and Se are reported to have lower MDA concentrations than rats and mice exposed to Cd alone (Trabelsi et al., 2013; Wang et al., 2013). Likewise, mussels (*Mytilus edulis*) exposed to both Cu and Se are reported to have less DNA damage than when exposed to Cu alone (Trevisan et al., 2010). Furthermore, Se and Zn have shown antioxidant activity in Cd-exposed zebra fish (*Danio rerio*; Banni et al., 2011). Selenium has also been shown to protect rainbow trout (*Oncorhynchus mykiss*) from oxidative stress when this fish is exposed to Cd and chromium (Talas et al., 2008). Lastly, the authors of a study showing that pike (*Esox lucius*) exposed to multiple trace elements from uranium-mining suffered less cellular damage than do pike from nearby uncontaminated lakes (Kelly and Janz, 2009), hypothesized that Se in the effluent may be responsible for this protective effect.

We conclude that low Se exposure reduces antioxidant activity in yellow perch and that the subsequent increase in reactive oxygen species induces the oxidation of metal-binding proteins, which leads to the release of Cd and Zn that then bind to sensitive cell fractions. High Cd concentrations in these cell fractions further exacerbate the lipid peroxidation induced by low Se exposure. We suggest that a minimum Se concentration of 300 nmol g^{-1} (24 µg g^{-1}) in the liver of yellow perch is required to protect this fish from lipid peroxidation and consequent higher metal concentrations in sensitive cell fractions. Although this recommended Se concentration should protect fish from oxidative stress, further studies are needed to determine if this concentration of Se induces teratogenic effects (Presser and Ohlendorf, 1987; Lemly, 1993) in yellow perch.

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CINQUIÈME ARTICLE

UPTAKE AND SUBCELLULAR DISTRIBUTIONS OF CADMIUM AND SELENIUM IN TRANSPLANTED AQUATIC INSECT LARVAE

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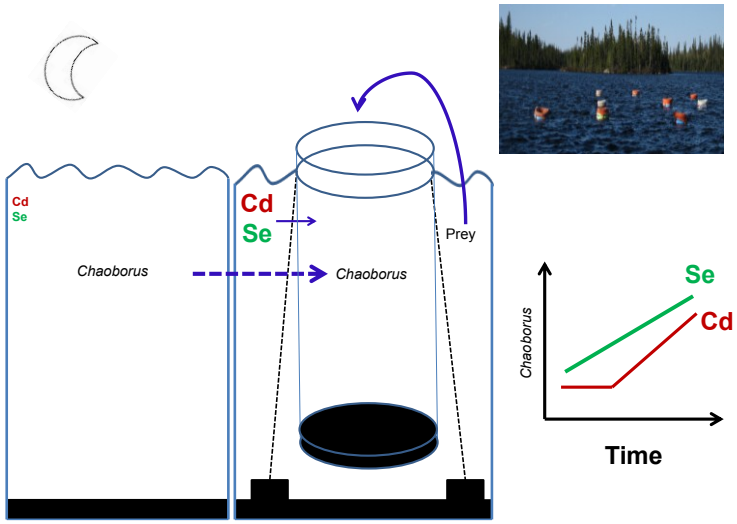

Keywords: Transplantation, Subcellular partitioning, Cadmium, Selenium, *Chaoborus*, Metallothionein.

Abstract

We transplanted larvae of the phantom midge *Chaoborus punctipennis* from a lake having lower concentrations of Cd and Se (Lake Dasserat) to a more contaminated lake (Lake Dufault) located near to a metal smelter in Rouyn-Noranda, Quebec. Transplanted individuals were held in mesh mesocosms for up to 16 days where they were fed with indigenous contaminated zooplankton. Larval Cd and Se burdens increased over time, and came to equal those measured in indigenous *C. punctipennis* from contaminated Lake Dufault. Larval Se burdens increased steadily, whereas those of Cd showed an initial lag phase that we explain by a change in the efficiency with which this insect assimilated Cd from its prey. We measured Cd and Se in subcellular fractions and found that larvae sequestered the majority (60%) of the incoming Cd in a detoxified fraction containing metal-binding proteins, whereas a minority of this non-essential metal was in sensitive fractions (20%). In contrast, a much higher proportion of the essential element Se (40%) was apportioned to metabolically-active sensitive fractions. Larvae took up equimolar quantities of these elements over the course of the experiment. Likewise, Cd and Se concentrations in wild larvae were equimolar, which suggests that they are exposed to equimolar bioavailable concentrations of these elements in our study lakes.

Introduction

Lakes near metal-smelting operations are usually contaminated by several metals.[1] A case in point is the region of Rouyn-Noranda (Quebec, Canada), where the concentrations of copper (Cu), zinc (Zn), cadmium (Cd) and selenium (Se) are elevated in the water, sediments and animals of many nearby lakes.[2-4] Of these elements, Cd is thought to be the major contributor to toxic effects in invertebrates[5] and fish[6] in these lakes, whereas Se has been reported to influence the uptake of Cd by aquatic invertebrates.[7]

Because larvae of the phantom midge *Chaoborus* are tolerant to trace elements, they have been used to monitor bioavailable Cd[8] and Se[3] in lakewater from this region. Although Se is an essential element, *Chaoborus* larvae do not regulate its concentrations (as they do those of Cu and Zn)[9] such that their Se concentrations are related to those in water and in their planktonic prey.[3] At high concentrations, both Cd[5-6] and Se[10] can be toxic to aquatic animals; Se can also protect animals from

oxidative stress[11] caused by high concentrations of metals such as Cd in their cells.[12-14] These interactions are not well understood in aquatic animals such as *Chaoborus*, especially under conditions of changing Cd and Se exposure.

To address this knowledge gap, we measured and compared Cd and Se concentrations in indigenous *Chaoborus punctipennis* larvae from 12 lakes situated near metal-smelters in Rouyn-Noranda and Sudbury (Ontario). From these lakes, we chose a low-metals lake and a high-metals lake and transferred *C. punctipennis* larvae from the former to the latter. Larvae were held in mesh mesocosms that allowed the free passage of contaminated lakewater and algae, but restrained the contaminated zooplankton prey that we added to the mesocosms.

We measured Cd and Se uptake over 16 days, both in whole larvae and in various subcellular fractions, to determine: (i) at what rates this insect takes up these elements; (ii) if the Cd and Se contents of transplanted larvae attain that of those in indigenous larvae within the duration of the experiment; and (iii) to which subcellular fractions the incoming Cd and Se are bound. Measurements of subcellular partitioning allowed us to assess the likelihood that these elements would either cause toxic effects or be safely detoxified.[15]

Methods

Study Sites. In June 2011, we transplanted larvae of the phantom midge *Chaoborus punctipennis* from Lake Dasserat, situated upwind from the Horne metal smelter in Rouyn-Noranda, Quebec, to Lake Dufault located < 1 km from the smelter and adjacent to several tailings ponds (Supporting Information, Table S1).[16] Although aerial emissions from the smelter were substantially reduced in the late 1980s,[17] trace-element concentrations in the latter lake have remained high in both water and plankton (Supporting Information, Table S1) due in part to continuous inputs from the drainage basin.[16] To compare Cd and Se concentrations in free-living larvae, we also measured these elements in *C. punctipennis* larvae, collected in 2007, from 12 other lakes situated near either Rouyn-Noranda (Marlon, Opasatica) or the metal-smelting center of Sudbury, Ontario (Clearwater, Crooked, Crowley, Hannah, McFarlane, McCharles, Raft, Ramsey, Silver, Tilton).[18] In addition, we collected bulk

zooplankton (53-125 μm) from 7 Sudbury-area lakes (Crooked, Crowley, Hannah, Laurentian, McFarlane, McCharles, Ramsey). Lakewater collection (diffusion samplers) and analyses (pH, DOC, trace elements; Supporting Information, Table S1) were performed using previously published methods.[3,19]

Invertebrate Collection and Experimental Design. *Chaoborus* larvae and zooplankton crustaceans were collected at night by hauling a 64 μm mesh-aperture plankton net horizontally in the epilimnion (1-3 m depth). Samples were transported in clean plastic bags filled with lakewater to the laboratory, where fourth-instar *Chaoborus* larvae were removed and identified to species.[20]

Chaoborus punctipennis from Lake Dasserat were grouped into nine lots of ~300 larvae each and held in plastic containers at 4 °C. The following day, one lot of larvae was added to each of nine mesocosms anchored at a water depth of 4 m in Lake Dufault. The cylindrical (1.2 m height by 0.5 m diameter) mesocosms were constructed of 64 μm mesh-aperture netting that allowed the free passage of lake water and phytoplankton but not the larger planktonic crustaceans on which late-instar *Chaoborus* larvae feed.[21] Lake Dufault sediments (without *Chaoborus* larvae) were placed in the bottom of each mesocosm as a refuge for transplanted *C. punctipennis* larvae because they feed in the water column at night then burrow in the sediment during the day to avoid predators.[22] Since *Chaoborus* larvae do not feed when in sediments[23] and do not take up Cd from water,[21] they must take up Cd[22] and Se[24] from the prey they consume in the water column at night.

To feed *C. punctipennis* larvae in mesocosms, we collected crustacean prey by hauling a 64 μm mesh-aperture plankton net horizontally in the epilimnion of Lake Dufault at night. The collected zooplankton were sieved using a 500 μm mesh-aperture sieve to eliminate the large invertebrates on which *C. punctipennis* cannot feed.[25,26] In the laboratory, prey density in plankton subsamples was estimated under a microscope. Based on these estimates, sufficient prey was added to each microcosm to attain densities of ~660 ± 15 (n = 5) prey per *C. punctipennis* larva. This procedure was repeated every 2-3 days. By this means, we maintained prey numbers well in excess of measured larval consumption.[21]

Larvae were collected from mesocosms at night, when they were in the water column, using a 100 μm mesh-aperture plastic sieve placed on the end of a wooden pole. Three replicate samples of 2-12 pooled larvae were collected from Lake Dasserat (day 0) and from three randomly-selected mesocosms on days 3, 6, 9, 11, 13, and 15 for total Cd and Se analyses. Subcellular trace element partitioning was measured in larvae collected both from Lake Dasserat (day 0) and from the mesocosms on days 6, 12 and 16 (30 larvae per sample; n = 3-5 samples). Lastly, larvae were collected from Lake Dufault by hauling a 64 μm mesh-aperture plankton net horizontally in the epilimnion (1-3 m depth) on day 9 of the experiment. We assumed that trace element concentrations in day 9 larvae were representative of those in Lake Dufault larvae throughout the duration of our experiment.

Larvae for total Cd and Se analyses were placed on acid washed (15% HNO_3; v/v) pieces of pre-weighed Teflon sheeting held in acid-washed 1.5-mL polypropylene micro-centrifuge tubes (Fisher Scientific) and then frozen at -20 °C. Larvae for the measurement of subcellular trace-element distributions were held in acid washed, pre-weighed, 1.5-mL micro-centrifuge tubes, frozen in liquid nitrogen and then held at −80 ^{o}C.

Subcellular Trace-Element Partitioning. We used a subcellular trace-element partitioning procedure, optimized for *Chaoborus*,[19, 27] that separated larvae into six operationally-defined fractions: (1) “debris” (nuclei and cellular debris); (2) “granules” (NaOH-resistant fraction); (3) mitochondria; (4) “organelles” (lysosomes and microsomes); (5) heat-denatured proteins (HDP) including enzymes; and (6) peptides and heat-stable proteins (HSP) including metallothionein and glutathione. The subcellular partitioning procedure (Supporting Information, Methods) is based on a series of differential centrifugations as well as an NaOH digestion and a heat treatment.

Cadmium and Selenium Analyses. All micro-centrifuge tubes, Teflon sheeting and other labware were soaked in 15% nitric acid (v/v; Omnitrace grade, Fisher Scientific), rinsed seven times with ultrapure water (18 MΩ cm) and dried under a laminar-flow hood to prevent inadvertent trace element contamination. Samples for total Cd and Se analyses in whole larvae and in centrifugation pellets were freeze-dried (72 h; FTS Systems TMM, Kinetics Thermal Systems), weighed (XS205 Dual Range Analytical Balance, Mettler Toledo) and digested in 100 µL of nitric acid (PlasmaPure Plus, SCP Science) per mg dry weight for two days at room temperature and then heated at 65 ºC for 6 h. After cooling, 40 µL per mg dry weight of hydrogen peroxide (H_2O_2; Optima grade, Fisher Scientific) was added and samples were held for 1 d in this solution after which time the digestate volume was completed to 1 mL per mg dry weight with ultrapure water.

Total Cd and Se concentrations were measured using an inductively-coupled plasma - mass spectrometer (ICP-MS; Thermo Elemental X Series). Samples of a certified reference material (lobster hepatopancreas, TORT-2, National Research Council of Canada) were concurrently subjected to the same digestion procedure and analyzed. Mean element recovery (n = 7) in samples of TORT-2 was 96 ± 3% and 90 ± 9% (± SD) for Cd and Se, respectively. For the subcellular partitioning study, a mass balance calculation was carried out by comparing element burdens estimated from the 100-μL aliquots removed from the first larval homogenate to the sum of Cd and Se values measured in the various subcellular fractions. Mean (± standard deviation) recoveries were close to 100% (Cd, 93 ± 13 %; Se, 96 ± 19 %).

Statistical Analyses. Relationships between temporal changes in Cd and Se concentrations in whole insects were initially examined in bivariate scatterplots. When these indicated a relationship, regression models were tested when the necessary assumptions (normality with the Shapiro-Will test and homoscedasticity using the Levene test) were satisfied. A t-test for independent samples was used to identify significant differences in dry weight, total trace element concentration, or total trace element quantity between indigenous larvae collected from Lake Dufault on day 9 and transplanted larvae collected on day 15 of the experiment. At the subcellular level, student t-tests were applied to compare trace element quantities in

detoxified and metal-sensitive trace-element compartments on each sampling day. Temporal differences in the quantities of trace-elements in each subcellular fraction were assessed using the non-parametric Kruskal-Wallis test, followed by the Tukey-Kramer HSD test on ranks. Percentage data were arcsine transformed prior to applying parametric tests (ANOVA followed by HSD test). Statistical analyses were performed using STATISTICA version 6.1 (StatSoft, Tulsa) and JUMP 9.0.0 (SAS Institute Inc.). A probability level of 0.05 was used as the threshold for statistical significance.

Results and Discussion

Cadmium and Selenium Concentrations in Wild *Chaoborus* Larvae and their Prey. Cadmium and Se concentrations in *C. punctipennis* larvae from Lake Dufault (96 and 62 nmol g^{-1}, respectively) were higher than those in larvae from Lake Dasserat (27 and 14 nmol g^{-1}, respectively; Supporting Information, Table S1). These values fell close to the regression line for Cd and Se in this insect species from 12 other eastern Canadian lakes (Figure 1), which suggests that our two study lakes are representative of those in this region. In transferring larvae between these two lakes, our goal was to monitor larval Cd and Se as their values increased from those in Lake Dasserat to those in Lake Dufault. We note that the relationship between Cd and Se concentrations in larvae is approximately linear (Figure 1). Furthermore, because these insects are likely to take up most of their Cd[21] and Se[28] from their zooplankton prey, there was also a linear relationship between these elements in zooplankton crustaceans from a subset of these lakes (Supporting Information, Figure S1).

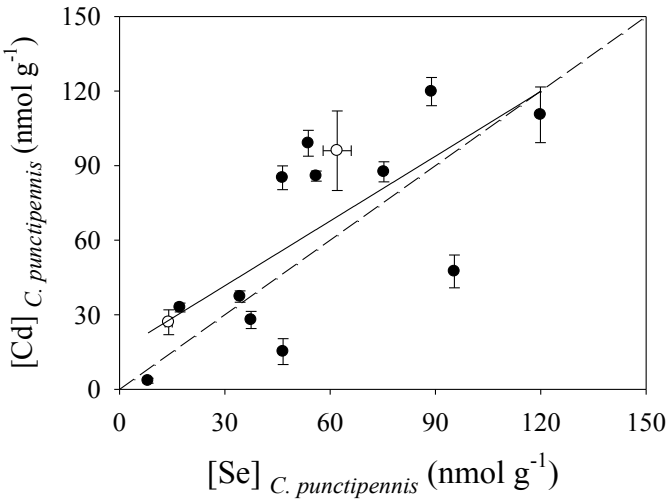

Figure 1. Linear relationship (solid line; r^2 = 0.52, P = 0.003) between mean (± SD, n = 4-6) Cd and Se concentrations (nmol g^{-1}) in *Chaoborus punctipennis* (closed symbols) collected from 12 eastern Canadian lakes and comparative values for Lakes Dasserat and Dufault (open symbols). The broken line is the 1 to 1 line.

Temporal Changes in Larval Mass and Trace Elements. The mean dry weight (dw) of *C. punctipennis* larvae in mesocosms (Supporting Information, Figure S2) increased significantly from day 3 to day 15 (P = 0.02, r^2 = 0.79), which corresponds to a larval growth rate of 0.015 ± 0.004 (± standard error (SE)) mg $larva^{-1}$ day^{-1}. This value is very close to that reported previously for this species (0.013 ± 0.01 mg $larva^{-1}$ day).[22] Since transplanted larvae gained weight in the mesocosms, the zooplankton densities offered were clearly sufficient for larval growth. Note that the weight of larvae from Lake Dasserat (day 0) was higher than that of larvae in mesocosms on the first sampling day (3). However, this day 0 value is likely an artifact because, whereas all larvae collected from mesocosms were used to measure larval weight, those on day 0 were selected from a large number of larvae from which we inadvertently selected the largest larvae. Furthermore, *Chaoborus* larvae held in the laboratory show no measurable loss of weight over 3 days (data not shown).

We estimated the growth rate constant (k_g) for transplanted *C. punctipennis* larvae using the equation

$$W = W^0 e^{k_g t} \qquad (1)$$

where W^0 is the initial weight (mg dw larva^{-1} at day 3, that is, ignoring the day 0 value). Resolving this equation by least square analysis yields a growth rate constant of 0.03 ± 0.01 d^{-1} (n = 18; P = 0.01), which is close to the value of 0.02 ± 0.01 d^{-1} previously reported for *C. punctipennis*[21] larvae held in mesocosms at a prey to predator ratio (675) similar to that used in our experiment (660).

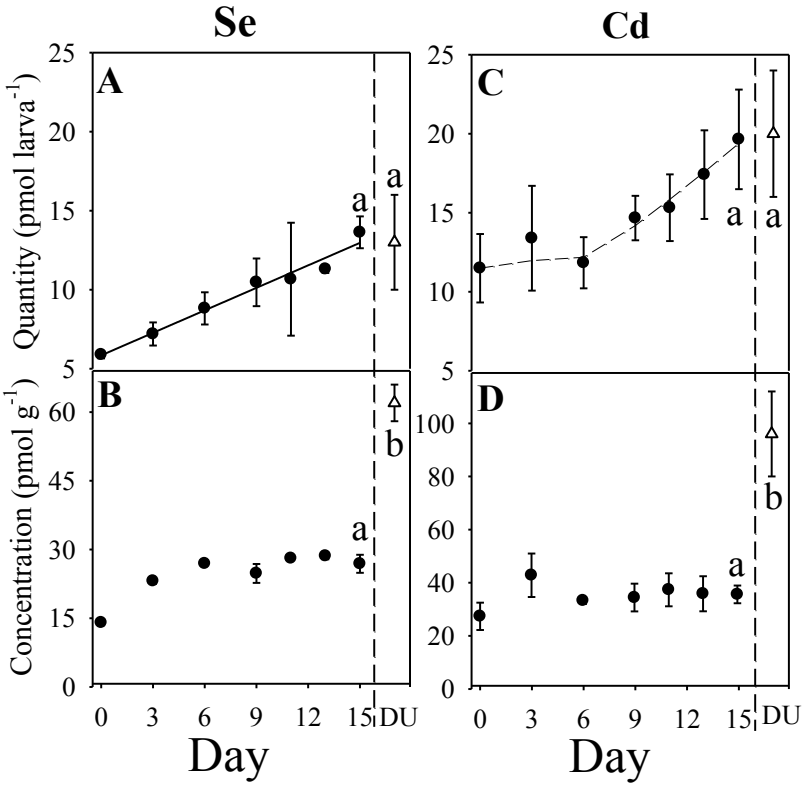

Figure 2. Temporal changes in the mean (± SD, n = 3-5) quantities (upper panels) and concentrations (lower panels) of Se (left panels) and Cd (right panels) in transplanted *Chaoborus punctipennis* larvae (closed circles) and in indigenous *C. punctipennis* larvae from Lake Dufault (DU, open triangles). The modeled dashed lines in panel C were generated using equation 3 (see text). Different lower-case letters indicate significant differences (Student's t-test, $P \leq 0.05$).

The Se content of *C. punctipennis* larvae transplanted to Lake Dufault increased linearly over time (r^2 = 0.79; P = 0.0001) such that by the end of the experiment it equaled that of indigenous Lake Dufault larvae (Figure 2A). The Cd content of larvae transplanted to Lake Dufault remained stable until day 6 and thereafter increased over time until it too reached that of indigenous Lake Dufault larvae by the end of the

experiment (Figure 2C). Note that the time needed to equal the Cd and Se contents of indigenous larvae would likely have been longer had prey ingestion rates been lower (as in the study of Munger et al.).[21]

There was no change over time in the concentrations of Cd and Se in transplanted larvae because increases in larval Cd and Se contents (Figure 2A,C) were matched by increases in larval weight (Supporting Information, Figure S2). For this reason, we consider temporal changes in trace element quantities rather than concentrations. Because indigenous Lake Dufault larvae were smaller (Supporting Information, Figure S2), but contained as much Cd and Se as did transplanted larvae at the end of the experiment (Figure 3A,B), their concentrations of these elements were higher than those in transplanted larvae (Figure 2B,D). Larvae from contaminated Lake Dufault weighed less than those from Lake Dasserat (Supporting Information, Figure S2), likely because there was less zooplankton food available in this lake due to the effects of metals on these planktonic crustaceans.[29,30] The fact that larval Cd content showed no increase during the first week of the experiment (Figure 2C), contrasts with the observation that the Cd content of *C. punctipennis* larvae increased steadily during the first week of a similar transplantation experiment.[21] To explore the reasons for this difference, we used a biodynamic model[31] to describe temporal changes in *Chaoborus* Cd concentrations ($[Cd]_{Chaoborus}$) as follows:

$$\frac{d[\mathrm{Cd}]_{Chaoborus}}{dt} = AE \times IR \times [\mathrm{Cd}]_{\mathrm{prey}} - k_e[\mathrm{Cd}]_{Chaoborus} - k_g[\mathrm{Cd}]_{Chaoborus} \qquad (2)$$

where the first term represents Cd uptake from prey, the second term represents physiological Cd loss and the third term represents Cd dilution by larval growth. Equation 2 ignores Cd uptake from water because *Chaoborus* larvae have been shown to take up all their Cd from food.[21, 32] Cadmium uptake from prey is represented as the product of the assimilation efficiency (*AE*; unitless), which is the proportion of the ingested Cd that crosses the gut membrane, the rate at which prey are ingested (*IR*; g of prey g^{-1} larval weight d^{-1}), and the Cd concentration in prey from Lake Dufault ($[Cd]_{prey}$; Table 1). The term k_e (d^{-1}) represents the rate constant for physiological Cd loss.

Since we maintained prey concentrations in the mesocosms in excess of larval needs, we assumed that the rate at which *C. punctipennis* larvae ingested prey (*IR*) was constant during the experiment. This supposition is supported by the fact that larval growth increased linearly throughout the experiment (Supporting Information, Figure S2) and by the absence of a lag in the accumulation of Se (Figure 2A). We conclude that the initial lag in Cd accumulation cannot be explained by an initially low ingestion rate. However, some studies have shown that increases in the concentration of metal-binding proteins in invertebrates and fish can increase the efficiency with which these animals assimilate Cd.[33, 34] With this possibility in mind, we hypothesize that a low initial (days 0-6) concentration of metal-binding proteins in the transplanted larvae led to a low Cd assimilation efficiency and Cd content, but that the concentration of metal-binding proteins, and thus AE, increased after day 6 leading to an increase in Cd content after this time (Figure 2C).

To test the plausibility of this hypothesis, we estimated Cd assimilation efficiencies separately for days 0-6, when larval Cd content did not vary, and days 6-15, when it increased, using the integrated form of equation 2, that is,

$$Q_{Cd-Chaoborus} = \frac{W_0 \cdot AE \cdot IR \cdot [Cd]_{prey}}{k_g + k_e}(e^{k_g t} - e^{-k_e t}) + Q^0_{Cd-Chaoborus} e^{-(k_g + k_e)t} \qquad (3)$$

where the quantity of Cd in *C. punctipennis* larvae is given by $Q_{Cd\text{-}Chaoborus}$. For this purpose we used an efflux rate constant (k_e) of 0.018 ± 0.005 d^{-1} and an ingestion rate (*IR*) of 0.9, as reported for a field experiment with similar prey densities.[21] This k_e value is comparable to values estimated for some mayflies,[35] but low compared to those reported for other aquatic insects.[35] We used our estimate of the growth rate constant (0.03 ± 0.01 d^{-1}) obtained from the data in Figure 1. The concentration of Cd in prey (zooplankton) was measured in Lake Dufault (66 ± 3 nmol g^{-1}). Using these values in equation 3 yielded an estimated Cd AE of 2.3% for days 0-6 and 4.6% for days 6-15. Model lines based on these estimates fit closely the measured data (Figure 2C; $r^2 = 0.95$; $P < 0.0001$), which suggests that changes in Cd assimilation efficiency during the experiment could explain the measured changes in larval Cd content. Although these AE estimates are low in comparison to those reported some predatory invertebrates,[36] they are close to that reported previously for *C. punctipennis* offered

similar prey densities in a transplant experiment (2%)[21]. At lower prey densities, AE values for this species can reach 18%.[21, 22] Note that some other *Chaoborus* species can assimilate up to 58% of the Cd that they ingest.[22]A low Cd AE could help to explain the persistence of *C. punctipennis* in metal-contaminated lakes.

The reason that the Se content of *C. punctipennis* larvae did not follow the same time-course as Cd can be explained by the fact that this essential element is a constituent of many types of invertebrate proteins and thus its uptake would be less influenced by the concentrations of metal-binding proteins. Indeed, the fact that Se tends to be more efficiently assimilated than does Cd is consistent with the variety of metabolic processes in which it is involved. For example, another member of the same insect order, the midge *Chironomus riparius*, is reported to assimilate the majority of the Se that it ingests (55-97%),[37] as do crustaceans from several groups (mysids, copepods and amphipods: 45-80%).[38] These high values for Se contrast markedly with the low Cd assimilation efficiencies reported for *C. punctipennis* in this and previous studies (2-18%).[22] We note that Se is reported to reduce oxidative stress,[11] which can result in greater metal binding to metallothionein[39] and consequently a higher efficiency of Cd assimilation.[40] This potential interaction between the two elements suggests that it would be useful to measure ambient Se concentrations when attempting to predict the bioaccumulation and effects of Cd.

Subcellular Partitioning of Cadmium and Selenium. To determine how the Cd and Se accumulated by transplanted *C. punctipennis* larvae were stored in their cells, and if the subcellular Cd distributions in transplanted larvae were similar to those in chronically exposed animals, we measured these elements in various operationally-defined subcellular fractions.[19] Considering internal trace element "speciation" can be more effective for demonstrating potential effects than are measurements of total concentrations of contaminants. [15]

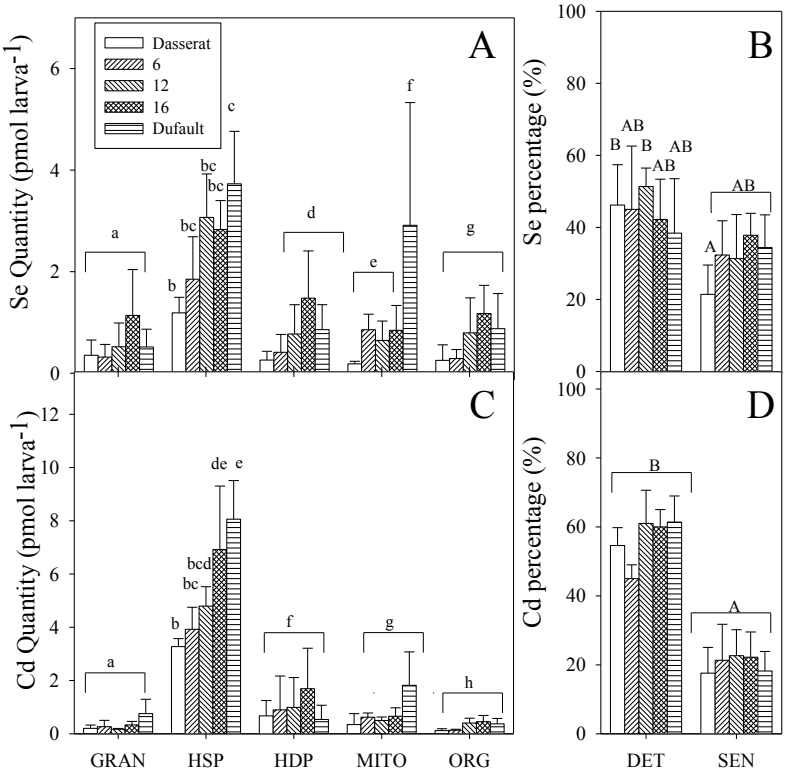

Figure 3. Temporal changes in the mean (± SD, n = 3-5) quantities (A, B) and percentages (C, D) of Se (upper panels) and Cd (lower panels) in subcellular fractions and compartments of *Chaoborus punctipennis* larvae collected either directly from Lake Dasserat (day 0), or transplanted from Lake Dasserat and held in mesocosms in Lake Dufault for 6, 12 or 16 days, or collected from Lake Dufault. Fractions (A, B) include: GRAN = granules, HSP = heat-stable proteins, HDP = heat-denatured proteins, MITO = mitochondria and ORG = organelles, but exclude debris. Fractions (excluding debris) have been grouped into two compartments (C, D) depending on whether the trace elements they contain have been detoxified (DET = HSP + GRAN) or could cause toxicity (sensitive; SEN = HDP + MITO + ORG). Different letters indicate a significant difference (Kruskal-Wallis test followed by Tukey-Kramer HSD test, $P \leq 0.05$) between times for a given fraction (A and C) or between times and compartments (B and D).

The highest quantities of larval Cd and Se were found at all times in the HSP (heat-stable protein) fraction (Figure 3A,C; $P < 0.05$), that is, the fraction containing metal-binding proteins and peptides. In proportional terms, 30-40% of the Se and 52-59% of the Cd (excluding cell "debris") were found in this fraction. The Se in this

fraction is likely present as either seleno-cysteine in metal-binding proteins or covalently bound to sulfur in cysteine residues.[41] The high proportion of Cd in the HSP fraction is typical for *C. punctipennis* in nature.[19] Cadmium in this fraction is likely bound to metallothionein,[42] which could contribute to the ability of this species to maintain viable populations in Cd-contaminated waters.[8, 19]

Larvae from the more contaminated lake (Dufault) had significantly more Cd (P = 0.004; Figure 3C) and Se (P = 0.007; Figure 3A) in the HSP fraction than did indigenous larvae from the less contaminated lake (Dasserat). The mean quantity of Se (Figure 3A) and Cd (Figure 3C) in the HSP fraction of transplanted larvae tended to increase over time (P = 0.0007), although these differences were non-significant for Se (Figure 3A; P = 0.07). The quantity of Cd in the HSP fraction did not show a significant increase until day 16. This time lag is consistent with the fact that in chronically metal-exposed *Chaoborus* there appears to be a threshold body concentration below which larvae do not activate their detoxification machinery and above which larvae induce metal-binding ligands to prevent toxicity.[19] After 16 days in the mesocosms, the quantity of Cd in the HSP fraction of transplanted *Chaoborus* larvae reached that of indigenous individuals from Lake Dufault (Figure 3C).

The quantities of Cd and Se in the other subcellular fractions showed no significant change over time (Figure 3A,C). In mitochondria, the quantity of Se was significantly higher (P = 0.008) in larvae from the contaminated lake (Figure 3A), which is consistent with the fact that mitochondria can generate high concentrations of reactive oxygen species[43] and Se is known to protect Cd-exposed animal cells from oxidative stress.[44, 45]

We grouped the various subcellular fractions into two compartments according to the likelihood that Cd, or an excess of Se, in these compartments would exert a toxic effect (sensitive compartment) or not (detoxified compartment).[15] The proportion of Se in a given compartment was constant over time (Figure 3B) and for a given time there was no significant difference ($P > 0.05$) in the proportion of Se in the detoxified and sensitive compartments, with the exception of larvae from Lake Dasserat that had a significantly (P = 0.04) smaller proportion of their Se in the sensitive compartment than in the detoxified compartment (Figure 3B). Some aquatic

insects[37] and crustaceans[46] maintain an even higher proportion of their Se in the sensitive compartment, which is consistent with the essential role that this element plays in animal metabolism. However, its presence in sensitive cell fractions can result in toxic effects if its concentration exceeds the narrow range between what is required by an animal and what is toxic.[28] In absolute terms, the quantity of Se in the potentially-sensitive compartment (mitochondria + HDP + "organelles") tended to increase throughout the experiment (data not shown) such that by day 16 it was significantly higher (P = 0.01) than that in larvae from Lake Dasserat (day 0) and equal to that in larvae from contaminated Lake Dufault (P = 0.70). A similar trend was observed for the quantity of Se in the detoxified compartment (HSP + granules), although the difference between the values observed on day 16 and those obtained in Lake Dasserat was not significant (P = 0.06).

In contrast to Se, the proportion of Cd in the sensitive compartment was significantly lower than that in the detoxified compartment at all times and in both study lakes (Figure 3D). Maintaining Cd at low concentrations in the sensitive compartment is clearly a priority for animals since this element has no essential role to play in their cells and thus can cause toxicity if it exceeds the cells' capacity to bind or eliminate this metal.[47] There was no significant temporal change in the proportion of Cd in either compartment, with that in the sensitive compartment remaining at approximately 20% of the total (Figure 3D). The Cd content of the detoxified compartment increased throughout the experiment such that the value at day 16 was about 2-times higher than that at day 0 (P = 0.008) and not significantly different (P = 0.48) from that of indigenous Lake Dufault larvae.

Overall, our subcellular partitioning results suggest that larvae exposed to low ambient concentrations of Cd and Se are able to manage higher concentrations of these elements as well as insects that have been chronically exposed to high concentrations of Cd and Se.

Relationships Between Cadmium and Selenium in *Chaoborus* Larvae. Relationships between Cd and Se in the metal-sensitive compartment (Figure 4B, open symbols; r^2 = 0.97, P = 0.002), as well as in its component fractions (P < 0.004 and r^2 = 0.96-0.99 for the mitochondria and "organelles" fractions), were

approximately linear, whereas the comparable relationship for the detoxified compartment was curvilinear (Figure 4B, solid symbols; r^2 = 0.99; P = 0.01). Consequently, the relationship for whole transplanted larvae was slightly curvilinear (r^2 = 0.89, P = 0.01, Figure 4A).

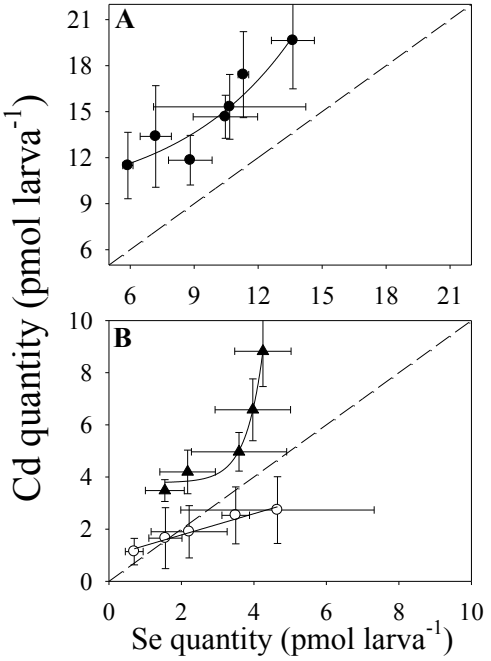

Figure 4. Relationships between the quantities (pmol larva^{-1}) of Cd and Se in A: whole larvae, and B: detoxified (HSP + NaOH-resistant fractions; solid symbols) and potentially-sensitive (HDP + MITO + ORG; open symbols) compartments of indigenous and transplanted *Chaoborus punctipennis* larvae. Complete fraction names are given in the caption of Figure 3. The broken line is the 1 to 1 line.

The total quantities of Cd and Se taken up by transplanted larvae (Figure 4A) were approximately equal (slope of 1.0 (± 0.2 (SE), r^2 = 0.84, P = 0.004) for linear relationship). Likewise, approximately equimolar relationships were measured between the concentrations of Cd and Se in wild *C. punctipennis* larvae (0.9 (± 0.2 (SE); Figure 1) and in their prey (1.0 (± 0.2 (SE); Supporting Information, Figure S1). These relationships suggest either that the distribution of these elements is linked at the subcellular level or that *C. punctipennis* larvae are exposed to similar concentrations of Cd and Se in our study lakes. The former possibility seems unlikely

because the subcellular distributions of the two elements differed somewhat (Figures 3 and 4); measurements of Cd and Se in cytosolic fractions of various molecular weights would be useful for exploring this possibility. The latter possibility is supported by the fact that Cd and Se concentrations in wild *Chaoborus* larvae from our study area are related to those of bioavailable Cd^{8} and Se^{3} respectively, in lake water. Consequently, the ratio of Cd to Se in *Chaoborus* larvae from regions in which the bioavailable concentrations of Cd and Se differ widely is likely to differ from 1.

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ASSOCIATED CONTENT

Details of the protocol used for the determination of subcellular trace element distributions in *Chaoborus* larvae are given as supporting information. Also included are data on our study lakes (Dufault and Dasserat; Table S1), the relationship between Cd and Se concentration in zooplankton from seven lakes (Figure S1) and changes in the weight of *Chaoborus* larvae during the transplantation experiment (Figure S2). This information is available free of charge via the Internet at http://pubs.acs.org/.

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Uptake and Subcellular Distributions of Cadmium and Selenium in Transplanted Aquatic Insect Larvae

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Supporting Information

Contents

Method Used for the Subcellular Partitioning of *Chaoborus* Larvae. Larval samples were thawed on ice in Tris buffer adjusted to pH 7.4 with HCl (25 mmol L^{-1}, OmniPur, EM Science, affiliate of MERCK KGaA, Darmstadt, Hesse, Germany) at a ratio 1:2 (insect wet weight: buffer volume; mg : µL) and each sample was homogenized using a Pellet Pestle (Kontes, Vineland, NJ, USA) for 2 s at 30 s intervals for a total of 5 min. A 100-µL aliquot was removed from the homogenate for mass-balance quality-control measurements and the remainder was centrifuged at 800 x g for 15 min at 4 ^{o}C. The resulting supernatant was removed and the pellet was re-suspended in homogenization buffer to a final ratio of 1:4. The sample was then homogenized a second time for 40 s using an Analog vortex mixer (Fischer Scientific) to rupture remaining whole cells. This second homogenate was also centrifuged at 800 x g. The two supernatants were combined and placed on ice until further separation.

The final pellet was suspended in 500 µL of ultrapure water, heated for 2 min at 100 ºC and digested with an additional 500 µL of 1N NaOH (99.998%, Sigma-Aldrich) at 65 ºC for 1 hour. Centrifugation of the digestate at 10,000 x g for 10 min at ambient temperature was performed to separate the NaOH-resistant fraction (granule-like fraction) from the solubilized fraction containing cellular debris and nuclei.

The combined supernatants from both homogenization steps were centrifuged at 10,000 x g for 30 min at 4 ^{o}C to yield the mitochondrial fraction. The remaining supernatant was centrifuged at 100,000 x g for 60 min at 4 ^{o}C, giving a pellet containing other organelles (lysosomes and microsomes). The resulting supernatant was held at 80 ºC for 10 min, left on ice for 1 hour and then centrifuged at 50,000 x g for 10 min at 4 ^{o}C to obtain a pellet containing heat-denatured proteins (HDP) and a supernatant containing heat-stable proteins (HSP). High-speed centrifugations (≥ 50,000 x g) were performed using a Beckman TLA-100 centrifuge equipped with a TLA-100.3 rotor (Beckman Counter), whereas lower-speed centrifugations were performed using an IEC Micromax centrifuge (Thermo IEC, Arlington, MA, USA). Each supernatant was acidified with nitric acid (final [HNO_3] 10%; v/v; Optima grade, Fisher Scientific) and kept at 4 ^{o}C until Cd and Se analyses. Pellets were frozen at −80 ^{o}C until drying and trace element analysis as described in the text.

Table S1: Dissolved organic carbon ([DOC]), free cadmium ion ($[Cd^{2+}]$; calculated with the chemical equilibrium model WHAM 6),[45] selenium ([Se]) and calcium ([Ca]) concentrations and pH in water from Lakes Dasserat and Dufault. Also given are Cd and Se concentrations in larvae of *Chaoborus punctipennis* and in their prey (zooplankton) as well as the quantities of these trace elements in individual larvae and their dry weight. Ratios of values for the two lakes are also shown. Values (except pH) are means ± standard deviations (n = 2-7).

Variables	Lake Dasserat (DA) (48°16'N, 79°23'W)	Lake Dufault (DU) (48°18'N, 79°00'W)	DU/DA
Lakewater			
pH	7.6	7.7	
[DOC] (mg L^{-1})	5.1 ± 0.2	3.8 ± 0.1	1.3
$[Cd^{2+}]$ (nmol L^{-1})	1.4 ± 0.2	5.1 ± 0.2	3.6
[Se] (nmol L^{-1})	0.9 ± 0.3	9.5 ± 5.1	10.6
[Ca] (mmol L^{-1})	0.21 ± 0.01	0.36 ± 0.01	1.7
Invertebrates			
[Cd] zooplankton (nmol g^{-1})	---	66 ± 3.1	---
[Se] zooplankton (nmol g^{-1})	63 ± 5.0	93 ± 2.4	1.5
[Cd] *Chaoborus* (nmol g^{-1})	27 ± 5.0	96 ±16	3.6
[Se] *Chaoborus* (nmol g^{-1})	14 ± 1.0	62 ±4.0	4.4
Cd *Chaoborus* (pmol $larva^{-1}$)	11 ± 2.0	20 ± 4.0	1.8
Se *Chaoborus* (pmol $larva^{-1}$)	5.9 ± 0.2	13 ± 3.0	2.2
Chaoborus weight (mg $larva^{-1}$)	0.4 ± 0.1	0.2 ± 0.1	0.5

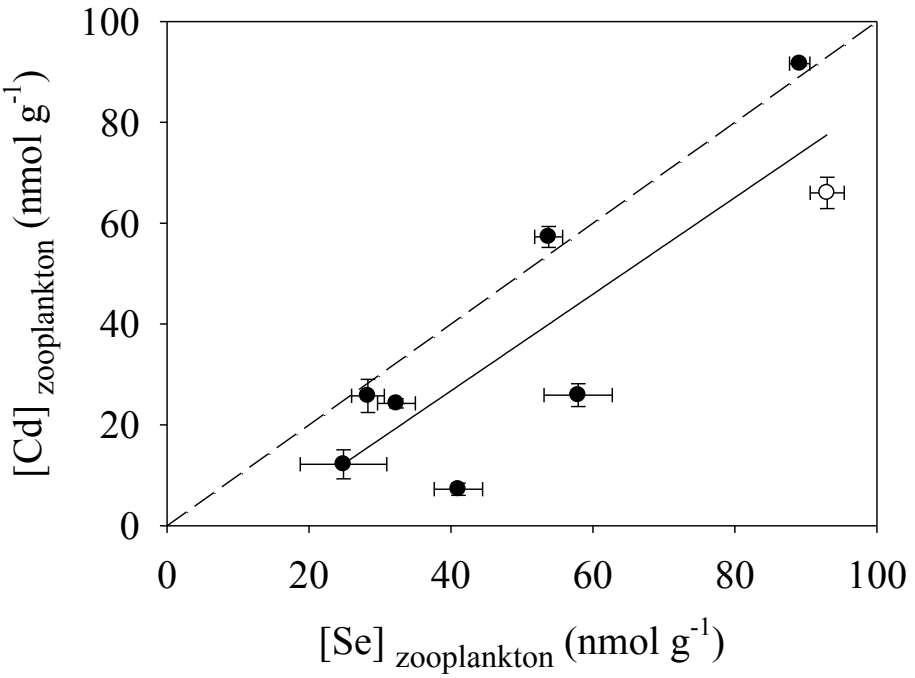

Figure S1: Linear relationship (solid line; r^2 = 0.74, P = 0.007) between mean (± SD, n = 3) Cd and Se concentrations (nmol g^{-1}) in zooplankton prey (53-125 μm) collected from 7 eastern Canadian lakes (solid symbols). The open circle is the value for Lake Dufault. The broken line is the 1 to 1 line.

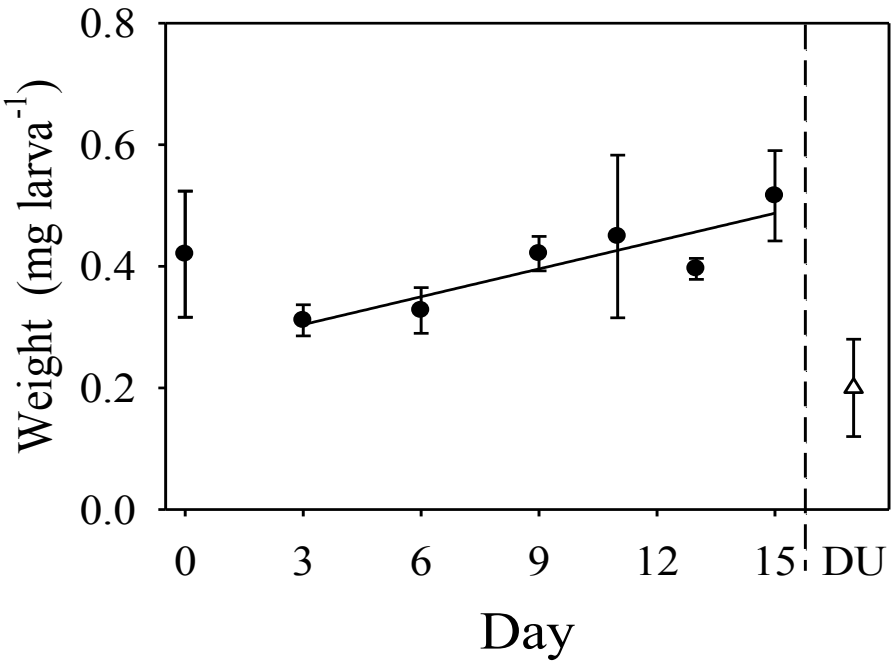

Figure S2. Temporal changes in mean individual dry weights (± SD, *n* = 3-5) of *Chaoborus punctipennis* larvae transplanted from Lake Dasserat to mesocosms in Lake Dufault (closed circles). The comparable value for indigenous Lake Dufault larvae (DU, open triangle) collected on day 9 is given on the right.