SELENIUM UPTAKE, TROPHIC TRANSFER, AND TOXICITY IN BOREAL LAKE ECOSYSTEMS

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By

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ABSTRACT

Selenium (Se) is emerging as a contaminant of concern, particularly in mine and agriculture influenced areas of Canada and the United States. Due to the high site- and species-specificity of Se bioaccumulation, site-specific biodynamic modelling is the accepted approach to predict Se accumulation in aquatic systems, and fish tissue-based guidelines for the protection of aquatic life are preferred over water quality guidelines. To date, few studies have assessed Se bioaccumulation in cold-water systems, such as Canadian boreal forest lakes. These lakes, which comprise a large proportion of Canada's freshwater, are also associated with several anthropogenic activities that can contribute to the excess release of Se to aquatic systems. Further, concern about excess Se has generally focused on the teratogenic effects on egg-laying vertebrates, with relatively little attention paid to aquatic invertebrates. The goal of this research was to improve the current understanding of Se biodynamics and toxicity in Canadian boreal lakes, with the ultimate goal of informing future ecological risk assessments of Se in Canada. Limnocorrals in two lakes located at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) were used to conduct small scale whole-ecosystem experiments to study bioaccumulation and toxicity of Se. In 2017, Se was added as selenite to six limnocorrals (two treatment groups, each in triplicate) to achieve mean measured water Se concentrations of 1.0 and 8.9 µg/L and three limnocorrals were untreated controls (mean measured Se = 0.12 µg/L). Distribution coefficients (k_ds) ranged from 7,772 L/kg dry mass (dm) in the 8.9 µg/L treatment to 23,495 L/kg dm in the 0.12 µg/L treatment, and trophic transfer factors (TTFs) for benthic macroinvertebrates ranged from 0.49 for Gammaridae to 2.3 for Chironomidae. Selenium accumulated in fathead minnow ovaries to concentrations near or above the current British Columbia Ministry of the Environment and US Environmental Protection Agency criteria (11 and 15.1 µg/g dm for fish ovary/egg, respectively) in the 1.0 and 8.9 µg/L treatments. Chironomidae and Gammaridae densities and biomass were significantly lower in the 8.9 µg/L Se treatment relative to the 1.0 µg/L Se treatment and the control, and invertebrate diversity significantly declined in the 1.0 µg/L and 8.9 µg/L Se treatments relative to the control (0.12 µg/L Se group). In 2018, a gradient approach was used in which three limnocorrals were controls (0.08-0.09 µg Se/L), and mean measured concentrations in Se-treated limnocorrals were 0.4, 0.8, 1.6, 3.4, 5.6, and 7.9 µg/L. Total Se (TSe) bioaccumulation by organisms was generally non-linear over the gradient of water Se concentrations used, and taxonomic differences in TSe accumulation by algae (phytoplankton <

periphyton) and invertebrates (Heptageniidae = Chironomidae > zooplankton) were observed. Zooplankton and benthic macroinvertebrate communities shifted according to Se exposure. Cladocera and Heptageniidae biomass and density decreased with increasing Se treatment. Overall, this work contributes to the understanding of Se trophic dynamics and toxicity in cold-water Canadian boreal lakes. These data showed how Se bioaccumulation changes with increasing aqueous exposure and with different taxa, and provided field-derived kinetic models for the saturable uptake of Se by algae and invertebrates. The levels of Se observed in algae, invertebrates and fish tissues suggest that, depending upon aqueous Se speciation, such exposures have the potential to cause Se accumulation in fish to levels of concern in cold-water, boreal lake systems. Further, these studies demonstrated that Se can have impacts on aquatic invertebrates at environmentally relevant exposure levels, and that future ecological risk assessments should consider the impacts of Se on both vertebrates and invertebrates.

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DEDICATION

This thesis is dedicated to my loving grandparents, John and Marjorie Graves

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LIST OF ABBREVIATIONS

$½C_{max}$	One half of the maximum concentration
AE	Assimilation efficiency
AFDM	Ash free dry mass
ACS	American chemical society
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BC MoE	British Columbia Ministry of the Environment
BMI	Benthic macroinvertebrate
BR	Beta regression
C	Carbon
C_a	Algal concentration
CCME	Canadian Council of Ministers of the Environment
C_{con}	Consumer concentration
CF-IRMS	Continuous flow – isotope ratio mass spectrometry
chl *a*	Chlorophyll *a*
C_i	Invertebrate concentration
CI	Confidence interval
C_{max}	Maximum concentration
C_o	Initial concentration
C_{prey}	Prey concentration
Cu	Copper
C_w	Water concentration
DO	Dissolved oxygen
DOC	Dissolved organic carbon
dm	Dry mass
EC	Effect concentration
EF	Enrichment function
EPT	Ephemeroptera, Plecoptera, Trichoptera
Fe	Iron
glm	Generalized linear model

glmm	Generalized linear mixed model
GSH	Glutathione
GSI	Gonadosomatic index
GSSG	Glutathione disulfide
H_0	Null hypothesis
HDPE	High density polyethylene
HF	Hydrofluoric acid
HNO_3	Nitric acid
ICP-MS	Inductively coupled plasma – mass spectrometry
IISD-ELA	International Institute for Sustainable Development – Experimental Lakes Area
IR	Ingestion rate
K	Fulton's condition factor
k_d	Distribution coefficient
k_e	Efflux rate
k_g	Growth rate
K_M	Substrate concentration at $½C_{max}$
k_u	Uptake rate
LC	Lethal concentration
LMM	Linear mixed model
LR	Linear regression
LSI	Liversomatic index
MM	Michaelis-Menten kinetics
Mn	Manganese
MS222	Tricaine methanesulfonate
N	Nitrogen
NaCl	Sodium chloride
Na_2SeO_3	Sodium selenite
Ni	Nickel
NLR	Non-linear regression
OM	Organic matter

OrganoSe	Organoselenium
PCA	Principal component analysis
PES	Polyethersulfone
PO_4	Phosphate
PRC	Principal response curve
PVDF	Polyvinylidene fluoride
RMSE	Root mean square error
RO	Reverse osmosis
S	Sulfur
Se	Selenium
Se^0	Elemental selenium
SD	Standard deviation
Se(0)	Selenium in 0 oxidation state
Se(-II)	Selenium in -2 oxidation state
SeCys	Selenocysteine
Se(IV)	Selenium in +4 oxidation state
SeMet	Selenomethionine
SeO_3^{2-}	Selenite
SeO_4^{2-}	Selenate
Se(VI)	Selenium in +6 oxidation state
SO_4^{2-}	Sulfate
SRM	Standard reference material
t	Time
TDN	Total dissolved nitrogen
TDP	Total dissolved phosphorous
TOC	Total organic carbon
TSe	Total selenium
TTF	Trophic transfer factor
US EPA	United States Environmental Protection Agency
WQC	Water quality criterion
YSI	Yellow springs instrument

PREFACE

This thesis is written in a manuscript-style format. Chapters 2 and 3 have been published in peer-reviewed journals, chapter 4 is currently in review, and chapters 5 and 6 are in preparation for publication. As a result, there is some repetition of information in the Introduction and Materials and Methods sections of each chapter. Supplementary materials that were included in the publication or submission of chapters 2 and 4 are included in an Appendix at the end of this thesis. These manuscripts have been adapted to produce a consistent format in this thesis.

Graves SD, Liber K, Palace V, Hecker M, Doig L, Janz DM. 2020. Trophic dynamics of selenium in a boreal lake food web. *In review*: Environ Sci Technol.

Graves SD, Liber K, Palace V, Hecker M, Doig L, Janz DM. 2019b. Effects of selenium on benthic macroinvertebrates and fathead minnow (*Pimephales promelas*) in a boreal lake ecosystem. Ecotox Environ Saf 182:109354.

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The author contributions for these manuscripts are as follows:

Stephanie Graves (University of Saskatchewan) conducted all field experiments, sample processing and analyses, statistical analyses, and drafted each of the manuscripts.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Selenium (Se) is increasingly being recognized as a global pollutant, and the presence of elevated Se in aquatic systems is largely due to the inadvertent release associated with many anthropogenic activities (Mosher and Duce 1987; Lemly 2004; Fordyce 2013). In the worst-case scenarios, elevated Se in aquatic systems has led to population-level impacts on fish due primarily to increased deformities in developing fish and resulting declines in recruitment (Lemly 2002; Young et al. 2010). Though high Se bioaccumulation and instances of Se toxicity to fish in aquatic ecosystems have been recorded in Canada, the guidelines for protection of aquatic life vary from province to province and the federal water quality guideline for Se (set at 1 µg/L for all systems) does not acknowledge the very site- and species-specific nature of Se as a contaminant.

The high spatial variability in Se bioaccumulation among systems results in difficulty understanding and predicting the fate and effects of the element. Factors such as water chemistry characteristics, Se speciation, and taxonomic differences in Se uptake and/or retention contribute to this variability (Presser and Luoma 2010; Ponton et al. 2020), and a better understanding of Se ecotoxicology depends on consideration of these factors. Thus, my thesis is part of a larger, multi-student project called the Ecological Risk Assessment of Selenium (ERASe) that was developed to address some of these research needs. This project aimed to use both laboratory and field approaches to better understand and characterize Se enrichment by primary producers, and trophic transfer to primary and secondary consumers (invertebrates and fish). It also aimed to characterize resulting toxic effects in fish using multiple exposure routes (maternally transferred Se versus direct microinjections to eggs) and seeks to explore molecular-level perturbations caused by Se. Ultimately, the ERASe project was developed with the intent to inform predictive models of Se bioaccumulation and effects, and to provide site-specific information on bioaccumulation that will be useful for conducting ecological risk assessments in Canadian boreal lake ecosystems.

1.2 Properties and sources of selenium

Selenium is non-metal with a molecular weight of 78.97 g/mol and the atomic number 34 from Group VIA on the periodic table of elements. Selenium has physico-chemical properties similar to sulfur, which is directly above it on the periodic table and, like sulfur, Se has four main oxidation states: VI, IV, 0, and -II (Bodnar et al. 2012). This element occurs naturally as six

different isotopes, the most common being ^{80}Se (50% of all Se in nature) and ^{78}Se (23.5% of all Se in nature).

1.2.1 Natural sources of selenium

Selenium occurs naturally in the earth's crust, and is considered a trace element, with concentrations typically around 0.05 to 0.09 µg/g in geological materials (Taylor and McLennan 1985). In sedimentary rocks, such as sandstone and limestone, which cover most of the earth's surface, Se concentrations do not exceed 0.1 µg Se/g. Higher Se concentrations (up to 300 µg/g) can be found in shales, phosphatic rocks, coals and organic-rich deposits (Tamari et al. 1990). Soils enriched with Se often originate from marine sedimentary deposits and have Se levels ranging from 0.1 to 2 µg/g, while the global average Se concentration in soil is around 0.4 µg/g (Fordyce 2007). In some regions, highly seleniferous soils have concentrations of over 1200 µg Se/g, although Se-deficient soils are much more widespread than Se-rich soils (Haygarth 1994). Selenium concentrations in soil are correlated with Se concentration in the underlying geological materials, and processes such as geological weathering of rocks, rock-water interactions, biological activity, and volcanism contribute to the natural movement of Se from rocks to other compartments (i.e. water, atmosphere). These natural processes account for the annual global atmospheric release of 18 to 25 gigagrams (Gg) Se/yr (Feinberg et al. 2020). Similar to the variation in Se concentrations in soil, water Se concentrations vary according to underlying geology and surrounding soil. In uncontaminated waterbodies, Se concentrations are typically between 0.1 and 0.2 µg/L (Conde and Alaejos 1997; Winkel et al. 2015).

1.2.2 Anthropogenic sources of selenium

In addition to the natural cycling of Se in the environment, anthropogenic activities have increased the release of this element from geologic sources and ultimately have led to higher Se emissions, both to the atmosphere and directly to surface water (Mosher and Duce 1987). These anthropogenic activities, including coal and petroleum fuel combustion, and the extraction and processing of metals, account for approximately 40% of atmospheric selenium while natural flux from volcanic eruptions, and emissions from marine and terrestrial organisms account for approximately 55% of global atmospheric selenium (Mosher and Duce 1987; Wen and Carignan 2007). All of these natural and anthropogenic activities combined lead to an estimated annual flux of 13-19 gigagrams (Gg) of Se in the atmosphere (Mosher and Duce 1987, Nriagu and Pacyna 1988; Wen and Carignan 2007). More recent models have estimated similar fluxes of Se from

anthropogenic activities (10.9 Gg Se/yr), but they estimate that total annual global Se emissions are much higher (approximately 29 to 36 Gg Se/yr) due to greater emissions from natural processes (Feinberg et al. 2020).

Selenium has had several applications, first being used in the 20th century to remove colour from glass or to add a red pigment. Selenium has photoelectric and semi-conductor properties, and it has been used in electronics such as photocopiers (Bodnar et al. 2012; Mehdi et al. 2013). Currently, Se is used by industries as a catalyst in metallurgy and organic synthesis, an antioxidant in inks, in lubricating oils, as well as in anti-dandruff and anti-fungal pharmaceuticals (Bodnar et al. 2012; Mehdi et al. 2013). Though there are many uses of Se, the main anthropogenic sources to the environment are inadvertent release during combustion of fossil fuels, and the extraction and processing of metals such as copper, lead, zinc and uranium (Lemly 1985). Other anthropogenic sources are the agricultural use of phosphate fertilizers, application of sewage sludge and manure to land, and from the use of Se-containing fungicides and pesticides (Lemly 1994).

The main anthropogenic activities mobilizing Se in the environment are the extraction, processing, and combustion of coal, which account for the annual release of approximately 7300 tons of Se (Lemly 1985; Yudovich and Ketris 2006). Because Se is naturally enriched in coal, virtually every step of the coal mining, transport and combustion processes can contribute to the release of Se (Lemly 2004). During mining preparation and at storage sites, rain or water used to wash coal prior to transport can leach Se from recently mined coal and enter waterbodies through precipitation runoff (Ensminger 1981). Selenium levels can be elevated in solid wastes, such as fly ash, that accumulate when coal is burned to produce electricity. This solid waste can contain highly concentrated Se relative to the surrounding soils and is disposed of largely by deposition into a wet-slurry or dry ash basin (Ensminger 1981; Lemly 2004). From here, rain can leach Se from dumping sites and overflow of waste sites can lead to contamination of nearby waterbodies. This leachate is of particular concern because its alkaline pH and oxidation state promote the formation of water soluble and bioavailable Se oxyanions in water (Lemly 2004).

The estimated annual movement of Se from land to oceans is 14,000 tonnes/year (Nriagu 1989, 1991). Surface water Se concentrations from 0.035 to 330 µg/L have been recorded in areas with varying levels of natural and anthropogenic Se inputs, but the global average Se concentration in water is 0.4 µg/L, and most waterbodies have Se concentrations less than 3 µg/L (Wallschlager

and Feldmann 2010; Ponton et al. 2020). Groundwater generally has higher concentrations than surface water because of the greater contact with rock (Fordyce 2007). Nearby anthropogenic activities increase surface water Se concentrations, for example, surface water Se concentrations were 45-50 µg/L near a nickel-copper (Ni-Cu) smelter in Sudbury, Canada (Nriagu and Wong 1983; Lemly 2004). Selenium can be directly deposited through run-off from contaminated sites such as coal mining areas as discussed above, but it may also be deposited from the atmosphere. Selenium can volatilize from volcanoes, soil, sediments, organisms, and industrial activities (Wen and Carignan 2007; Buchs et al. 2013). Gaseous Se (selenides) can stay in the atmosphere for weeks and can be transported several thousand kilometers before being deposited through wet or dry deposition (Wen and Carignan 2007). Wet deposition of Se accounts for most Se removal from the atmosphere (81%) while dry deposition accounts for 19% of removal (Feinberg et al. 2020). Recent models have suggested that atmospheric deposition of Se to land may be more important than previously thought, with an estimated 870 mg/ha/yr being deposited (Feinberg et al. 2020).

1.3 Selenium speciation and partitioning in aquatic systems

There are four categories of Se speciation: inorganic Se, volatilized and methylated Se, protein and amino acid Se, and non-protein amino acids and biochemical intermediates (Maher et al. 2010). Selenium exists in one of four main oxidation states as Se(VI), Se(IV), Se(-II) or Se(0). In oxidized systems, Se is most commonly found as the oxyanions selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}), while anaerobic conditions favour the more reduced forms, elemental Se (Se^0) and selenides (Se(-II)). Once deposited into water, Se can exist in all four of these oxidation states, and each of these Se species has different properties (Lemly 1994). Selenate is the fully oxidized form of Se, and its chemistry in water is similar to sulfate (Peak and Sparks 2002). It is very water-soluble due to generally low precipitation and adsorption potential and is typically present under high redox conditions (Fordyce 2007). In contrast, selenite is a weak acid and is present in moderate redox conditions (Papelis et al. 1995; Fernandez-Martinez and Charlet 2009). Selenite is also water soluble, but it's presence in water is more dependent on adsorption and desorption on oxyhydrides and organic matter (Fernandez-Martinez and Charlet 2009). Selenides are present under reducing conditions and occur in both inorganic and organic forms (Lenz et al. 2006). Biota form organic selenides when they take up Se and incorporate it into organic molecules in the place of sulfur (S). These selenides include methylated (organic) Se compounds (dimethylselenide, dimethyldiselenide; volatile), or the seleno-amino acids selenocysteine (SeCys) and

selenomethionine (SeMet) (Maher et al. 2010). Elemental Se is non-soluble and is generally present as a result of microbial reduction of inorganic Se (Fernandez-Martinez and Charlet 2009).

Selenium speciation is affected by the chemical and physical properties of a system including redox potential and pH. The speciation of Se, in turn, influences the partitioning, bioavailability, and movement of Se in aquatic systems (Sharma et al. 2015). The most common forms of Se in water (selenate and selenite) differ in their adsorption properties, therefore, Se partitioning to sediment depends on the oxidation state of Se (Maher et al. 2010). Selenite adsorbs to oxy-hydride minerals of iron (Fe) and manganese (Mn), whereas selenate adsorbs only weakly to Fe compounds and does not adsorb to Mn (Selim and Sparks 2001; Maher et al. 2010). Selenite and selenate adsorption both generally decrease with increasing pH, because the alkaline conditions change the surface charge of oxy-hydrides (Maher et al. 2010). Under highly reducing conditions, Se solubility is lower because of the reduction of Se oxyanions to insoluble elemental Se. The presence of competing anions such as carbonate, phosphate and sulfate decrease adsorption of selenite and selenate to mineral surfaces, therefore increasing the bioavailability of these oxyanions (Sharma et al. 2015). Organic matter (OM) and total organic carbon (TOC) content also determine the extent of Se adsorption and partitioning to sediment. Total organic carbon content is positively correlated with sediment Se concentrations (Janz et al. 2014), and in general %OM is positively correlated with adsorption of weak acids (such as selenite), particularly at lower pH. Freshwater sediments appear to be a sink for inorganic Se species, and sediment-associated organisms such as benthic algae and detritivores/benthic macroinvertebrates often have higher Se concentrations than pelagic organisms in the same system (Stewart et al. 2004; Muscatello et al. 2008). As such, characterizing the partitioning of added Se to sediment is important for understanding Se cycling and incorporation into food webs.

1.4 Selenium bioaccumulation and trophic transfer

1.4.1 Selenium essentiality

Selenium is an essential element required by almost all organisms, except for higher plants and yeast, and 30 different seleno-protein families have been identified (Kryukov et al. 2003; Kryukov and Gladyshev 2004; Castellano et al. 2005; Zhang and Gladyshev 2005). The function of all Se-containing molecules are not known, but Se is known to be incorporated into selenocysteine (an amino acid) and subsequently inserted into antioxidant enzymes (i.e., glutathione peroxidases and thioredoxin reductases), or transport proteins (i.e., selenoprotein P)

(Behne et al. 2000; Reilly 2006). The minimum Se concentration required for normal physiological function varies among organisms. In humans, 0.6 µg Se/kg/d is recommended, while in fish optimal growth occurs at Se concentrations ranging from 3.5 to 35 µg/kg/d among species (Hilton et al. 1980; Rayman 2002; Lin and Shiau 2005). In algae, the optimal Se level ranges from 0.7 µg/L to 18 µg/L in a variety of studied species (Price et al. 1987; Harrison et al. 1988). The essentiality of Se for most organisms makes uptake and trophic transfer of Se difficult to predict, because uptake is an active process and essentiality varies among species. In addition, Se uptake by consumers occurs primarily via dietary pathways, and concentrations in organisms are not easily predicted based on aqueous Se levels. For these reasons, knowledge of ecological and physiological factors affecting Se accumulation are essential to the accurate prediction of Se bioaccumulation.

1.4.2 Selenium uptake by primary producers

Incorporation of Se into the food web occurs mainly by primary producers, and this assimilation at the base of the food web represents the largest accumulation step in the food chain (Orr et al. 2006; Muscatello et al. 2008; Presser & Luoma 2010; Kuchapski and Rasmussen 2015; DeForest et al. 2017). Therefore, primary producer enrichment is one of the most important factors determining Se exposure to higher trophic levels. Uptake rates and pathways vary among Se species, and they also vary both inter- and intra-specifically among organisms. Currently, there is relatively little known about the specific mechanisms of Se uptake and the carrier proteins involved. Most studies have focused on the uptake, biotransformation and trophic transfer by algae as this is the primary energy source for several aquatic consumers. Comparatively little is known about other organisms such as bacteria or fungi and their contributions to the trophic transfer of Se.

1.4.2.1 Bacteria

Bacteria are an essential component of periphyton, but their role in food web transfer of Se has been much less studied than algae. They have been shown to take up aqueous Se rapidly and incorporate it into amino acids and proteins (Ogle et al. 1988). The uptake of selenate occurs through a common membrane carrier for selenate and sulfate, and there is a different uptake mechanism for selenite, which does not seem to be related to sulfite (Ogle et al. 1988). Bacteria can also remove Se from water via respiratory reduction, and some species use selenite or selenate as the terminal electron acceptor in respiration (Nancharaiah and Lens 2015). Bacteria may reduce

Se to Se^0, dimethylselenide or selenoamino acids, with selenite being more readily reduced and incorporated into amino acids than selenate (Hudman and Glenn 1984). The relative contribution of bacterial Se uptake and incorporation into food webs, compared to algae, remains a large knowledge gap in the understanding of Se trophic transfer.

1.4.2.2 Algae

In algae, SeMet and selenate uptake are active processes, whereas selenite uptake is less clear but appears to have both high-affinity (active) and low-affinity (passive) transport mechanisms at least in some algal species (Fisher and Wente 1993; Fournier et al. 2006; Araie et al. 2011; Vriens et al. 2016). In the green algae *Chlamydomonas reinhardtii* exposed to 200, 500, 1,000, and 2,000 μg/L SeMet, selenate, and selenite for 1 hr, selenate and SeMet uptake became saturated, whereas selenite uptake remained linear over the range of tested concentrations (Fournier et al. 2006). Other studies have found that selenite, along with selenate and SeMet, can be fitted with Michaelis-Menten saturation curves. Such studies also demonstrated that SeMet has a higher uptake rate than selenite or selenate, indicating higher bioavailability of SeMet compared to selenite or selenate (Baines and Fisher 2001; Fournier et al. 2006). The saturation of Se uptake appears to vary among species and among studies, for example, *C. reinhardtii* exposed to selenite in two different studies showed different uptake curves (one linear and one saturable) (Riedel et al. 1991; Fournier et al. 2006).

While relatively little is known about the mechanisms of Se uptake in algae, transport of selenate appears to occur through a sulfate transporter, and research has shown that a similar membrane carrier may exist for selenite and phosphate (El Kassis et al. 2007; Vriens et al. 2016). Selenate and sulfate compete for uptake both at absorption sites and at activation enzymes once taken up into cells (Ogle et al. 1988). This competition results in an inverse relationship between sulfate concentrations and selenate accumulation in algae (Lo et al. 2015; Ponton et al. 2020). In a similar way, inverse relationships have been observed between selenite uptake and phosphate concentrations (Vriens et al. 2016). As discussed above, the uptake kinetics of selenite appear more variable than other Se species, and likely indicate both active and passive uptake mechanisms in algae (Araie et al. 2011; Vriens et al. 2016). SeMet is taken up most readily by algae, followed by selenite and then selenate, and SeMet appears to be more bioavailable than both selenite and selenate, but occurs in water at much lower concentrations in water. In field-grown periphyton, SeIV accumulation was 2- to 31-fold higher than for SeVI at a given aqueous Se concentration

(Markwart et al. 2019). With some exceptions (for selenite), dead algal cells appear to take up negligible amounts of Se, indicating that Se uptake for the most part is a non-passive, carrier-mediated process (Riedel et al. 1991; Stewart et al. 2010).

Once Se is taken up by plants and algae, selenite and selenate are both readily converted to organo-selenium compounds such as SeMet and SeCys, and incorporated into proteins (Shrift 1954; Fisher and Reinfelder 1991; Riedel et al. 1996). Following uptake, Se has the same conversion pathway as sulfur. Once in plant cells, it is hypothesized that selenate is reduced to selenite by adenosine phosphoselenate, and then further reduced to selenides. SeCys is synthesized from selenides by cysteine synthase, which is converted to SeMet, and SeMet and SeCys are then incorporated into proteins (Sors et al. 2005; Wallenberg et al. 2010). Once reduced to these organoSe compounds, intracellular Se can be present in proteins (as selenoamino acids), or in lipids and carbohydrates (Stewart et al. 2010). Due to this rapid reduction of assimilated Se and formation of organoSe, consumers are mainly exposed to reduced, organic forms of Se (Stewart et al. 2010).

As discussed above, the uptake rates of selenate, selenite, and SeMet differ, often exhibiting saturation kinetics, and it is difficult to generate predictable relationships between Se concentrations in water and biota. In addition, the degree of inter-specific variability in Se uptake is extremely high (Riedel et al. 1991; Fisher and Wente 1993; Baines and Fisher 2001; Wang and Dei 2001). In monocultures of several marine algal species exposed to 0.15 and 4.5 nM selenite, distribution functions (k_ds; the concentration of Se in algae divided by the concentration in water) ranged from 30 to 10^6, and higher k_ds were observed at the lower exposure concentration (Baines and Fisher 2001). In field-grown periphyton exposed to 5 µg SeIV/L, organic matter-normalized k_ds ranged from 3,244 L/Kg dm in communities dominated by Bacillariophyta to 76,599 L/Kg dm for communities dominated by Cyanophyta where high amounts of iron oxides were also present (Markwart et al. 2019). The main reasons proposed for the differences in uptake among species are 1) inter-specific differences in Se requirements, and 2) inter-specific differences in ability to regulate Se uptake (Baines and Fisher 2001). In some species there is “luxury” or excess uptake of Se, i.e. uptake additional to what is optimal for physiological function (Harrison et al 1998). Inter-specific variability in Se is particularly high compared to other essential metals where the variability in uptake differs by less than one order of magnitude among species (after cell volume correction) and differences are predicted by cell size (Fisher and Reinfelder 1995). Selenium

accumulation varies within taxonomic group and even within species, among seasons, and among growth phases of algae. Diatom Se uptake was measured in different physiological phases and higher Se uptake was observed for cells in a log growth stage compared to cells in stationary phase (Riedel et al. 1996). In addition, spatial variability in Se uptake within a species has been observed, suggesting that Se requirements and uptake may be influenced by an organism's surrounding environment (Margalef 1978).

1.4.3 Selenium trophic transfer to invertebrates

Invertebrates take up Se mainly through dietary pathways, however, depending on the species and the conditions, direct uptake from water may be significant (Roditi et al. 2000; Tsui and Wang 2007; Presser and Luoma 2010). Although inter-specific differences in Se accumulation are not as variable as algae/periphyton, there is considerable difference in uptake of Se among invertebrate taxa due to both dietary and physiological factors (Presser and Luoma 2010; Stewart et al. 2010). As discussed above, algae display large inter-specific variability in Se accumulation. Therefore, where and what invertebrates feed on (i.e. phytoplankton versus benthic algae) will influence their exposure to Se. Physiological processing of Se, including the assimilation efficiency, ingestion rate and efflux rate of Se from tissues, will also determine Se body burdens of invertebrates.

Assimilation efficiencies of most studied invertebrates range from 70% to 90%, but some can have AEs as low as 20% (Stewart et al. 2010). Efflux rates can also vary between organisms, for example, Se rate loss for clams is 8-10 times slower than crustaceans, and some copepod species appear to have higher Se efflux rates than mayflies (Riedel and Cole 2001; Schlekat et al. 2002; Stewart et al. 2004). These rate loss differences may be due to differences in an organism's ability to recycle Se in proteins (Stewart et al. 2010).

The direct aqueous uptake of Se mentioned above may be important to consider in organisms such as bivalves where assimilation efficiency (AE) from the diet is low (Stewart et al. 2010). In such species, aqueous uptake may contribute relatively larger portions of Se to the total body burden. In *Daphnia magna*, aqueous uptake of Se was estimated to comprise 20 to 40% of their total Se body burden (Roditi and Fisher 1999; Tsui and Wang 2007). Direct aqueous uptake may occur to meet physiological Se requirements when relatively low levels of Se are present in the diet, or when there is more organic Se (SeMet; preferred form of Se) in the water (Stewart et al. 2010).

Trophic transfer factors (TTFs), calculated by dividing the Se concentration in a consumer by the Se concentration in their diet, are used to describe the amount of Se transferred from diet to consumer. Field-derived TTFs for freshwater invertebrates range from 0.9 for amphipods to 3.2 for caddisflies (Saiki et al. 1993; Harding et al. 2005), meaning that invertebrates may have Se concentrations up to 3-fold higher than their diet, but generally do not have Se concentrations lower than their diet. Freshwater aquatic insects tend to have relatively high TTFs compared to zooplankton and other benthic macroinvertebrates (reviewed in Presser and Luoma 2010), suggesting that organisms feeding on aquatic insects may be expected to have greater Se exposure than those feeding on amphipods or zooplankton in the same system.

It is important to note that much of what is currently known about Se accumulation in invertebrates is based on marine organisms, and there is relatively little information about freshwater invertebrates. In addition, there are fewer data available for invertebrates compared to higher trophic level organisms, and in most field-based studies, invertebrates from several taxonomic groups are pooled to obtain sufficient mass for analyses. As a result, inter-specific differences in invertebrate Se accumulation have rarely been assessed. More recently, studies have simulated simple food webs in the laboratory to more accurately represent uptake and trophic transfer pathways for Se (Conley et al. 2013; Raes 2020). Augmenting field studies with these lab-based food webs is particularly useful since determining species-specific TTFs of field-collected invertebrates can be difficult (Conley et al. 2013; Raes 2020). Because there are few data on freshwater invertebrate Se TTFs, building a database of TTFs for a number of macroinvertebrate taxa will be one focus of this thesis.

1.4.4 Selenium trophic transfer to fish

Similar to invertebrates, uptake of Se in fish is primarily dietary and in the form of organic Se compounds, and direct Se uptake from water is low (uptake rate; k_u = 0.01 L/g/d; Zhang and Wang 2007; Stewart et al. 2010). Like invertebrates, Se accumulation in fish is variable among species and this is primarily due to differences in diet (where and what fish are eating) and physiology (assimilation efficiency, ingestion rate, and efflux rate). As such, to effectively evaluate the exposure risk of Se to fish, quantitative estimates of the above factors are necessary. In addition to diet and physiological differences among fish, inter-specific variability in Se accumulation and toxicity due to different life history characteristics, habitat, migration activity,

spawning type, and tissue distribution of Se have been observed (Baines and Fisher 2001; Stewart et al. 2004; Zhang and Wang 2007; Mathews and Fisher 2008).

Once ingested by fish, organic Se forms may be incorporated into proteins, or Se may be methylated and excreted. Selenomethionine is incorporated non-specifically into proteins as a replacement for methionine (Schrauzer 2000). In contrast, selenocysteine is specifically incorporated into selenoproteins, and is not incorporated into proteins as a replacement for cysteine (Stadtman 1996).

The range of TTFs measured for fish is small relative to k_ds for algae, with values generally between 0.5 to 1.5, although much higher TTFs can be observed based on feeding habits (TTF up to 10 for benthivorous fish has been observed; Muscatello et al. 2008; Presser and Luoma 2010). The half-life of Se in fish is usually 3 to 4 weeks, but it can vary based on experimental conditions (laboratory versus field), diet, and food abundance (Presser and Luoma 2010). The biomagnification potential of Se is debated because TTFs range from values that indicate no biomagnification (i.e. TTF<1) to values that do (TTF>1). In addition, Se concentrations do not appear to increase with fish size or age, as seen with several other contaminants (Gantner et al. 2009). The extent of Se exposure in fish is more dependent on the amount of Se being enriched at the base of food web rather than biomagnification by subsequent trophic levels, and high levels of Se can accumulate in fish without much biomagnification occurring.

1.5 Selenium toxicity to organisms

1.5.1 Mechanisms of action

Selenium is noted for having a particularly small range between essentiality in toxicity. For instance, in fish, the optimal level of Se ranges from 3.5 to 35 µg/kg/d whole body while the threshold for toxic effects is currently set at 10 µg/g dm in eggs (Lemly 1993, Muscatello et al. 2006). In addition, Se appears to be a "double-edged sword" because at physiologically optimal concentrations, it is involved in the antioxidant response, but at higher levels, it can cause the production of reactive oxygen species and oxidative stress (Janz et al. 2010). Initially, the primary mechanism of Se toxicity was thought to be misfolded proteins and impaired protein function, caused by the unintended replacement of sulfur with Se in amino acids (Maier and Knight 1994; Lemly 1997). More recently, the importance of this mechanism has been questioned because of the nature of Se incorporation into the amino acids SeMet and SeCys. Although the Se molecule in SeMet is incorporated non-specifically, it is "insulated" by a methyl group, therefore the

structure and function of the amino acid is presumably unaffected by Se replacing S (Mechaly et al. 2000; Egerer-Sieber et al. 2006). In contrast, selenocysteine incorporation into proteins is highly specific and highly regulated within cells. Therefore, neither SeCys nor SeMet should affect protein structure or function (Stadtman 1996). Recent studies have suggested that there is still evidence of a role of protein misfolding in Se toxicity (Kupsco and Schlenk 2016), but oxidative stress has been more recently proposed as the primary cause of teratogenicity and embryo toxicity associated with Se. In eggs of oviparous vertebrates, the most sensitive organisms to Se toxicity, SeMet is the dominant form of Se. This SeMet may be metabolized to more reactive Se forms such as methylselenol, which is hypothesized to cause the production of reactive oxygen species and oxidative stress (Fan et al. 2002; Palace et al. 2004). With reference to a specific mechanism for this generation of oxidative stress, an interaction with glutathione has been identified (Hoffman 2002; Spallholz and Hoffman 2002). Glutathione (GSH) is an antioxidant that, in conjunction with glutathione peroxidase (which contains Se), reduces reactive oxygen species. At high levels of Se, birds show lower ratios of reduced GSH to oxidized GSH (GSSG), which is an indicator of increased oxidative damage (Hoffman 2002). A reaction of glutathione with Se can also lead to the formation of selenopersulfides and thiyl radicals (Spallholz and Hoffman 2002), which both lead to the production of reactive oxygen species (Lin and Spallholz 1993; Arteel and Sies 2001). Recent studies have provided even more evidence for this mechanism of action, by showing that pre-treatment with an antioxidant prevents SeMet from causing deformities in exposed larvae (Arnold et al. 2016).

1.5.2 Effects on fish

In female fish, SeMet is incorporated into vitellogenin, an egg yolk precursor protein, which is transported from the liver to developing ovaries, where yolk absorption causes the release of Se and subsequent exposure of developing embryos (Kroll and Doroshov 1991; Janz et al. 2010). The characteristic effects of Se on larvae include spinal, fin, and craniofacial deformities, as well as edema (Lemly 1993; Maier and Knight 1994; Hamilton 2003; Lane et al. 2019). While it is accepted that Se causes these characteristic deformities in larvae, effects of Se on fertility or hatchability of fish have generally not been observed (Holm et al. 2005; Muscatello et al. 2006, Lane et al. 2019).

Fish sensitivity to Se toxicity is greatest during egg development because toxicity occurs mainly through maternal transfer of Se to eggs (Janz et al. 2010). This maternal transfer varies

between species, depending on life history characteristics and spawning type (i.e. synchronous versus asynchronous versus multiple spawners), which influence ovary development time and length of egg Se exposure (Stewart et al. 2010). Exposure can also depend on fish habitat use, such as movement in and out of high Se exposure areas. For example, fish feeding in lentic areas may be exposed to higher Se than those feeding in lotic areas of the same system. In addition, inter-specific variability in tissue distribution of Se has been observed; deBruyn et al. (2008) found that rainbow trout (*Oncorhynchus mykiss*) had a significantly higher ratio of egg:muscle Se compared to brook trout (*Salvelinus fontinalis*) in the same systems. Correlations between Se levels in different tissues (muscle, ovary, liver, muscle plug, egg, larvae) show that some species, such as bluegill sunfish (*Lepomis macrochirus*) and razorback sucker (*Xyrauchen texanus*) have strong tissue Se relationships (R^2 range 0.72 to 1.00) while other species such as mountain whitefish (*Prosopium williamsoni*) and cutthroat trout (*Oncorhynchus clarkii*) have weaker tissue Se correlations (R^2 range 0.28 to 0.85) (deBruyn et al. 2008).

1.5.3 Effects on aquatic invertebrates

A considerable knowledge gap in the Se literature concerning toxicity of Se to invertebrates exists. While there is widespread consensus that egg-laying vertebrates are most sensitive to Se exposure, little information about toxicity to invertebrates is available. Most invertebrate toxicity studies to date have involved short-term, waterborne toxicity tests. The 48-h LC_{50} values for two Chironomid species were 48.2 and 42.5 mg selenite/L (Call et al. 1983; Maier and Knight 1993). For *Hyalella azteca*, the lowest 48-h LC_{50} was 70 µg selenite/L (Adams 1976). When toxicity levels are instead considered in terms of internal Se concentrations, body burdens that are associated with Se toxicity in invertebrates are more similar to those that are a concern for fish. For instance, the LC_{95} for *Daphnia magna* was 42 µg/g dm (Hansen et al. 1993) and the LC_{50} for *Chironomus decorus* was 63 µg/g dm (Maier and Knight 1993), while egg Se EC10s for field-exposed fish range from 20.4 µg/g dm for northern pike (*Esox lucius*) to 54 µg/g dm for Dolly Varden (*Salvelinus malma*) (Muscatello et al. 2006; McDonald et al. 2010).

Many field studies investigating the impacts of Se on invertebrates occur in mine-influenced areas, such that it is not always possible to tease apart the impact of Se from other stressors. For instance, in the Canadian Rockies, the diversity of more sensitive taxa (Ephemeroptera, Plecoptera, Trichoptera; EPT) declined in streams with Se concentrations up to 100 µg/L Se, but no change in density of more tolerant taxa (Chironomidae) were observed

(Kuchapski and Rasmussen 2015). In that study, the mine-affected streams also had higher conductivity and alkalinity relative to reference sites, which are additional stressors potentially confounding the observed toxicity results (Kuchapski and Rasmussen 2015). In Belews Lake, North Carolina, where aqueous Se concentrations up to 10 µg Se/L caused the extirpation of most fish species (Cumbie and Van Horn, 1978), zooplankton diversity did not change (Marcogliese et al. 1992). The density of several cladoceran (and some copepod) species did decline, but those declines were attributed to changes in fish community structure, and not direct toxicity to invertebrates (Marcogliese et al. 1992). In mesocosms within Clay Lake, Ontario, Canada, Se was added one time to obtain nominal concentrations of 1, 10, and 100 µg selenite/L. Little accumulation of Se in invertebrates was observed, and as a result, no impacts on zooplankton communities were seen (Turner and Rudd 1983; Saiki et al. 1985). The variability in results of the relatively few field-based studies conducted demonstrate the need for more field-based studies to assess the effects of excess Se on invertebrates.

1.6 Water quality guidelines for the protection of aquatic life

The current Canadian Council of Ministers of the Environment (CCME) Se water quality guideline for the protection of aquatic life was established in 1987 and is set at 1 µg/L for freshwater (CCME 2003). More recent understanding of Se ecotoxicology, specifically the site-specific nature of Se accumulation in aquatic food webs, led the British Columbia Ministry of the Environment (BC MoE) to implement fish tissue-based guidelines for the protection of aquatic life. They have set a fish egg-ovary tissue safety guideline at 11 µg/g dry mass (dm), and an interim Se guideline for fish muscle at 4 µg/g dm. The water quality criterion is 2 µg/L, with an alert concentration of 1 µg/L (BC MoE 2014). The United States Environmental Protection Agency (US EPA) have also recently amended their Se water quality criteria, with fish egg-ovary, whole body and muscle tissue safety levels set at 15.1, 8.5, and 11.3 µg/g dm, respectively (US EPA 2016). Chronic (30-day average) exposure guidelines are set at 1.5 µg/L for lentic water and 3.1 µg/L for lotic water (US EPA 2016).

1.7 Areas of selenium exposure concern in Canada

In Canada, Se exposure levels exceeding the toxicity threshold for fish recently set by the US EPA (11.3 µg/g dm muscle) and BC MoE (4 µg/g dm muscle; interim guideline) have been documented recently in areas downstream of coal, uranium, and metal mining operations, even when aqueous Se concentrations do not exceed current CCME guidelines (set at 1 µg/L). In the

Elk River watershed in British Columbia, an area with coal mining activity, Se concentrations of lentic waters ranged from 0.5 to 543 µg/L and total selenium (TSe) concentrations in several salmonids ranged from 4.71 to 14.31 µg/g dry mass (dm) (Kuchapski and Rasmussen 2015). In northern Saskatchewan, lakes downstream of a uranium mining operation were reported to have concentrations of 0.7 to 2.7 µg/L in water, with fish accumulating mean whole body TSe concentrations of 14.98 µg/g dm (spottail shiners; *Notropis hudsonius*) and 17.02 µg/g dm (northern pike; *Esox lucius*) (Muscatello et al. 2008). In eastern Canadian lakes downstream of mining operations, Se in lentic waters ranged from 0.45 to 1.44 µg/L, and in yellow perch (*Perca flavescens)* mean TSe concentration was 27 µg/g dm in muscle tissue (Ponton and Hare 2015). Despite these incidences of high Se exposure in contaminated Canadian ecosystems, relatively little is known about the bioaccumulation and trophic transfer of Se in these systems. In order for future risk assessments in Canada to be effective, more information is needed to understand at what aqueous levels Se will be a concern, and to understand the factors that govern Se uptake and transfer specifically in these systems.

Lentic systems tend to be at comparatively higher risk for Se exposure compared to lotic systems because of their relatively longer water residence times, characterized by low-flow, low oxygen (reducing conditions), and generally higher biological activity. These conditions lead to higher proportions of more bioavailable forms of Se (i.e. more organoSe and selenite than selenate) and greater assimilation of Se into the food web (reviewed in Ponton et al. 2020). Further, higher retention times in lentic environments leads to more recycling of Se between compartments, for example, Se in organisms will eventually cycle back to the sediment/detrital layer once organisms die. Higher Se concentrations in slow moving waters compared to adjacent lotic areas in the same system have been observed in the Elk Valley watershed (Orr et al. 2006). Selenium concentrations in biota collected from lentic sites were higher compared to lotic sites despite no significant difference in aqueous Se concentrations between sites. Similar observations were made in the Jordan River drainage basin (Great Salt Lake, Utah), where Se concentrations were 2- to 5-fold higher in ponds than in creeks within the same basin (Hillwalker et al. 2006). Because of the higher potential for Se accumulation in lentic systems, this thesis will focus on understanding Se accumulation in lake ecosystems.

1.8 Canadian boreal lake characteristics

Canadian boreal lakes are defined as lakes within the boreal forest of Canada, which extends east to west from Newfoundland and Labrador to the far border of Yukon. Lakes in the boreal region can be split based on water hardness – hardwater lakes are in western Canada and softwater lakes are in central/eastern Canada. Softwater lakes are abundant, with approximately 700,000 in eastern Canada (Schindler et al. 1996). Lake catchments are mostly composed of a mixture of coniferous (spruce and pine) and deciduous (trembling aspen and white birch) trees, and bogs and fens are typical as well (Brunskill and Schindler 1971; Schindler et al. 1996). Depending on the location, lakes can have cold-water fish species (salmonids, whitefish species) or warm water species (northern pike, walleye *Sander vitreus*, smallmouth bass *Micropterus dolomieu*) (Wells et al. 2010).

There is large variation in the chemical and physical characteristics of boreal lakes, resulting in differences in nutrient content and food web structure which may lead to large variation in Se accumulation between lakes. Boreal lakes range from oligotrophic to eutrophic, shallow to >170 m deep, with large ranges in water chemistry variables such as DOC and water clarity (Brunskill and Schindler 1971; Schindler et al. 1996). Some lakes may rely primarily on pelagic energy sources, while others are shallow with large littoral zones where benthic energy sources dominate primary production (Schindler et al. 1996; Wells et al. 2010).

Approximately 180 million acres of the boreal forest are impacted by industries including forestry, road building, mining, and oil and gas exploration/production. More specifically, there are approximately 7000 abandoned mines, 105 active mines, and over 155,000 active oil and gas wells in the boreal forest, making it very relevant in terms of potential for Se exposure (Wells et al. 2010).

1.9 Biodynamic model of selenium accumulation

Because uptake of Se is an active process and transfer through food webs occurs mainly through the diet, environmental and physiological factors need to be considered to accurately predict Se exposure to fish. Currently, the best model for predicting Se bioaccumulation in higher trophic levels is a biodynamic model developed by Presser and Luoma (2010). This model focuses on the physiological factors that control Se uptake and considers diet preferences of organisms. The model uses measured particulate matter concentrations and either field or laboratory derived TTFs for primary and secondary consumers to determine accumulation of Se in fish and birds.

Model parameters include assimilation efficiency (AE; the proportion of total Se consumed that is absorbed by the consumer), ingestion rate (IR; the amount of Se being consumed per unit time), growth rate (k_g; unless the consumer of interest is in high growth phase this parameter is assumed to be negligible) and efflux rate (k_e; the rate that Se is lost from the organism). The equation for calculating the trophic transfer function for an organism is: TTF = (AE x IR)/(k_e+k_g). If no experimental data are available for an organism, which is the case for many freshwater organisms, then the TTF is calculated by dividing the concentration of Se in a consumer (C_{con}) by the concentration of Se in its prey (C_{prey}), TTF=(C_{con}/C_{prey}). If the consumer is known to feed on multiple prey items, the TTF for each prey type and the proportion each of prey type being consumed can be used to calculate a more accurate Se concentration. Therefore, knowledge of food web dynamics is critically important when estimating TTFs in field studies. This biodynamic model was tested by using a database of TTF values from invertebrates and fish and empirically measured particulate matter (periphyton/organic matter/sediment samples) to calculate predicted concentrations in invertebrates and fish. It was validated using 29 datasets from several sites including rivers, streams, lakes, reservoirs and estuaries and the R^2 values for predictions versus observations at these sites were 0.92 for invertebrates and 0.89 for fish (Presser and Luoma 2010). Due to the high site-specificity of Se accumulation, one of the main goals of the present study was to improve the prediction of Se exposure in Canada by increasing the applicability of the above models specifically to boreal lake ecosystems.

1.10 Statement of purpose, objectives and hypotheses

Selenium has recently been recognized as a contaminant of concern in Canada and water quality guidelines have been set for the protection of aquatic life. However, due to the highly site-specific nature of Se and the high variability in uptake and transfer through food webs, these guidelines may not be protective of all systems. There is a need to characterize Se accumulation and toxicity to lower trophic level organisms, particularly in boreal forest lakes, where many industrial activities occur in Canada. The goals of my research were to use *in-situ* mesocosms (limnocorrals) spiked with environmentally relevant levels of selenium to characterize the movement of Se through representative boreal lake food webs, identify lake- and taxa-specific differences in Se accumulation, and use these data to better predict Se accumulation in organisms. I also aimed to assess the response of aquatic organisms, particularly benthic macroinvertebrates and zooplankton, to these Se additions.

The overall objectives of my thesis were to:

1. Characterize partitioning of selenium in compartments of a boreal lake ecosystem (Chapters 2 and 4)
2. Determine site-specific k_ds and TTFs for biota within two boreal lakes (Chapters 2 and 4)
3. Assess the response of benthic macroinvertebrates and zooplankton to excess Se exposure (Chapters 3 and 5)
4. Compare Se bioaccumulation in mesocosms to Se bioaccumulation in other systems (Chapter 6)

The specific objectives, hypotheses, and predictions for each chapter were as follows:

In chapter 2, I wanted to characterize the partitioning of selenium into compartments of a boreal lake ecosystem (water, sediment, periphyton, invertebrates and fish) spiked with environmentally relevant levels of selenite, estimate k_ds and TTFs for biota, and calculate a mass balance for Se in filtered water, surface sediment, and biota. In this chapter I tested the hypothesis that Se partitions to environmental compartments (i.e., water, sediment and biota) in a concentration-response manner. I predicted that Se concentrations in water, sediment, and biota would increase with increasing amounts of Se added to water, but that k_ds and TTFs would decrease as a result of saturation of uptake or increased efflux rates.

In chapter 3, my goal was to determine the effect of Se exposure on benthic macroinvertebrate (BMI) abundance, biomass and taxonomic richness. I also wanted to explore effects of Se exposure on fathead minnow condition, liversomatic index and gonadosomatic index, and the association between Se exposure and autotrophic endpoints (chlorophyll *a* and organic matter content of algae). In this chapter, I hypothesized that selenium exposure has negative impacts on invertebrates. I predicted that benthic macroinvertebrate density, biomass, and diversity would decrease with increasing Se concentration as a result of toxicity to the organisms.

In chapter 4, I wanted to characterize Se bioaccumulation in food web compartments along a gradient of aqueous exposure concentrations, determine if there are differences in accumulation among different taxa, and use stable isotope values to explain the variability in fish Se bioaccumulation. I tested the hypothesis that Se exhibits saturable uptake kinetics in algae, invertebrates, and fish, and predicted that all organisms and tissues would fit Michaelis-Menten uptake kinetics due to the saturation of Se uptake or increased efflux rate of Se at higher exposure concentrations. I also tested the hypothesis that taxonomic groups differ in Se bioaccumulation

due to differences uptake capacities. For this hypothesis I predicted that there would be taxonomic differences in Se accumulation within the same habitat and trophic level due to physiological differences in uptake/excretion and/or differences in dietary Se exposure. Finally, I tested the hypothesis that fish diet changes with increasing Se exposure due to decreased biomass of food items sensitive to Se. I predicted that stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ in fish tissues would shift to reflect less feeding on organisms that decreased in biomass due to Se exposure.

In chapter 5, I aimed to assess community-level responses of benthic macroinvertebrates and crustaceous zooplankton to Se additions. I tested the hypothesis that Se exposure impacts the survival of invertebrates. I predicted that both zooplankton and benthic macroinvertebrate community composition would change with increasing Se concentration, such that Se-exposed communities would shift to be dominated by Se-tolerant species. I also predicted that the biomass, density, and diversity of organisms would decrease with increasing Se concentration as a result of Se toxicity.

In chapter 6, I wanted to compare Se bioaccumulation in several compartments between two study lakes, use the mesocosm Se data collected from both lakes to develop predictive models of Se bioaccumulation and trophic transfer for lentic ecosystems, and test the predictive ability of the models using existing literature data for algae, invertebrates and fish. For this chapter, I tested the hypothesis that Se accumulation in compartments is influenced by lake water chemistry characteristics (i.e., lake productivity). I predicted that Se accumulation in compartments would be higher in a mesotrophic lake relative to an oligotrophic one due to greater biological activity leading to the production of more reduced Se species and consequently higher uptake. I also hypothesized that the experimental mesocosm Se data could be used to predict Se bioaccumulation in other systems. I predicted that observed and predicted Se concentrations would be similar, provided that taxa of interest and the systems being compared were similar.

CHAPTER 2: DISTRIBUTION OF EXPERIMENTALLY ADDED SELENIUM IN A BOREAL LAKE ECOSYSTEM

Preface

In this chapter I focused on understanding the distribution and assimilation of Se following additions to limnocorrals in a representative boreal lake ecosystem. This is one of few studies to experimentally assess Se distribution at the whole-ecosystem scale. In this experiment, I added environmentally relevant concentrations of Se to *in situ* enclosures at the IISD-ELA and measured TSe bioaccumulation in periphyton, sediment, benthic macroinvertebrates, zooplankton, and female fathead minnow muscle and ovary tissues. Selenium bioaccumulation, enrichment and trophic transfer of Se was compared among two treatments and the control. A mass balance was done to determine the sinks for Se in this representative boreal lake. The results of this study show that in a mesotrophic lake exposed to excess Se as selenite, Se bioaccumulation in biota can exceed tissue guidelines for the protection of aquatic life at relatively low exposure levels. Differences in accumulation are evident between invertebrate taxa even in the same habitat or at the same exposure concentrations, and both enrichment and trophic transfer decrease with increasing exposure concentration.

This study was published in Environmental Toxicology and Chemistry with co-authors Karsten Liber (University of Saskatchewan), Vince Palace (IISD-ELA), Markus Hecker (University of Saskatchewan), Lorne Doig (University of Saskatchewan), and David Janz (University of Saskatchewan):

Graves SD, Liber K, Palace V, Hecker M, Doig LE, Janz D. 2019a. Distribution of experimentally added selenium in a boreal lake ecosystem. Environ Toxicol Chem 38:1954-1966.

2.1 Abstract

Human activities have increased the release of selenium (Se) to aquatic environments, but information about the trophic transfer dynamics of Se in Canadian boreal lake systems is limited. In the present study, Se was added as selenite to limnocorrals (2 m diameter, 3000 L *in situ* enclosures) in a boreal lake in northwestern Ontario to reach nominal concentrations of 1 µg Se/L and 10 µg Se/L in triplicates each for 77 d, and three additional limnocorrals were controls with no Se added. Total Se (TSe) concentrations were determined in water, sediment, periphyton, benthic macroinvertebrates, zooplankton and reproductively mature female fathead minnows (*Pimephales promelas*; added on d 33) collected throughout (and at the end of) the exposure period. Mean measured water Se concentrations in the control, 1 and 10 µg/L treatments were 0.12, 1.0 and 8.9 µg/L. At the end of exposure (day 77), distribution functions (k_ds) ranged from 7,772 L/kg dry mass (dm) in the 8.9 µg/L treatment to 23,495 L/kg dm in the 0.12 µg/L treatment, and trophic transfer factors (TTFs) for benthic macroinvertebrates ranged from 0.49 for Gammaridae to 2.3 for Chironomidae. Selenium accumulated in fathead minnow ovaries to concentrations near or above the current US EPA criterion (15.1 µg/g dm for fish ovary/egg) in the 1.0 and 8.9 µg/L treatments, suggesting that, depending upon aqueous Se speciation, such exposures have the potential to cause Se accumulation in fish to levels of concern in cold-water, boreal lake systems.

2.2 Introduction

Selenium (Se) is a contaminant of global concern, with elevated inputs of Se to aquatic environments being the result of several anthropogenic activities, such as coal mining and combustion, irrigation of seleniferous soils, and oil and gas extraction in areas with naturally high geological Se sources (Lemly 1985; Yudovich and Ketris 2006). Although there are natural sources of Se to aquatic environments, such as geological weathering of rocks and volcanic eruptions, human activities have led to an estimated 43% increase in the atmospheric load of Se (Nriagu 1989). Selenium is an essential nutrient for most organisms, but it exhibits a narrow range between essentiality and toxicity, and it is a contaminant of concern in aquatic environments where excess uptake of Se can lead to teratogenicity in egg laying vertebrates, such as fish and birds (Spallholz and Hoffman 2002; reviewed in Janz et al. 2010). While Se is increasingly being recognized as a contaminant of concern, its distribution in aquatic food webs is relatively under-studied in northern regions such as Canada, making the prediction, risk assessment and management of Se in northern aquatic systems difficult.

Selenium is typically released from anthropogenic point sources as the two main inorganic oxyanions (selenate and selenite), with higher proportions of selenate dominant in agricultural drainage and mining discharge, and higher proportions of selenite in oil waste and coal fly ash effluent (Presser and Luoma 2010). After release to aquatic systems, the oxidation state of Se in waterbodies depends to a large extent on the hydrological and biogeochemical characteristics of the receiving system. The more reduced oxyanion, selenite, tends to be associated with the reducing conditions and higher biological growth and activity in lentic environments (Nishri et al. 1999; Simmons and Wallschlager 2005). Globally, the speciation of Se in freshwater lakes is variable and relatively understudied, with the total percentage of selenite in aqueous Se samples ranging from 9 to 100% in Canada and Europe (Niedzielski et al. 2003; Pelechaty et al. 2004; Ponton et al. 2013). Selenium bioaccumulation in aquatic biota is highly site-specific and is dependent on food web pathways, with several biogeochemical, hydrological, and ecological factors affecting the speciation, partitioning, and bioavailability of Se to organisms (Stewart et al. 2004; Presser and Luoma 2010; Sharma et al. 2015). As well, Se concentrations in higher trophic level organisms such as fish do not necessarily reflect water Se concentration because diet is the primary route of Se exposure for animals (Young et al. 2010). As such, there is a need to

characterize Se bioaccumulation in several types of habitats and in different taxa in order to better understand the potential for differences in Se accumulation across different ecosystem types.

Currently, the Canadian Council of Ministers of the Environment (CCME) Se water quality guideline for the protection of aquatic life is set at 1 µg/L for all freshwater systems (CCME 2003), which does not necessarily reflect the site-specificity of Se exposure. The United States Environmental Protection Agency (US EPA) recently amended their Se criterion and recognized the importance of tissue-based guidelines because of the site-specific nature of Se accumulation in aquatic food webs. Egg-ovary and muscle tissue Se levels for the protection of fish populations are set at 15.1 and 11.3 µg/g dry mass (dm), respectively (US EPA 2016). Chronic (30 day average) exposure guidelines are set at 1.5 µg/L for lentic water and 3.1 µg/L for lotic water (US EPA 2016). The British Columbia Ministry of the Environment (BC MoE) have also implemented water (2 µg/L) and fish tissue (4 µg/g dm for whole body and 11 µg/g dm for ovary) guidelines for the protection of fish populations (BC MoE 2014). In Canada, Se bioaccumulation exceeding the toxicity threshold for fish tissues set by the US EPA and BC MoE (see above; there are currently no federal tissue-based guidelines in Canada) have been documented recently in areas downstream of coal, uranium, and metal mining operations, even in cases where aqueous Se concentrations have not exceeded the current CCME guideline (1 µg/L; Muscatello et al. 2008; Ponton and Hare 2013; Kuchapski and Rasmussen 2015). Despite these incidences of high Se exposure in contaminated Canadian ecosystems, relatively little is known about the bioaccumulation and trophic transfer of Se in these northern systems. In addition, the boreal forest region in Canada contains over 1.5 million lakes, and approximately 180 million acres of this boreal forest are impacted by industries including forestry, road building, mining, and oil and gas exploration/production, making the Canadian boreal forest very relevant in terms of potential for Se exposure (Wells et al. 2010).

There is currently a need to better understand the fate of Se in Canadian boreal lakes in order to characterize expected bioaccumulation factors and determine Se exposure risks to aquatic organisms in this region. The objective of the present study was to investigate the environmental distribution of Se in a representative boreal lake at two ecologically relevant aqueous selenite concentrations. Selenite, rather than selenate, was the form of Se chosen for additions in the present study because it poses a greater risk to aquatic life due to its higher bioavailability relative to selenate (Besser et al. 1993; Conley et al. 2013), and higher proportions of selenite can be observed

in slow-moving waters with high residence times such as the lake studied herein (Nishri et al. 1999; Ponton et al. 2013). More specifically, we added Se as selenite to 2 m diameter, 3000-L limnocorrals to achieve nominal aqueous concentrations of 1 and 10 µg Se/L in order to 1) determine total Se bioaccumulation in biota at each exposure level, 2) determine resulting enrichment functions for periphyton and trophic transfer factors (TTFs) for invertebrates and fish at each exposure level, and 3) determine the mass balance for Se in filtered water, surface sediment, and biota (periphyton, invertebrates and fish) after 77 d of exposure.

2.3 Materials and Methods

2.3.1 Study area

The International Institute for Sustainable Development - Experimental Lakes Area (IISD-ELA) is located southeast of the city of Kenora, in western Ontario, Canada. The lakes and their watersheds are devoted to scientific research and are relatively unaffected by human impacts. Lake 114 is located within the IISD-ELA and was chosen for this research based on the extensive monitoring data available for this lake, and the presence of the target fish species (fathead minnow, *Pimephales promelas*) within the lake. Lake 114 has a total area of 12.1 ha and a maximum depth of 5 m. Generally, the lake pH is 6.6 ± 0.2, conductivity is 17 ± 2.9 µS/cm, and dissolved organic carbon (DOC) is 7.1 ± 0.50 mg/L. Total dissolved nitrogen (TDN) in the lake is 638 ± 309 µg/L and total dissolved phosphorus (TDP) is 3.0 ± 0.7 µg/L. Water selenium levels in similar boreal shield lakes range from 0.04 to 0.23 µg/L (Belzile et al. 2005). In the ELA region, lakes are ice-covered from approximately November to April, and in 2017 the air temperature ranged from -33°C in January to 30°C in July.

2.3.2 Limnocorral set-up and experimental design

In May 2017, nine limnocorrals (circular enclosures; 2 m diameter, ~1 m depth) were deployed in Lake 114 (Figure A.2.1). These limnocorrals were sealed to the lake bottom with a continuous row of sandbags and enclosed approximately 3,000 L of water as well as the natural sediment and biota of the lake. Minnow traps were set in each limnocorral prior to the beginning of the experiment to remove endemic fish and amphibians. Artificial substrate samplers were deployed initially in the lake proper to maximize colonization time for periphyton and benthic macroinvertebrates, and then were transferred to limnocorrals after two weeks of colonization. Within each limnocorral, nine strips of five 4.8 x 4.8 cm unglazed clay tiles were suspended in the water column 30 cm from the surface for periphyton collection, and nine Hester-Dendy samplers

(benthic macroinvertebrate samplers) were suspended at the sediment-water interface. One 0.01 m^3 wire basket filled with cobble from the lake (Merritt and Cummins 1996) was placed on the sediment within each limnocorral to increase benthic macroinvertebrate biomass collection at the end of the Se exposure period. One 10 x 40 cm strip of limnocorral curtain material was suspended in the water column in each enclosure in order to estimate the mass of Se in periphyton on enclosure walls. The limnocorrals were set up in groups of 3 at three different sites within the lake based on similarity in sediment characteristics. Limnocorrals were separated due to the lack of sufficiently similar habitat to set up all nine corrals at any one site within the lake.

Within each of the three sites, limnocorrals were randomly assigned as a control (no Se added), 1 µg/L and 10 µg/L aqueous Se treatments. From June 1 to August 17, 2017, limnocorrals were spiked with selenite to maintain nominal concentrations of approximately 1 or 10 µg/L aqueous TSe. Details of limnocorral spiking are presented in the Supplemental Information. Limnocorrals were re-spiked four times (on d 7, 28, 42 and 56) throughout the exposure period to maintain nominal concentrations of Se in water. On d 32, minnow traps were set in L114 proper overnight and on d 33, 50 reproductively mature female fathead minnows in pre-spawning condition (morphometric data are reported in a companion manuscript; Graves et al. 2019b) were collected from the traps and kept in aerated coolers of lake water for transport to the limnocorrals. Five size-matched female fathead minnows were added to each limnocorral. Three female fathead minnows were also collected on d 33 for baseline TSe measurements. Minnows were intentionally added after periphyton and invertebrate TSe concentrations had reached approximate steady state, so that accurate measures of time to Se steady state for fish could be assessed. As well, fish consumption of biota could potentially decrease biomass of invertebrates.

2.3.3 Sampling and processing

A summary table of sample collection days and matrices collected is included in the supplemental information (Table A.2.1). Surface water from each limnocorral was collected on days -2, 0, 3, 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, and 77 of the exposure period. An integrating water sampler constructed in-house (composed of 1 m of 5.0-cm-inner diameter flexible polyvinyl chloride tubing with a one-way valve) was used to collect 1 L of water, from each enclosure at each sampling time, which incorporated the top 0.75 m of the water column. Water from each limnocorral was transferred to an acid-washed 1-L Nalgene bottle and transported to the laboratory on ice. In the lab, the 1-L sample was mixed, and an 8-mL subsample was taken using a 5-mL

syringe and filtered through a 0.45-µm polyethersulfone (PES) membrane. The subsample was acidified to 2% HNO_3 using 69% ultrapure HNO_3 (Fisher Scientific, Ottawa, ON, Canada) and samples were stored at 4°C until Se analysis. On surface water collection days, dissolved oxygen (DO) concentration and temperature were measured within each limnocorral using a Yellow Springs Instruments (YSI) Professional 2030 probe (Yellow Springs, OH).

From the remaining 1-L water sample, processing for water chemistry was done on days -2, 7, 14, 33, 49, 63 and 77. Total dissolved nitrogen and phosphorus (TDN and TDP) were analyzed at the IISD-ELA chemistry laboratory following filtration of 200 mL of limnocorral water through a pre-ashed 0.45-µm Whatman grade GF/C glass microfiber filter. Another 150 mL of water were filtered through a pre-ashed 0.45-µm GF/C filter for dissolved organic carbon (DOC) and major ions. Analysis of total dissolved nutrients, dissolved ions, acidity, and DOC followed the methods of Stainton et al. (1977).

On d -2, 7, 14, 33, 49, 63, and 77, periphyton was scraped from one strip of clay tiles from each limnocorral using an acrylic scraper, and loosened periphyton was then rinsed into a plastic sample bag. On day 77, periphyton from each strip of curtain material was collected in the same manner. Periphyton slurries were kept on ice for transport back to the laboratory, then transferred to acid-cleaned 50-mL Falcon tubes and topped up to 50 mL with reverse osmosis water. The slurry was centrifuged to pellet the periphyton, and water was decanted. Periphyton was stored at -20°C until further analysis.

Benthic macroinvertebrates were collected on days -2, 7, 14, 33, 49, 63 and 77 by removing one Hester-Dendy sampler from each limnocorral, placing it in a plastic sample bag with limnocorral water, and then placing it on ice for transport to the lab. In the laboratory, samplers were disassembled, and each piece was gently rubbed to dislodge all periphyton and invertebrates. The slurry of periphyton and invertebrates was transferred to a sorting tray, and all invertebrates were picked out, separated by family, counted, and then frozen at -20°C until further analysis. On day 77, wire basket substrates were removed from the limnocorrals and processed in the same manner. Invertebrates from the rock baskets were initially kept separate from invertebrates from the Hester-Dendy samplers. However, preliminary analyses showed no difference in TSe for the same taxa collected from the two substrate types; and therefore, invertebrates from Hester-Dendy samplers and rock baskets were subsequently pooled for analysis of day 77 samples.

Zooplankton was collected on d -2, 7, 14, 33, 49, 63, and 77 by making nine 1-m horizontal tows in the limnocorral using a 150-µm mesh plankton tow net. The net was rinsed using reverse osmosis water, and the composite zooplankton sample was rinsed into a plastic sample bag and transferred to the laboratory on ice. In the field laboratory, zooplankton samples were rinsed with reverse osmosis water through a 53-µm sieve, transferred to 50-mL falcon tubes, and frozen. Because of time constraints, zooplankton could not be live-sorted by taxon and were instead thawed and sorted in the laboratory upon return to the University of Saskatchewan. Zooplankton were sorted and analyzed for TSe by main taxa (cladocerans, and copepods sorted to two families: Cyclopoida and Calanoida) on days 33, 63, and 77. Preliminary analyses showed no discernible differences among these major taxonomic groups and therefore only zooplankton composite samples were analyzed for TSe on days -2, 7, 14, and 49.

Fathead minnow were re-captured from limnocorrals on days 49, 63, and 77 using minnow traps baited with dog food (enclosed in containers and covered with mesh so that the food could not be consumed) suspended in the water column. On days 49 and 63 one fish from each limnocorral was taken, and on day 77 the remaining 3 fish from each limnocorral were collected. Of the 45 fish added to the nine limnocorrals, 39 were recaptured at the end of the study. There were two mortalities observed during the experiment (both in 10-µg/L treatment limnocorrals). An additional two fish from controls and two fish from 10-µg/L treatment limnocorrals were not retrieved. Fish were anesthetized with buffered tricaine methanesulfonate (MS222; 1 mg/L), euthanized by spinal severance, and kept on ice for transport back to the laboratory. Immediately on arrival at the field laboratory, fish were weighed, fork length was measured, and fish were dissected. Muscle and ovary tissues were excised and stored at -20°C until further processing and Se analysis. All fish collection and handling procedures used in the present study were approved by the Animal Research Ethics Board at the University of Saskatchewan (protocol no. 20170046) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Sediment was collected on days -2, 33 and 77 using a sediment corer constructed in-house, composed of polyvinyl chloride pipe (~1.3 m long) with a ball valve at one end that, when closed, provided suction to recover the core. Acid-washed acrylic core tubes that were 28 cm long and 4.8 cm in diameter were used to collected cores on days -2 (nine baseline cores outside limnocorrals), 33, and 77 (one core per limnocorral). Total Se was measured in one baseline core, and all 9 cores were subsampled to determine loss on ignition and particle size (Heiri et al. 2001; Kettler et al.

2001). Cores were extruded on site immediately after collection, and the top 3 cm of sediment were taken (Orihel et al. 2015). In this lake, the top 3 cm of sediment is the readily mixing section of surface sediment that was expected to contain the majority of Se that partitioned to sediment during the experiment (Brunskill et al. 1971). Sediment was kept on ice for transport back to the field lab and was stored in the dark at 4°C until further analysis.

2.3.4 Analysis of total selenium (TSe)

Biota and sediment were prepared for total Se analysis using previously validated, in-house protocols. Samples were weighed, lyophilized, and weighed again dry. Sample weights were small (0.001 - 0.1 g), and in most cases the entire sample was used for total Se analysis. Where subsampling was done, the sample was first homogenized to a powder using a small glass stirring rod. All samples were microwave-digested in 1 mL 69% ultrapure HNO_3 and 2 mL American Chemical Society grade 30% H_2O_2 (Fisher Scientific, Ottawa, ON, Canada) and digested samples were diluted to 2% HNO_3.

Selenium in water, biota and sediment digests were measured separately using inductively coupled plasma - mass spectrometry (ICP-MS) at the Toxicology Centre, University of Saskatchewan. Quality assurance/quality control measures included an instrumental standard, a certified reference material, instrumental and method blanks, and sample duplicates. The instrumental standard reference material (SRM), natural water 1640a (National Institute of Standards and Technology, Gaithersburg, USA) was run with all samples with an analytical accuracy of 99.2 ± 4.5% (*n=71*). Sample duplicate mean percent difference was 5.86 ± 8.10% (*n=38*). A certified reference material (TORT-3, lobster hepatopancreas, National Research Council, Ottawa, Canada) was digested with biota and sediment samples with a mean (±standard deviation [SD]) percent recovery of 95.9 ± 6.75% (*n=30*). Ultra-pure water was also digested with samples as a method blank. Method blank Se concentrations averaged 0.03 ± 0.07 µg/L (*n=30*) and were generally lower for all analyzed samples than the instrumental quantification limit, which ranged from 0.06 to 0.10 µg/L among runs.

2.3.5 Mass balance of selenium

The instantaneous mass of Se was calculated at the end of the 77-d exposure period in six compartments: filtered water, periphyton, zooplankton, benthic macroinvertebrates, fathead minnow, and surface sediment. Detailed methods for mass balance calculations and masses of each

compartment used in mass balance calculations are presented in the supplemental information (Table A.2.2).

2.3.6 Statistical analyses

All statistical analyses were performed using the R software environment and associated base packages (R Core Team, 2014). Selenium concentrations were not normally distributed, and therefore generalized linear models (glm) were used to analyze the data with a gamma distribution and an inverse link. For all statistical analyses, alpha was set at 0.05 and data were presented as mean ± standard deviation (SD) unless otherwise stated.

Differences in sediment, periphyton, zooplankton, benthic invertebrate, and fish tissue total Se among treatments (within each time point) were assessed using glm with treatment group as the fixed categorical predictor variable. When significant differences were observed, a Tukey's multiple comparisons post hoc test was performed to determine where these differences occurred. For periphyton and sediment, only one sample was analyzed per limnocorral. For invertebrates, all individuals within each family were pooled for one replicate per limnocorral. Sample sizes varied for treatments and taxa based on which invertebrates were collected. In the 10-µg/L treatment, a decrease in invertebrate abundance and taxa present was observed, which affected the presence and abundance of invertebrates that could be collected (for example, mean biomass of Chironomidae larvae in the 10-µg/L treatment at day 77 was 4.66 ± 8.08 mg/m^2 compared to 65.1 ± 23.4 mg/m^2 in the control; Graves et al. 2019b). For benthic macroinvertebrates on days -2, 7 and 14, collected biomass was very low; and therefore, only composite samples of invertebrate taxa were analyzed for total Se. On days 33, 49, 63 and 77, benthic invertebrates were analyzed by taxon for total Se, and comparisons among treatments were made within each taxon. For zooplankton, samples were sorted to main taxa (Cladocera, Calanoida, and Cyclopoida) for three time points (days 33, 63, and 77) to determine if there were differences in total Se content among taxa. However, Cyclopoida were the only taxa present in the 10-µg/L treatment, and small sample masses limited the analysis of separated taxa, particularly in the 1- and 10-µg/L treatments. No differences in total Se concentrations were observed when multiple taxa were analyzed within a limnocorral (see below, Invertebrate total Se); therefore, all total Se values were pooled within limnocorrals to determine mean composite zooplankton total Se concentrations, and only composite samples were analyzed for the other time points (days -2, 7, 14, and 49). On days 49 and 63 there were 3 fish per treatment (except for one 8.9 µg/L limnocorral on day 63, where only

one fish was collected). On day 77, three individuals were analyzed per limnocorral (except for one control and one 8.9-µg/L treatment limnocorral, where two fish could not be retrieved from each), and values were pooled for each limnocorral so that there was an *n* of 3 per treatment for statistical analysis. A Kendall rank correlation test was performed to determine the relationship between fathead minnow muscle and ovary total Se for all individuals collected on day 77.

Differences in the distribution coefficient (k_d; Eq. 2.1) for sediment and periphyton, and trophic transfer factors (TTF; Eq. 2.2) for fish and benthic invertebrates among treatments were also analyzed using glm.

$$k_d \text{ (L/kg dm)} = \text{Sediment or algae TSe (µg/kg dm)/Water TSe (µg/L)} \quad (2.1)$$

$$\text{TTF} = \text{Consumer TSe (µg/g dm)/Diet TSe (µg/g dm)} \quad (2.2)$$

Sediment k_ds values were calculated using sediment total Se concentrations normalized to sediment organic matter content. Trophic transfer factors were calculated for several scenarios. For primary consumers (Chironomidae, Gammaridae, Ceratopogonidae, Planorbidae, Sphaeriidae, Ephemerellidae, Phryganeidae) TTFs were calculated using periphyton as the diet item. For predatory invertebrates (Aeshnidae, Corduliidae, Polycentropodidae, Dytiscidae, Hirudinea), TTFs were calculated using the mean total Se concentration of potential prey items as the diet total Se concentration (Tallman et al. 1984). For fish, TTFs were calculated based on muscle Se concentrations using zooplankton, Gammaridae, and Chironomidae as diet items. A fathead minnow TTF was also calculated with periphyton as the diet item because periphyton growth was substantial in the limnocorrals and fathead minnows are generally opportunistic feeders (Scott and Crossman 1973). For zooplankton, TTFs were not calculated because phytoplankton/particulate matter total Se was not measured. Differences in total Se and TTFs among invertebrate taxa within each treatment were analyzed using glm, as described above.

2.4 Results

2.4.1 Water chemistry

Water chemistry parameters were similar among the limnocorrals throughout the experimental period (Table 2.1). Temperature of surface water ranged from a minimum of 17°C in May to a maximum of 25°C in late July. Water total Se concentrations were variable throughout the exposure period within each limnocorral (Figure 2.1), but mean measured aqueous total Se concentrations were 0.12 ± 0.03, 1.0 ± 0.1, and 8.9 ± 2.7 µg/L for the control, 1.0- and 10-µg/L treatments, respectively. Mean measured aqueous total Se concentrations were within 13% of

Table 2.1: Limnocorral volume (measured on day 77) and water chemistry parameters (mean and minimum – maximum values) measured on days -2, 7, 14, 33, 49, 63 and 77 of the experimental period.

Treatment	Volume (L)	DO (mg/L)	pH (SU)	DOC (mg/L)	SO_4 (mg/L)	TDN (µg/L)	TDP (µg/L)
Control (1)	3140	8.8 (8.1 – 9.5)	6.4 (6.3 – 6.5)	6.77 (5.98 – 7.81)	1.34 (1.18 – 1.47)	323 (297 – 369)	5.72 (4.70 – 6.97)
Control (2)	3200	8.7 (8.2 – 9.5)	6.3 (6.0 – 6.4)	6.85 (6.38 – 7.42)	1.87 (1.46 – 2.15)	321 (297 – 367)	5.19 (4.35 – 5.67)
Control (3)	2857	8.6 (8.1 – 9.4)	6.3 (6.2 – 6.6)	6.62 (6.33 – 6.94)	0.80 (0.62 – 0.90)	344 (299 – 406)	5.09 (4.75 – 5.72)
1.0 µg/L (1)	3140	8.9 (8.3 – 9.5)	6.3 (6.1 – 6.8)	6.55 (6.10 – 7.05)	1.90 (1.25 – 2.11)	304 (279 – 371)	5.13 (4.47 – 5.80)
1.0 µg/L (2)	3110	8.8 (8.2 – 9.5)	6.5 (6.3 – 6.6)	6.85 (6.33 – 7.09)	1.55 (1.25 – 1.73)	321 (285 – 373)	4.76 (4.12 – 5.53)
1.0 µg/L (3)	3300	8.7 (7.9 – 9.8)	6.4 (6.3 – 6.7)	7.57 (6.77 – 7.87)	1.07 (0.82 – 1.24)	361 (323 – 412)	5.83 (4.10 – 8.63)
10 µg/L (1)	3548	8.7 (7.4 – 9.5)	6.5 (6.4 – 6.9)	7.27 (6.89 – 7.83)	1.33 (1.26 – 1.44)	333 (319 – 360)	5.73 (5.07 – 6.25)
10 µg/L (2)	3391	8.9 (8.3 – 9.6)	6.4 (6.2 – 6.4)	6.52 (5.90 – 6.93)	1.71 (1.19 – 1.79)	303 (294 – 363)	4.20 (3.84 – 8.83)
10 µg/L (3)	2830	8.7 (8.2 – 9.4)	6.6 (6.2 – 6.6)	7.01 (6.53 – 7.35)	1.05 (1.01 – 1.13)	313 (272 – 343)	4.53 (3.86 – 5.13)

DO = dissolved oxygen, DOC = dissolved organic carbon, SO_4 = sulphate, TDN = total dissolved nitrogen, TDP = total dissolved phosphorus

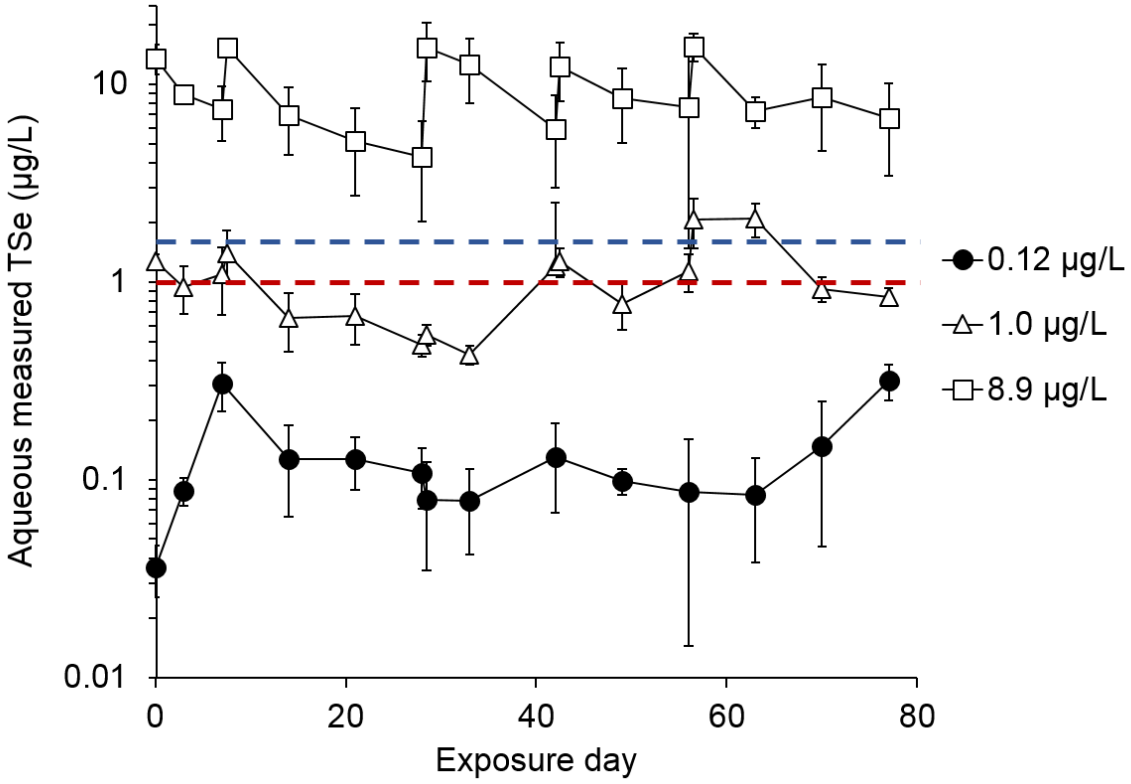

Figure 2.1: Mean (± SD) waterborne total selenium (TSe) measured at each sampling time point for 0.12-, 1.0- and 8.9-µg/L Se treatments throughout the 77-d exposure period. Red dotted line represents Canadian Council of Ministers of the Environment water Se guideline for the protection of aquatic life (1 µg/L) and blue dotted line represents US Environmental Protection Agency water Se guideline (1.5 µg/L for lentic systems).

target concentrations in the 1-µg/L treatment group and within 29% of target concentrations for the 10-µg/L treatment group. Treatment groups are referred to as mean measured aqueous total Se concentrations (0.12, 1.0 and 8.9 µg/L) throughout the *Results* and *Discussion* sections.

2.4.2 Sediment total selenium

Sediment total organic matter content ranged from 44 to 60% of total sediment dry mass. Inorganic sediment consisted mostly of particles in the >2000 µm (15 to 53%) and 53 to 2000 µm (45 to 74%) size fractions, with 0 to 19% of the particles in the <53-µm size fraction. No differences in particle size (glm, p=0.185) or organic matter content (glm, p=0.217) of baseline sediment were observed among the three sites where limnocorrals were deployed. On day 77, mean sediment total Se in 0.12-µg/L limnocorrals was 2.2 ± 0.38 µg/g dm and there was no significant difference between sediment total Se in the 1.0-µg/L group (3.7 ± 1.7 µg/g dm) and the 0.12 µg/L sediment total Se (glm, p=0.132; Figure 2.2A). Mean sediment total Se in the 8.9-µg/L treatment was 22 ± 4.7 µg/g dm on day 77 and was significantly greater than both 0.12- and 1.0-µg/L treatment sediment total Se (glm and Tukey, p<0.001; Figure 2.2A).

The distribution coefficient (k_d), normalized to sediment organic matter content, for sediment in 0.12 µg/L limnocorrals on day 77 was 35,102 ± 14,503 L/kg dm and was significantly greater than the k_d for sediment in the 1.0-µg/L treatment (7,162 ± 1,969 L/kg dm; glm and Tukey, p<0.001) and the 8.9-µg/L treatment (4,767 ± 645 L/kg dm; glm and Tukey, p<0.001; Figure 2.2B). There were no statistically significant differences between the k_d of the 1.0- or 8.9-µg/L treatments on day 77 (glm and Tukey, p=0.223; Figure 2.2B).

2.4.3 Periphyton total selenium

Periphyton total Se appeared to reach steady state by day 7 in the 1.0-µg/L treatment and by day 33 in the 8.9-µg/L treatment (Figure 2.3). Periphyton total Se differed significantly among each of the three treatments on days 14, 33, 49, 63, and 77 (glm and Tukey, p<0.008; Figure 2.3). On day 77, mean periphyton total Se in the 0.12-µg/L group was 3.0 ± 0.7 µg/g dm and had increased 4- and 24-fold to concentrations of 12 ± 2.5 µg/g dm and 71 ± 18 µg/g dm in the 1.0- and 8.9 µg/L treatments, respectively (Figure 2.3).

On day 77, mean periphyton total Se enrichment in the 0.12-µg/L group was 23,495 ± 5,816 L/kg dm and was significantly greater than enrichment in the 1.0-µg/L (11,777 ± 3,221 L/kg dm) and 8.9-µg/L (7,772 ± 2,333 L/kg dm) treatments (glm and Tukey, p<0.001; Table 2.2). There was no significant difference in k_d between the 1.0- and 8.9-µg/L treatments on day 77 (glm,

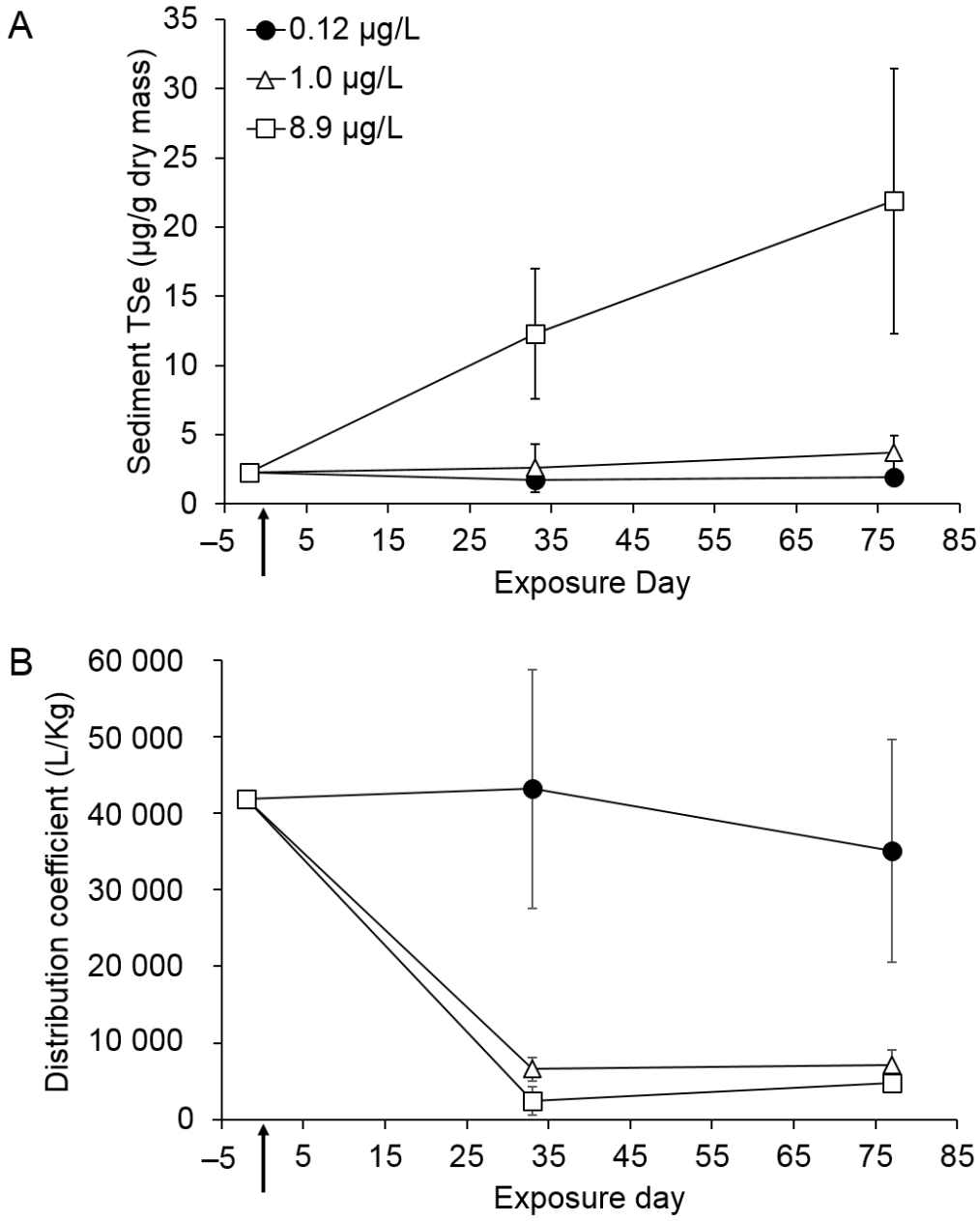

Figure 2.2: Mean (± SD) sediment total selenium (TSe; A) and associated distribution coefficients (k_d; B) for 0.12- (black circles), 1.0- (white triangles) and 8.9-µg/L (white squares) Se treatments on days 33 and 77. Day -2 represents baseline sediment Se concentration in Lake 114 proper. Date of first Se application is shown by an arrow.

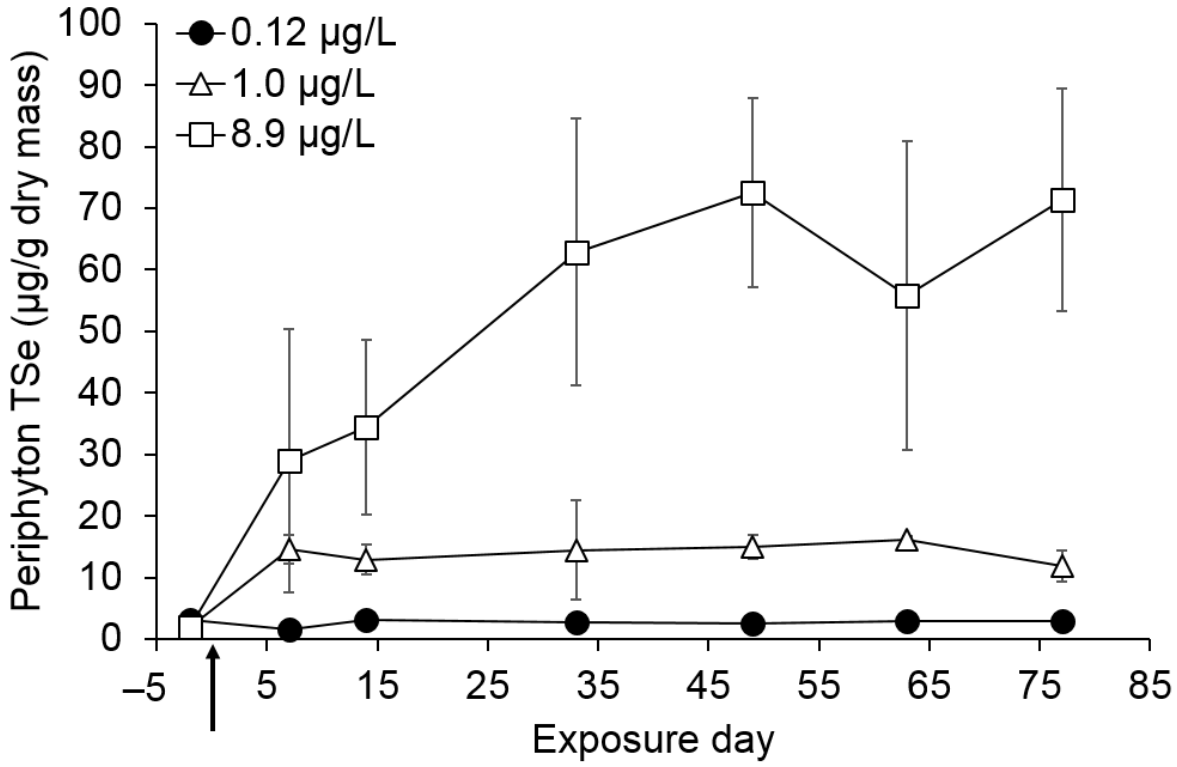

Figure 2.3: Periphyton total selenium concentrations (TSe; mean ± SD) for 0.12- (black circles), 1.0- (white triangles) and 8.9 µg/L (white squares) Se treatments on day 7, 14, 33, 49, 63, and 77. Day -2 represents baseline periphyton Se concentration in each treatment. Date of first Se application is shown by an arrow.

Table 2.2: Distribution functions (L/kg dm; mean ±SD) of periphyton on days 7, 14, 33, 49, 63, and 77 from 0.12-, 1.0- and 8.9-µg/L treatments.

Exposure Day	0.12 µg/L[b]	1.0 µg/L	8.9 µg/L
7	14,862 ± 5,649A	12,986 ± 1,880A	4,934 ± 3,534B
14	18,000 ± 8,816A	12,859 ± 3,593A	3,420 ± 1,616B
33	19,908 ± 4,745A	19,144 ± 10,025A	7,687 ± 3,578B
49	19,958 ± 7,189A	19,406 ± 3,038A	7,177 ± 1,849B
63	24,750 ± 3,582A	16,848 ± 2,358A	6,236 ± 3,178B
77	23,495 ± 5,816A	11,777 ± 3,222B	7,772 ± 2,333B

[a]Different letters denote significant differences among treatments within time points.

p=0.138; Table 2.2). The mean periphyton total Se enrichment in the 1.0-µg/L treatment once steady state was reached (day 7, 14, 33, 49, 63, and 77 pooled) was 15,018 ± 5,175 L/kg dm and for the 8.9-µg/L treatment (d 33, 49, 63, and 77 pooled) was 7,528 ± 2,109 L/kg dm.

2.4.4 Invertebrate total selenium

Invertebrate taxa collected from limnocorrals during the experiment included midge larvae (Chironomidae, Ceratopogonidae), amphipods (Gammaridae), dragonfly larvae (Aeshnidae, Corduliidae), caddisfly larvae (Polycentropodidae, Phryganeidae), predatory beetle larvae (Dytiscidae), freshwater clam (Sphaeriidae), freshwater snail (Planorbidae), mayfly larvae (Ephemerellidae), leech (Hirudinea), copepods (Cyclopoida, Calanoida), and cladocerans. Benthic macroinvertebrate total Se values for days 33, 49, 63, and 77 ranged from 1.5 to 154 µg/g dm across all treatments and taxa for primary consumers and from 1.5 to 135 µg/g dm for secondary consumers (Table 2.3). The TTFs ranged from 0.21 to 2.3 across all treatments and taxa for primary consumers, and from 0.31 to 2.3 across all treatments and taxa for secondary consumers (Table 2.4). The most abundant benthic macroinvertebrate taxon collected throughout the exposure period was Chironomidae. Chironomidae total Se was significantly different among 0.12-, 1.0-, and 8.9-µg/L treatments on days 33, 49, 63, and 77 (glm and Tukey, $p<0.001$; Figure 2.4). Zooplankton composite total Se was significantly greater in the 8.9-µg/L treatment relative to the 0.12 - and 1.0-µg/L treatments on days 7, 14, 33, 49, 63, and 77 (glm and Tukey; $p<0.021$; Figure 2.5). Zooplankton total Se was significantly greater in the 1.0-µg/L treatment compared to the 0.12-µg/L treatment on days 14, 33, 49, 63, and 77 (glm and Tukey, $p<0.019$; Figure 2.5). Total Se in zooplankton appeared to reach steady state by day 14 in the 1.0-µg/L treatment, and by day 49 in the 8.9-µg/L treatment. Although our ability to analyze separate zooplankton taxonomic groups for total Se was limited in the present study due to insufficient sample mass at several collection days, we did not observe any differences in total Se accumulation among cladocerans, cyclopoids or calanoids in the 0.12-µg/L group, with total Se concentrations ranging from 1.6 to 3.6 µg/g dm for cyclopoids, 2.2 to 3.5 µg/g dm for cladocerans, and 2.4 to 2.9 µg/g dm for calanoids. In the 1.0-µg/L treatment, calanoid concentrations ranged from 9.0 to 22 µg/g dm and cyclopoid concentrations ranged from 11 to 18 µg/g dm. In the 8.9-µg/L treatment, only cyclopoids were present with concentrations ranging from 91 to 193 µg/g dm.

Table 2.3: Total selenium concentrations (mean ± SD) for benthic macroinvertebrates (primary and secondary consumers) collected on days 33, 49, 63, and 77 from 0.12-, 1.0- and 8.9-µg/L treatments.

Day	Taxon	0.12 µg/L	1.0 µg/L	8.9 µg/L
Primary Consumers				
33	Chironomidae	2.6	12 ± 1.6	88 ± 22
33	Gammaridae	2.4	3.2 ± 1.8	
33	Sphaeriidae	1.5		
33	Ephemerellidae	2.8		
33	Phryganeidae			17
49	Chironomidae	4.0 ± 0.29	18 ± 4.9	59
49	Gammaridae			28
63	Chironomidae	5.2 ± 2.3	20 ± 6.3	42
63	Gammaridae	4.2 ± 3.0	7.2 ± 0.98	51 ± 4.3
77	Chironomidae	3.6 ± 1.7	20 ± 8.5	154 ± 57
77	Gammaridae	1.6 ± 0.17	8.4 ± 2.5	41 ± 9.8
77	Ceratopogonidae	2.3	17	
77	Ephemerellidae	3.0 ± 2.6		
77	Sphaeriidae		11 ± 2.0	
77	Planorbidae	4.9		
Secondary Consumers				
33	Polycentropodidae	3.3 ± 1.7	15	60
33	Aeshnidae			38
49	Polycentropodidae	3.0	11 ± 1.3	
63	Corduliidae	4.3		
77	Polycentropodidae		11	135
77	Aeshnidae	1.5 ± 0.08	10	
77	Hirudinea	2.1		34
77	Dytsicidae	3.0		

Table 2.4: Trophic transfer factors (TTF; mean ± SD) for benthic macroinvertebrates collected on days 33, 49, 63, and 77 from 0.12-, 1.0- and 8.9-µg/L treatments. Secondary consumer TTFs represent mean of TTFs calculated for all potential diet items.

Day	Taxon	0.12 µg/L	1.0 µg/L	8.9 µg/L
Primary Consumers				
33	Chironomidae	1.1	1.0 ± 0.77	1.7 ± 0.40
33	Gammaridae	0.96	0.21 ± 0.03	
33	Sphaeriidae	0.58		
33	Ephemerellidae	0.90		
33	Phryganeidae			0.31
49	Chironomidae	2.1 ± 0.45	1.2 ± 0.19	0.93
49	Gammaridae			0.44
63	Chironomidae	1.8 ± 0.65	1.24 ± 0.43	0.75
63	Gammaridae	1.4 ± 0.68	0.45 ± 0.09	1.2 ± 0.82
77	Chironomidae	0.96 ± 0.41	1.8 ± 0.93	2.3 ± 0.06
77	Gammaridae	0.63 ± 0.24	0.76 ± 0.27	0.49 ± 0.12
77	Ceratopogonidae	0.66	1.6	
77	Ephemerellidae	1.0 ± 0.68		
77	Sphaeriidae		1.1 ± 0.23	
77	Planorbidae	2.3		
Secondary Consumers				
33	Polycentropodidae	1.6 ± 0.15	2.3 ± 1.4	0.84
33	Aeshnidae			0.37
49	Polycentropodidae	0.71	0.73 ± 0.28	
63	Corduliidae	0.68		
77	Polycentropodidae		1.0 ± 0.32	2.3 ± 1.4
77	Aeshnidae	1.1 ± 0.22	1.2 ± 1.13	
77	Hirudinea	0.70 ± 0.46		0.30
77	Dytsicidae	1.3 ± 0.32		

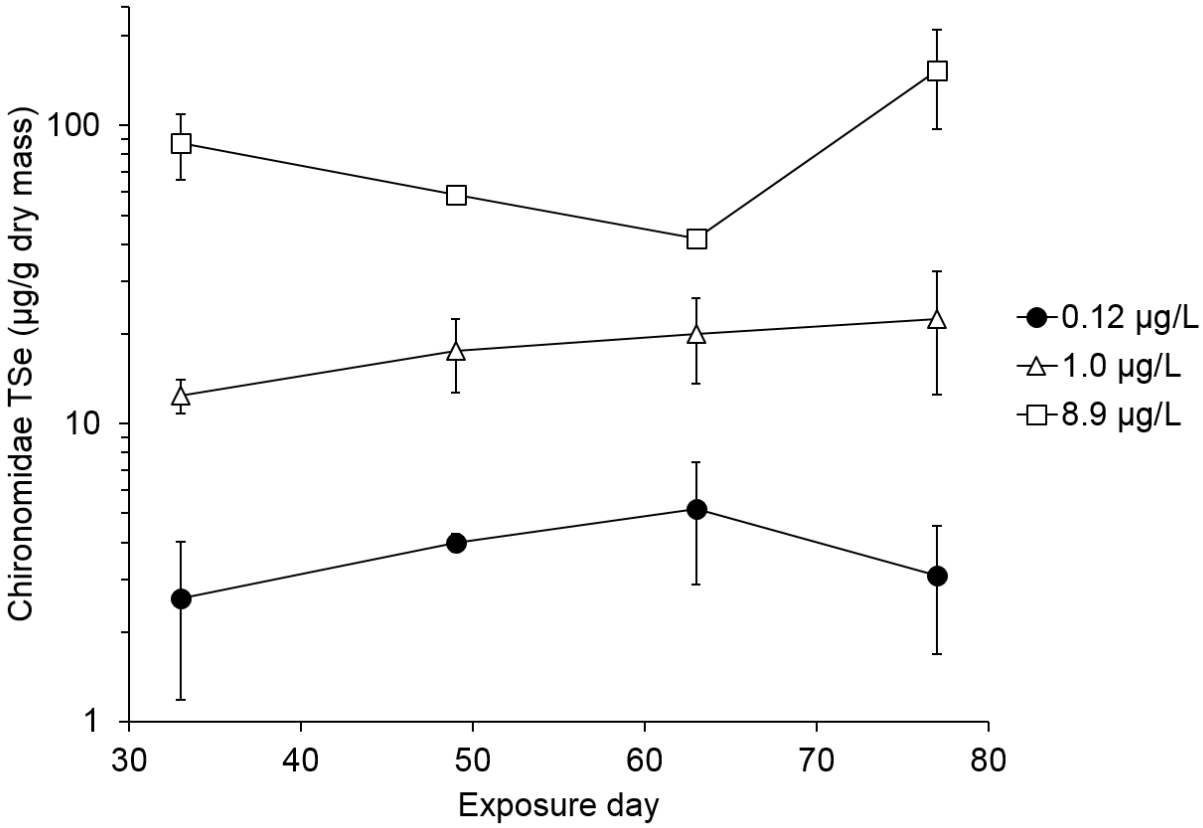

Figure 2.4: Chironomidae total selenium concentrations (TSe; mean ± SD) for 0.12- (black circles), 1.0- (white triangles) and 8.9-µg/L (white squares) Se treatments on days 33, 49, 63, and 77.

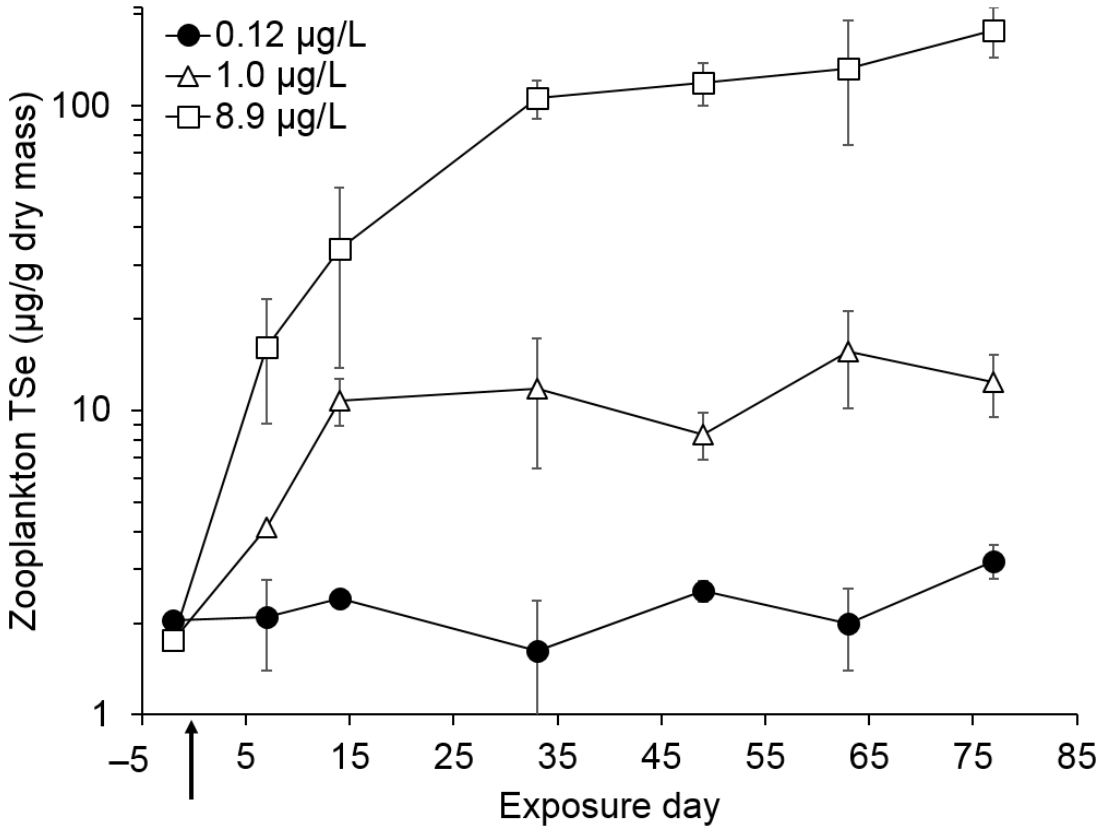

Figure 2.5: Composite zooplankton total selenium concentrations (TSe; mean ± SD) for 0.12- (black circles), 1.0- (white triangles) and 8.9-µg/L (white squares) Se treatments on days 7, 14, 33, 49, 63, and 77. Day -2 represents baseline composite zooplankton Se concentration in each treatment. Date of first Se application is shown by an arrow.

The invertebrate taxa that were present on day 77 in all treatments were Gammaridae, Chironomidae and Cyclopoida. Within the 0.12-µg/L group, there was no significant difference in total Se among Chironomidae, Cyclopoida or Gammaridae (glm, $p>0.080$). In the 1.0-µg/L treatment, total Se in Chironomidae was significantly greater than total Se in Gammaridae (glm and Tukey, $p=0.002$) but was not significantly different from total Se in Cyclopoida (glm and Tukey, $p=0.244$). Cyclopoida total Se was also greater than Gammaridae total Se in the 1.0-µg/L treatment (glm and Tukey, $p=0.049$). In the 8.9-µg/L treatment, Gammaridae total Se was significantly less than Chironomidae total Se (glm and Tukey, $p<0.003$) and Cyclopoida total Se (glm and Tukey, $p=0.004$). Cyclopoida and Chironomidae total Se were not significantly different in the 8.9-µg/L treatment (glm, $p=0.940$).

The corresponding mean TTF for Gammaridae on day 77 was 0.63 ± 0.24 for the 0.12-µg/L group and was not significantly different from the TTFs in the 1.0-µg/L (0.76 ± 0.27) and the 8.9-µg/L (0.49 ± 0.12) treatments (glm, $p>0.190$, Table 2.4). Mean Chironomidae TTF on day 77 was 0.96 ± 0.41 in the 0.12-µg/L treatment and was not significantly different from the Chironomidae TTF in the 1.0-µg/L (1.8 ± 0.93) or the 8.9-µg/L treatments (2.3 ± 0.06) (glm, $p>0.060$; Table 2.4). Chironomidae and Gammaridae TTFs were not significantly different from each other in the 0.12-µg/L group (glm, $p=0.564$; Table 2.4). In the 1.0- and 8.9-µg/L treatments, Chironomidae TTFs were significantly greater than Gammaridae TTFs (glm, $p<0.012$; Table 2.4).

2.4.5 Fish tissue total selenium

Background mean female fathead minnow muscle total Se concentration in fish collected from Lake 114 (outside of enclosures) was 2.0 ± 0.39 µg/g dm. After 44 d of exposure (day 77 of experimental period; fish were added on ayd 33), mean fish muscle total Se was 2.3 ± 0.27 µg/g dm in the 0.12-µg/L limnocorrals and significantly increased 2.3-fold (6.3 ± 2.1 µg/g dm) and 9-fold (21 ± 3.0 µg/g dm) in the 1.0- and 8.9-µg/L treatments, respectively (glm and Tukey $p<0.001$; Figure 2.6A).

Background mean female fathead minnow ovary total Se was 3.8 ± 0.17 µg/g dm in fish collected from Lake 114 proper. After 44 d of exposure (day 77 of experimental period), mean total Se in ovary tissue was 5.5 ± 1.8 µg/g dm in fish from 0.12-µg/L limnocorrals and significantly increased 2.3-fold (12 ± 2.7 µg/g dm) and 8.4-fold (46 ± 4.3 µg/g dm) in the 1.0- and 8.9-µg/L treatments, respectively (glm and Tukey, $p<0.001$; Figure 2.6B). Fathead minnow muscle and ovary total Se concentrations were significantly and positively correlated (Kendall correlation,

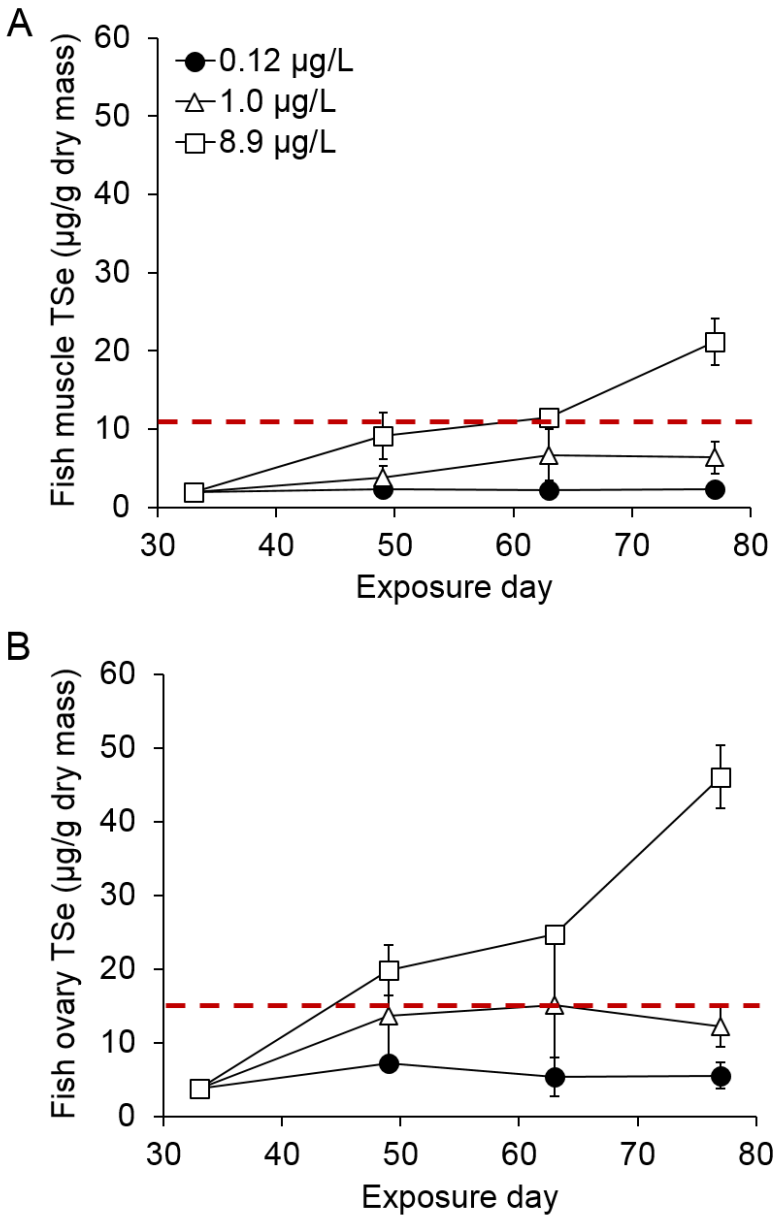

Figure 2.6: Mean (± SD) female fathead minnow muscle (A) and ovary (B) total selenium (TSe) concentrations for 0.12- (black circles), 1.0- (white triangles) and 8.9-µg/L (white squares) Se treatments on days 49, 63, and 77. Day 33 represents the mean total Se in muscle or ovary tissue of 3 female fathead minnows collected from Lake 114. Red dotted lines represent US Environmental Protection Agency fish muscle (11.3 µg/g dm) and egg/ovary (15.1 µg/g dm) tissue guidelines for the protection of fish populations.

$p<0.001$, $T=0.75$).

The TTFs for fathead minnow (based on muscle Se concentrations), calculated using zooplankton as the assumed diet after 44 d of exposure (day 77 of experimental period), were 0.96 ± 0.16 in the 0.12-µg/L treatment, and decreased significantly to 0.47 ± 0.20 and 0.15 ± 0.07 for the 1.0- and 8.9-µg/L treatments, respectively (glm and Tukey, $p<0.020$; data not shown). With Chironomidae used as the diet item, calculated TTFs for fathead minnow were 0.96 ± 0.30 for the 0.12-µg/L treatment and decreased significantly to 0.37 ± 0.16 (glm and Tukey, $p<0.001$) and 0.15 ± 0.05 (glm and Tukey, $p<0.001$) for the 1.0- and 8.9-µg/L treatments, respectively. With Gammaridae used as the diet item, calculated TTFs for fathead minnow were 1.4 ± 0.30 for the 0.12-µg/L treatment and decreased significantly to 0.87 ± 0.30 (glm and Tukey, $p=0.004$) and 0.45 ± 0.06 (glm and Tukey, $p<0.001$) for the 1.0- and 8.9-µg/L treatments, respectively. With periphyton used as the diet item, calculated TTFs for fathead minnow were 0.79 ± 0.13 for the 0.12-µg/L treatment and decreased significantly to 0.52 ± 0.10 (glm and Tukey, $p<0.001$) and 0.28 ± 0.04 (glm and Tukey, $p<0.001$) for the 1.0- and 8.9-µg/L treatments, respectively (data not shown).

2.4.6 Selenium mass balance

The total mass of Se quantified in filtered water, periphyton, zooplankton, benthic macroinvertebrates, fathead minnow, and surface sediment ranged from 2,582 to 3,594 µg among 0.12-µg/L limnocorrals with mean percentages of 13 ± 1.1%, 1.7 ± 1.3%, and 86 ± 0.25% in filtered water, periphyton and surface sediment compartments, respectively (Table 2.5). The total mass of Se quantified in fathead minnow, zooplankton and benthic macroinvertebrates was <1% of total Se quantified in 0.12-, 1.0- and 8.9-µg/L limnocorrals (Table 2.5). The relative proportions of Se in periphyton and water increased in the treatment limnocorrals, while the proportion of Se in surface sediment decreased in the treatment limnocorrals (Table 2.5). The mean percentage of total measured Se in periphyton was 3.9 ± 2.4% and 7.0 ± 4.2%, in sediment was 50 ± 8.7% and 49 ± 15%, and in water was 46 ± 6.5% and 45 ± 19% in the 1.0- and 8.9-µg/L treatments, respectively. The mass balances were variable among limnocorrals and accounted for 41 to 88% of the total Se added for the 1.0-µg/L treatment, and from 42 to 59% of the total Se added for the 8.9-µg/L treatment (Table 2.5).

Table 2.5: Mass balance of Se in 0.12, 1.0 and 8.9 µg/L treatment limnocorrals after 77 d of exposure.

Treatment	0.12 µg/L			1.0 µg/L			8.9 µg/L		
Replicate	1	2	3	1	2	3	1	2	3
Se Mass added (µg)	0	0	0	8064	9612	11699	94356	93888	95148
					Mass of Se measured (µg)				
Filtered Water	471	320	343	2763	2799	2442	37149	14344	15649
Periphyton	31	90	28	83	327	226	1238	4775	3616
Zooplankton	1.2	1.7	0.69	6.6	6.0	5.2	77	64	70
Benthic Macroinvertebrates	1.1	5.1	4.6	17	5.1	20	2.4	30	38
Fathead Minnow	1.6	1.3	0.42	3.4	6.4	2.7	9.2	2.7	8.8
Sediment	3088	2425	2203	4275	2597	2138	18445	29480	21315
						% of Se added			
Filtered Water				34	29	21	39	15	16
Periphyton				1	3	2	1	5	4
Zooplankton				0.08	0.06	0.04	0.08	0.07	0.07
Benthic Macroinvertebrates				0.21	0.05	0.17	0.003	0.03	0.04
Fathead Minnow				0.04	0.07	0.02	0.01	0.003	0.009
Sediment				53	27	18	19	31	22
Total				88	59	41	59	51	42

2.5 Discussion

To investigate the *in situ* distribution of Se in a boreal lake ecosystem at two environmentally relevant Se concentrations, continuous experimental additions of selenite were conducted to characterize distribution to water and sediment, enrichment by periphyton, and trophic transfer to benthic invertebrates, zooplankton, and fathead minnow. Significant accumulation of Se in sediment and biota after 77 d of exposure in both the 1.0- and 8.9-µg/L Se treatments was observed, and fish tissue Se concentrations approached or exceeded the US EPA and BC MoE tissue guidelines for the protection of aquatic life (BC MoE 2014; US EPA 2016). A concentration-dependent response in the enrichment of Se by periphyton and taxonomic differences in bioaccumulation of Se by benthic macroinvertebrates were observed. The results suggest that the current CCME guideline of 1 µg Se/L in water may not be protective of all freshwater systems across Canada, particularly in organic and nutrient-rich systems with high residence times and reducing conditions that may favour the production of more bioavailable forms of Se such as selenite, which can account for 9 to 63% of total Se in Canadian freshwater lakes (Ponton and Hare 2013).

Aqueous Se concentrations declined over time after spiking, likely as a result of several processes including assimilation by organisms at the base of the food web, assimilation by sediment associated microorganisms, and adsorption to inorganic and organic constituents in the water and sediment such as iron and organic carbon (Bruggeman et al. 2007; Luoma and Rainbow 2008; Peel et al. 2017). In the present study, Se was added as selenite, which is the more bioavailable of the two water soluble oxyanions of Se and represents a higher and more immediate uptake scenario than exposure to selenate (Fisher and Wente 1993; Luoma and Rainbow 2008). The mass balance showed that sediment accumulated the greatest mass of Se of the the compartments considered, indicating that sediment was a significant sink for Se in the present study. We did, however, observe lower partitioning of Se to sediment in treatment limnocorrals compared to controls, which may indicate that steady-state concentrations were not reached in the lake surface sediments after addition of Se or that partitioning of Se is influenced by the form of Se added (100% selenite in the present study). Partitioning to sediment could be due to selenite adsorption to sediment organic matter, assimilation and reduction by sediment-associated microorganisms, as well as detritus from dead and decaying organisms that have accumulated Se. Because Se in sediment did not appear to reach steady state after 77 d, removal of Se to sediment

appears to be a slow process. However, detritivores have been shown accumulate greater Se concentrations relative to other invertebrate types, suggesting that the sediment-detrital pathway can be an important source of Se accumulation in benthic aquatic food webs, particularly in boreal lake food webs with lower nutrients and lower lake productivity (Canton and Van Derveer 1997; Orr et al. 2006; Muscatello et al. 2008; Janz et al. 2014). The k_d values measured for sediment in the 0.12-, 1.0- and 8.9-µg/L treatments herein were indeed within the range of values for lakes and reservoirs summarized by Presser and Luoma (2010), and were in general greater than those measured for rivers and creeks.

In the present study, the mass balance calculations for filtered water, periphyton, zooplankton, benthic macroinvertebrates, fathead minnow, and sediment accounted for 41 to 88% of total added Se within each limnocorral. A portion of unmeasured Se was likely bound to particulate matter and taken up by phytoplankton in the water column, but this would likely account for a relatively small mass of Se compared to sediment or water compartments. Leakage from limnocorrals over the experimental period was not measured in the present study; however, a previous study using similar-sized enclosures measured sodium loss over time and estimated that water residence time in enclosures was 5.6 d, suggesting that leakage and porosity of the enclosure could contribute to water (and, subsequently, Se) loss (Baulch et al. 2003). As well, a portion of the added Se was likely methylated by algae or bacteria to volatile Se-containing compounds such as dimethylselenide and dimethyldiselenide (Vriens et al. 2015; Luxem et al. 2017), but the amount of volatilized Se was not measured in the present study. The present mass balance focused on accounting for masses of Se in the largest compartments of the boreal lake ecosystem, but future studies should focus on refining the mass balance to include Se adsorption to particulates, assimilation by phytoplankton, and volatilization from freshwater lakes.

Periphyton enriched Se by up to 4 orders of magnitude in the present study and this represented the largest accumulation step in the food web. Enrichment decreased as aqueous Se concentration increased, likely due to the high uptake efficiency of Se occurring at lower source concentrations (DeForest et al. 2016). Relative to lakes in northern Saskatchewan with similar water Se concentrations, periphyton bioaccumulation of Se was greater in the present study and is likely due to the form of aqueous Se added (selenite in the present study). For example, with water Se concentrations of <0.1, 0.7, and 2.7 µg/L, mean (± standard error) periphyton Se concentrations were 0.29 ± 0.05, 1.01 ± 0.26, and 3.75 ± 0.64 µg/g dm, respectively (Muscatello et al. 2008).

Periphyton Se in the 1-µg/L treatment herein was more comparable to particulate matter Se from the Salton Sea (8.2-16 µg/g dm), where the aqueous Se concentration was 0.92 µg/L (Leblanc and Schroeder 2008). Periphyton Se concentrations in the 8.9-µg/L treatment herein were greater than particulate matter concentrations measured in Belews Lake, North Carolina (30.5 µg/g dm) and Kesterson reservoir, California (32.0 µg/g dm) when water concentrations were 10.9 µg/L and 9.0 µg/L, respectively (Cumbie 1984; Lemly 1985; Saiki and Lowe 1987).

Macroinvertebrates in the present study accumulated high levels of Se, and taxonomic differences in Se accumulation were observed, although trophic transfer factors appeared to be unchanged among exposure levels within taxa. In the 1-µg/L Se treatment, accumulation of Se in macroinvertebrates was comparable to previous studies; invertebrate total Se at Barns Lake in the Elk Valley watershed ranged from 3.4 µg/g dm in caddisflies to 9.6 µg/g dm in mayflies when water total Se was 0.5 µg/L (Orr et al. 2006). However, in the 8.9-µg/L treatment herein invertebrate total Se concentrations were greater in the present study compared to Belews Lake, where mean invertebrate Se was 51 µg/g dm for zooplankton/phytoplankton and 93 µg/g dm for insects when water Se concentrations were 10 µg/L (Cumbie 1984; Lemly 1985). The higher concentrations of Se in biota observed in the present study relative to other systems at similar water total Se concentrations are likely attributable to Se having been added as 100% selenite, which is the more bioavailable inorganic water-soluble form of Se.

Similar to previous studies, detritivores such as Chironomidae appeared to accumulate the highest Se concentrations among invertebrate taxa, with amphipods (Gammaridae) accumulating some of the lowest Se concentrations (Saiki et al. 1993; Orr et al. 2006; Muscatello et al. 2008). For example, Chironomidae, Amphipoda and zooplankton were reported to have TTFs of 2.7, 0.94, and 1.5, respectively (Saiki et al. 1993). The benthic invertebrates in the present study were collected from and compared within the same substrates, indicating that invertebrates occupying the same habitat can differ in Se by 2- to 3-fold depending on diet and/or physiological differences in the assimilation or efflux of Se. As well, this implies that fish tissue concentrations could likely also vary by 2- to 3-fold in the same habitat depending on food preferences. This influence of food web pathway on Se accumulation was also reported by Stewart et al. (2004), who observed that bivalves in San Francisco Bay had a 10-fold slower rate loss of Se than crustaceous zooplankton, resulting in higher concentrations of Se in predators of bivalves relative to organisms feeding on zooplankton within the same system. Interestingly, in the present study Cyclopoida and

Chironomidae accumulated very high Se concentrations, particularly in the 8.9-µg/L treatment (up to 200 and 193 µg/g dm, respectively), showing that at high exposure concentrations of selenite these organisms may lack an adequate mechanism to regulate Se efflux or decrease assimilation of Se. It is important to mention that in the present study high accumulation of Se in the 8.9-µg/L treatment led to decreases in the abundance and diversity of invertebrates (Graves et al. 2019b), which limited our ability to collect several invertebrate taxa from the 8.9-µg/L treatment. This decrease in invertebrates appeared to limit the food availability for fish in the 8.9-µg/L treatment.

Fish accumulated Se to levels above the US EPA's fish tissue guidelines after exposure to 1.0 µg/L and 8.9 µg/L Se and associated Se enrichment of food organisms for a total of 44 d. Although mean TSe concentrations in fish ovaries from the 1.0-µg/L treatment did not exceed tissue guidelines (15.1 µg/g dm for ovary tissue; US EPA 2016) individual fish did have ovary Se concentrations of up to 27 µg/g dm. In the 8.9-µg/L treatment, all fish exceeded US EPA (2016) and BC MoE (2014) muscle and ovary tissue guidelines. As mentioned above, invertebrate abundance decreased in the 8.9-µg/L treatment, which likely altered fish diet and decreased overall food availability in this treatment, impeding the estimation of time to steady state Se concentration for these fish (Graves et al. 2019b). In addition, the Se uptake curve for the 8.9-µg/L treatment indicates that fish did not reach steady-state Se concentrations after the 44-d exposure period. Given that the half-life of Se in fish is approximately 3 to 4 weeks (Presser and Luoma 2010), it can take up to 100 d to reach steady state (Bennett et al. 1986; McIntyre et al. 2008). Previously measured fathead minnow TTFs were approximately 1.0; for example in Miller's Lake, Colorado, where particulate matter Se concentrations were 4.6 µg/g, Chironomidae concentrations reached 18.8 µg/g dm and fathead minnow Se ranged from 8.7 to 16.6 µg/g dm (Birkner 1978). Fathead minnow TTFs were also approximately 1.0 in Belews Lake, when the aqueous Se concentration was 10 µg/L the mean invertebrate Se was 51 µg/g dm for zooplankton and 93 µg/g dm for insects, and fish muscle total Se was approximately 70 µg/g for bluegill sunfish (*Lepomis macrochirus*), redear sunfish (*Lepomis microlophus*), warmouth (*Lepomis gulosus*), and pumpkinseed sunfish (*Lepomis gibbosus*; Cumbie 1984; Lemly 1985).

It is important to note that although female fathead minnow in the present study appeared to be in spawning condition on day 33 when they were added to enclosures (gonadosomatic index (GSI) of fish ranged from 6.0 to 9.1%; Graves et al. 2019b), on day 49 only fish in the control enclosures appeared to be in spawning condition (GSI ranged from 3.8 to 16.9%), and on day 63

and 77, all fish appeared to be in post-spawning condition (GSI ranged from 0.12 to 1.8% among fish). Given that egg maturation is correlated with GSI (Smith 1977), this indicates that fathead minnow at these later time points had less mature and therefore less vitellogenin-rich eggs, possibly confounding attainment of steady state Se concentrations in ovaries. However, despite being in postspawning condition at the end of the 77-d exposure period, there was a strong positive correlation between muscle and ovary tissue Se concentrations. This relationship between tissue Se concentrations has been observed for different fish species at reproductive maturity, such as bluegill sunfish and razorback sucker (*Xyrauchen texanus*) (R^2 range 0.72 to 1.00) whereas other species such as mountain whitefish (*Prosopium williamsoni*) and cutthroat trout (*Oncorhynchus clarkii*) show weaker tissue Se correlations (R^2 range 0.28 to 0.85) (deBruyn et al. 2008), indicating potential differences in the toxicokinetics of Se among fish species.

The present study showed that fish exposed to aqueous selenite concentrations at levels similar to the current CCME water quality guideline for the protection of aquatic life (1 µg/L) can exceed tissue guidelines for the protection of fish populations established by the US EPA and there is potential for adverse effects particularly in developing embryos. In Herrington Lake, Kentucky, adverse effects in wild young of the year largemouth bass (*Micropterus salmoides*) with whole-body Se concentrations ranging from 5.9 to 8.5 µg/g dm have been observed, with spinal, craniofacial and fin abnormalities increasing to 25 times background deformity rates (Lemly 2017). However, these whole-body concentrations were observed where water Se concentrations were around 10 µg/L, sediment concentrations were 15 µg/g, and invertebrate concentrations were 27 µg/g (Downstream Strategies 2016). For rainbow trout (*Oncorhynchus mykiss*), a rate of 15% deformities in populations of wild fish were observed for eggs with 8.8 to 10.5 µg/g wet mass (wm) Se, which is approximately 22 to 27 µg/g dm assuming 61% moisture (Holm et al. 2005). For wild brown trout (*Salmo trutta*) eggs collected from Sage Creek in Southeast Idaho, Se concentrations ranged from 6.2 to 40.3 µg/g dm, and the calculated 10% effect concentrations for survival during the hatch to swim-up period and for deformities were 20.6 µg/g dm and 21.8 µg/g dm, respectively (Covington et al. 2018). Based on the effect concentrations observed in previous studies and the measured ovary Se concentrations in the present study, there is potential for adverse effects in wild fish to occur after exposure to 1 µg/L Se as selenite in water and associated elevated Se levels in fish prey.

The goal of the present study was to characterize the distribution of Se in a boreal lake food web in order to better understand exposure, bioaccumulation and trophic transfer of this contaminant of concern. We found that the extent of Se bioaccumulation was comparable to previously studied lake systems in the United States and Canada and that there is potential for fish to exceed tissue guidelines for the protection of fish populations at the current Canadian water quality guideline for Se.

CHAPTER 3: EFFECTS OF SELENIUM ON BENTHIC MACROINVERTEBRATES AND THE FATHEAD MINNOW IN A BOREAL LAKE ECOSYSTEM

Preface

This experiment was conducted concurrently with that of Chapter 2. As a consequence of the high bioaccumulation of Se in the aforementioned study, toxicity to benthic macroinvertebrates was observed. I observed decreased biomass and density of Gammaridae and Chironomidae, and decreased richness of benthic macroinvertebrate taxa. Overall health effects of Se on fathead minnow (measured as condition factor) and changes in primary production as a function of Se exposure were also observed. The results of this study show that some invertebrate taxa may be at risk for Se toxicity at relatively low selenite exposure concentrations, and that decreases in benthic macroinvertebrate biomass, density, and diversity may indirectly impact the overall health of adult fish in Se-exposed areas. Overall, this study suggests that invertebrates may be affected by Se exposure at levels lower than previously thought, and future ecological risk assessment of Se may need to consider toxicity and sensitivity of invertebrate taxa as well as fish.

This research was published in Ecotoxicology and Environmental Safety with co-authors Karsten Liber (University of Saskatchewan), Vince Palace (International Institute for Sustainable Development-Experimental Lakes Area), Markus Hecker (University of Saskatchewan), Lorne Doig (University of Saskatchewan), and David Janz (University of Saskatchewan):

Graves SD, Liber K, Palace V, Hecker M, Doig LE, Janz DM. 2019b. Effects of selenium on benthic macroinvertebrates and fathead minnow (*Pimephales promelas*) in a boreal lake ecosystem. Ecotox Environ Saf 182:109354.

3.1 Abstract

Selenium (Se) is a contaminant of concern in many aquatic ecosystems due to its narrow range between essentiality and toxicity in oviparous (yolk-bearing) vertebrates. The objective of the present study was to determine the effects of Se, experimentally added to *in situ* limnocorrals as selenite, on invertebrate communities and fathead minnow (*Pimephales promelas*) at environmentally realistic Se concentrations. Nine limnocorrals were deployed in a mesotrophic lake at the International Institute for Sustainable Development – Experimental Lakes Area in Ontario, Canada in May 2017. From June 1 to August 17, 2017, selenite was added to six enclosures to attain mean measured aqueous Se concentrations of 1.0 ± 0.10 or 8.9 ± 2.7 µg/L Se (in triplicate) and three limnocorrals were untreated controls (background mean aqueous Se = 0.12 ± 0.03 µg/L). Benthic macroinvertebrates were collected throughout and at the end of the exposure period using artificial substrates to determine density, dry biomass, diversity, and taxa richness at the family level. Reproductively mature female fathead minnows (added on d 33 of the study) were collected throughout and at the end of the exposure period. After 77 d, Chironomidae and Gammaridae densities and biomass were significantly lower in the 8.9 µg/L Se treatment relative to the 1.0 µg/L Se treatment and the control. Invertebrate diversity (measured as Shannon's and Simpson's indices) significantly declined in the 1.0 µg/L and 8.9 µg/L Se treatments relative to the control (0.12 µg/L Se group). Fulton's condition factor for fathead minnow was significantly less in the 8.9 µg/L treatment compared to 0.12 and 1.0 µg/L Se experimental groups. The results of this study indicated that exposure to relatively low aqueous selenite concentrations can negatively affect invertebrate density and biomass, as well as fish condition. More research is necessary to characterize the risk of selenite exposure to aquatic invertebrates under realistic field conditions, and future risk assessments may need to consider reduced food availability as a factor that may impair the health of higher trophic level organisms in areas with elevated selenite.

3.2 Introduction

Selenium (Se) is recognized as a contaminant of concern in many aquatic systems impacted by human activity, with fish and birds considered the most sensitive to Se toxicity, and therefore the most extensively researched organisms (Lemly 1997; Spallholz and Hoffman 2002; Janz et al. 2010). While Se is a naturally occurring trace element, human activities, particularly coal mining and combustion in Canada, have led to elevated levels of Se both in aquatic environments and the atmosphere (Lemly 1985; Nriagu 1989; Yudovich and Ketris 2006). Once released to aquatic environments, Se occurs mainly as the oxyanions selenate (Se(VI); predominates in lotic environments) and selenite (Se(IV); predominates in lentic environments) (Simmons and Wallschlager 2005; Hillwalker et al. 2006). The speciation of Se also depends on the source of contamination, for instance, selenate is dominant in agricultural drainage and mining discharge whereas selenite is associated with oil waste and coal fly ash effluent (Presser and Luoma 2010). Selenium bioaccumulation in aquatic food webs varies considerably depending on several biogeochemical, hydrological, and ecological factors in a system; in general, selenite is more bioavailable than selenate, and propensity to bioaccumulate increases with residence time and greater biogeochemical cycling of Se (Stewart et al. 2004; Presser and Luoma 2010; Sharma et al. 2015). Therefore, bioaccumulation tends to be greater in lentic relative to lotic systems. As well, organisms at the base of the food web can bioconcentrate, or enrich, Se by three to six orders of magnitude (Riedel et al. 1991; Fisher and Wente 1993; Baines and Fisher 2001). For consumers, diet is the main source of Se and as a result, Se concentrations in higher trophic level organisms do not often reflect water Se concentrations (Young et al. 2010).

Most organisms accumulate Se primarily through their diet, where it can be non-specifically incorporated into proteins as selenomethionine (SeMet; Wallenberg et al. 2010). One of the most well-documented endpoints for Se toxicity is teratogenicity in oviparous vertebrates, which is attributed to SeMet being incorporated into egg yolk during vitellogenesis. During hatching and development of embryos, it is hypothesized that SeMet in yolk proteins is metabolized, generating reactive oxygen species and causing oxidative stress that ultimately leads to deformities in developing fish and birds (Palace et al. 2004; Janz et al. 2010). While less studied, lower trophic level organisms may be susceptible to Se toxicity in a similar manner, where Se taken up by individuals is reduced to hydrogen selenide and further to elemental Se, which

generates reactive oxygen species causing oxidative stress and cellular damage (Spallholz 1994; Misra et al. 2010).

Effects of Se on aquatic macroinvertebrates have not been well characterized, although they have typically been considered relatively insensitive to Se (deBruyn and Chapman 2007). Further, Se toxicity to invertebrates has been understudied particularly in colder water systems, such as Canadian boreal lakes. Most studies have performed short-term, waterborne toxicity tests for invertebrates, yielding, for example, 48-h LC_{50} values of 48.2 and 42.5 mg selenite/L for *Chironomus decorus* and *Tanytarsus dissimilis*, respectively (Call et al. 1983; Maier and Knight 1993). However, waterborne toxicity tests typically do not consider dietary uptake of Se over longer periods of time. This is important because in natural systems, invertebrates have been shown to accumulate Se to extremely high levels, even when aqueous Se levels are relatively low, due to the high enrichment and incorporation of Se into the food web by algae and other organisms at the base of the food web (Muscatello et al. 2008; Ponton and Hare 2015; Markwart et al. 2019). Although invertebrates can to some extent take up Se directly from water, simplified food webs or field-based studies provide more realistic exposure scenarios for bioaccumulative contaminants like Se, to which higher trophic level organisms are exposed mainly through their diet and not via aqueous exposure (Young et al. 2010).

Adverse effects of Se on lower trophic level organisms such as invertebrates are a concern for the health and diversity of invertebrate populations, as well as the ecosystem functions they provide such as influencing energy flow and nutrient cycling (Wallace and Webster 1996; Covich et al. 1999). As well, invertebrates are considered an important food source for various fish species and declines in food availability could exacerbate toxic effects of Se, through decreased metabolic efficiency and decreased energy stores for metabolism of Se and associated reactive products. It is therefore important to consider the effects of Se on several components of a food web and the interactions between them, and not just on single organisms in laboratory-based scenarios.

Given the site-specific and variable nature of Se bioaccumulation and effects in aquatic ecosystems, and the lack of information on the effects of Se on aquatic macroinvertebrates, particularly in cold-water systems, the objective of the present study was to use a whole ecosystem approach to determine the effects of experimentally added selenite on benthic macroinvertebrates and concurrently on the health of adult female fathead minnow (*Pimephales promelas*). Selenium was added to six limnocorrals to obtain nominal concentrations of 1 and 10 µg/L selenite in

triplicate. Selenium was added to enclosures as selenite because it predominates under lentic conditions such as the lake studied herein, and it poses a greater risk to organisms due to its high propensity to bioaccumulate in aquatic organisms (Riedel et al. 1991; Conley et al. 2013; Franz et al. 2013). Three limnocorrals served as controls with no Se added. Benthic macroinvertebrate density, biomass, and indices of taxonomic diversity were determined and associations with autotrophic endpoints (periphyton chlorophyll *a* and organic matter content) were investigated. Resulting effects on the overall health of female fathead minnow were also assessed.

3.3 Methods

3.3.1 Experiment design and set-up

The present study was conducted using limnocorrals (2 m diameter, ~ 3000-L *in situ* enclosures) in Lake 114 at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA), which is located within the boreal shield ecoregion, in northwestern Ontario, Canada. Lake 114 is a small, shallow mesotrophic lake with a total area of 12.1 ha and a maximum depth of 5 m. IISD-ELA biweekly monitoring data (mean ± standard deviation) collected from May to August 2017 using a Van Dorn sampler at mid-depth within the epilimnion indicate that the lake pH is 6.6 ± 0.2, conductivity is 17 ± 3 µS/cm, and dissolved organic carbon (DOC) is 7.1 ± 0.5 mg/L. Total dissolved nitrogen in the lake is 638 ± 309 µg/L and total dissolved phosphorus is 3.0 ± 0.7 µg/L. Water selenium levels fluctuate slightly seasonally, but are on average approximately 0.1 µg/L. In the ELA region, lakes are ice-covered from approximately November to April, and in 2017 the ambient air temperature ranged from -33°C in January to 30°C in July.

Limnocorral set-up and treatment with Se are described in detail in Graves et al. (2019). Briefly, nine limnocorrals (2 m diameter, ~ 1 m depth) were installed in Lake 114 in May 2017. Exposure systems were sealed to the lake bottom with a continuous row of sandbags, and enclosed approximately 3,000 L of water, as well as the natural sediment and biota of the lake. Endemic fish were removed prior to the beginning of the experiment using minnow traps suspended in the water column within each enclosure. From June to August 2017 limnocorrals were spiked five times to maintain nominal concentrations of 1 or 10 µg/L selenium (as selenite; Na_2SeO_3) in triplicate, with three limnocorrals serving as untreated controls. The exposure period occurred from June 1 to August 17 for a total of 77 d. Artificial substrates (five 4.8 x 4.8 cm clay tiles for periphyton and Hester-Dendy samplers (seven 7 cm diameter plates) for benthic

macroinvertebrates) were deployed first in Lake 114 proper for two weeks for colonization and then transferred to limnocorrals to allow for collection of periphyton and benthic macroinvertebrates throughout the study. A total of nine tile samplers and nine Hester-Dendy samplers were deployed within each limnocorral. Tile samplers were suspended in the water column 30 cm from the water surface, and Hester-Dendy samplers were suspended at the sediment-water interface. On d 33, 50 reproductively mature female fathead minnow were collected from Lake 114 using minnow traps baited with dog food (enclosed in capsules with mesh covers so that they could not be eaten). Five female fathead minnow were added to each enclosure, and five additional female fish were kept for baseline morphometrics and Se analysis. The number of fish per limnocorral was decided *a priori* by estimating the fish biomass that could be supported in the enclosures given the productivity and food availability in this particular lake. Only female fathead minnow were added because the objective of the present study was to investigate the uptake and trophic dynamics of Se in a boreal lake, and it is currently thought that the toxicokinetic mechanism of Se is maternal transfer of Se via vitellogenin deposition to developing eggs (Janz et al. 2010).

3.3.2 Sample collection and processing

On d -2, 7, 14, 33, 49, 63 and 77, 1 L of water was collected from each limnocorral for water chemistry analysis as described in detail in Graves et al. (2019). Water from each limnocorral was transferred to an acid-washed 1-L high density polyethylene (HDPE) bottle and transported to the field laboratory on ice. An 8 mL subsample of water was transferred through a 0.45-µm polyethersulfone (PES) membrane filter to an acid-cleaned 8 mL bottle, acidified to 2% HNO_3 using ultrapure HNO_3, and stored at 4˚C for Se analysis. In addition to the water chemistry parameters described previously (Graves et al. 2019a), a 300-mL subsample was filtered through a pre-ashed 0.45-µm Whatman grade GF/C glass microfiber filter for phytoplankton chlorophyll *a* (chl *a*) analysis. Filters were placed in plastic petri dishes, dried in a desiccator, then wrapped in aluminum foil and frozen at -20˚C until further analysis.

Benthic macroinvertebrates were collected on d -2, 7, 14, 33, 49, 63 by removing one Hester-Dendy sampler from each limnocorral, placing it in a plastic sample bag with limnocorral water, and transporting it on ice to the lab. On d 77, the last three Hester-Dendy samplers were removed from each limnocorral. In the laboratory, samplers were disassembled, and each plate was gently rubbed to dislodge all periphyton and invertebrates. The slurry of periphyton and macroinvertebrates was transferred to a sorting tray and all invertebrates were picked out by eye,

identified to family, counted, lyophilized, and weighed for total dry biomass. All dried invertebrate taxa were then analyzed for total selenium content (described below in Total Selenium Analysis). Abundance and dry biomass were recorded for Gammaridae and Chironomidae for all sampling time points. Only Gammaridae and Chironomidae biomass and density were assessed because these taxa were the only ones present in all treatments. Density and dry biomass per m^2 were calculated by dividing abundance or biomass by the total surface area of a Hester-Dendy sampler (0.069 m^2). To correct for the three samplers collected on d 77, density and biomass were divided by 3 to obtain a density or biomass estimate per Hester-Dendy at that time point. To describe community composition, taxa richness (number of taxa at the family level), Shannon's index (Eq. 3.1; Shannon and Weaver 1949) and Simpson's index (Eq 3.2; Magurran 2004) were calculated:

$$\text{Shannon's diversity index (H)} = - \Sigma\, p_i \ln p_i \qquad (3.1)$$

$$\text{Simpson's diversity index (D)} = 1 - (\Sigma\, p_i{}^2) \qquad (3.2)$$

where p_i is the proportion of individuals found in Family i.

Periphyton was collected on d 77 by removing one suspended tile sampler from each enclosure and scraping the surface of the tiles into a plastic sample bag using an acrylic scraper. Reverse osmosis (RO) water was used to rinse periphyton into the bag. The slurry was kept on ice for transport back to the field laboratory. In the laboratory, the periphyton was transferred to an acid-cleaned 50 mL falcon tube and was topped up to 50-mL with RO water. Periphyton was subsampled for analysis of chl *a* and ash free dry mass (AFDM) by taking 15 mL subsamples of the 50-mL slurry for each analysis. The remaining slurry was centrifuged, water decanted, and then periphyton was stored at -20˚C for Se analysis. Subsamples for chl *a* were filtered onto pre-ashed 0.45-µm Whatman grade GF/C filters. Filters for chl *a* were folded in half with forceps, wrapped in aluminum foil, and frozen at -20˚C until further analysis. Chlorophyll *a* was measured in phytoplankton and periphyton using a Trilogy Fluorometer (Turner Designs, San Jose, California, USA) following extraction in hot ethanol using a previously validated in-house protocol at the University of Saskatchewan. Subsamples for AFDM were filtered onto pre-ashed and pre-weighed GF/C filters, dried in an oven at 103˚C for 1 h, weighed, and then ignited at 550˚C for 1h, cooled in a desiccator and weighed again (APHA 1995).

Fathead minnows were re-captured from limnocorrals on d 49, 63, and 77 using minnow traps suspended in the water column. On d 49 and 63, one fish from each enclosure was removed, and on d 77 the remaining 3 fish from each enclosure were collected. Fish (including baseline fish

from the lake) were anesthetized using 1 mg/L buffered tricaine methanesulfonate (MS-222), euthanized by spinal severance, and held on ice for transport back to the lab. Immediately upon return to the lab, fish were weighed, fork length was measured, and fish were dissected. Gonad and liver tissues were weighed, and muscle and ovary tissues were excised and stored at -20˚C for Se analysis. All fish collection and handling procedures used in this study were approved by the Animal Research Ethics Board at the University of Saskatchewan (protocol #20170046) and adhered to the Canadian Council on Animal Care guidelines for humane animal use. Biomarkers of overall health, including Fulton's condition factor (K; Eq 3.3), liversomatic index (LSI; Eq 3.4) and gonadosomatic index (GSI; Eq 3.5) were calculated as follows:

$$K = 100 \times (\text{body weight (g)}/\text{fork length (cm)}^3) \quad (3.3)$$

$$LSI = 100 \times (\text{liver weight (g)}/\text{body weight (g)}) \quad (3.4)$$

$$GSI = 100 \times (\text{gonad weight (g)}/\text{body weight (g)}) \quad (3.5)$$

3.3.3 Total selenium analysis

A detailed description of Se analysis is presented in Graves et al. (2019). Biota were lyophilized, weighed, microwave-digested in 1 mL 69% ultrapure HNO_3 and 2 mL ACS grade 30% H_2O_2 (Fisher Scientific, Ottawa, ON, Canada) and digested samples were diluted to 2% HNO_3. Total selenium in water and biota were measured separately using inductively coupled plasma – mass spectrometry (ICP-MS) at the Toxicology Centre, University of Saskatchewan.

3.3.4 Statistical analyses

Statistical analyses were performed using the R software environment, associated base packages, as well as the "multcomp", "lsmeans", and "lme4" packages (R Core Team 2014). Alpha was set at 0.05 for all statistical tests and data are presented as mean ± standard deviation. Differences in density and biomass of main benthic macroinvertebrate taxa (Chironomidae and Gammaridae) among treatments over time were assessed using generalized linear mixed models (glmm) with the following model structure: y ~ Treatment + Time + Treatment × Time + (1|Enclosure), where density and biomass were the dependent variables (y), treatment and day the fixed main effects, Treatment × Time the interaction effect, and (1|Enclosure) was the random effect that accounted for the repeated sampling of each enclosure over time. To determine the distribution that best fit the dependent variable, biomass and density were first plotted as normal, log normal, negative binomial, poisson, and gamma distributions. Chironomidae and Gammaridae biomass best fit gamma distributions with log links. Chironomidae and Gammaridae density best

fit Poisson distributions with log links. For this analysis, the interaction between treatment and time was the effect of interest (Paine 1996; Kreutzweiser et al. 2002). When a significant interaction between treatment and time was detected, pairwise comparisons of biomass or density averaged over time were assessed by treatment group using a least-squares means pairwise comparisons test. Significant differences between treatments at each sampling point were not assessed as this would inflate the overall error rate.

Differences in diversity indices, chl *a* and AFDM among treatments at d 77 were assessed using generalized linear models followed by Tukey's post hoc multiple comparisons. Generalized linear models were used to determine the relationship between Chironomidae biomass or abundance and chl *a* and AFDM. To determine the goodness of fit for those relationships, a pseudo-R^2 was calculated (1-residual deviance/null deviance). For fathead minnow, differences in condition factor, GSI, and LSI at d 49, 63, and 77 among treatments were assessed within each time point using generalized linear models followed by Tukey's post hoc test if significant differences among treatments were detected.

3.4 Results

3.4.1 Water chemistry

Water chemistry parameters for each limnocorral (mean and ranges for entire experimental period) are reported in the companion manuscript (Graves et al. 2019a). Briefly, temperature of surface water at 20 cm below the surface ranged from a minimum of 17°C in May to a maximum of 25°C in late July. Dissolved oxygen concentration ranged from 7.6 to 9.6 mg/L (91-106% saturation) over time, and pH ranged from 6.0 to 6.9 over time among the limnocorrals. Mean DOC ranged from 6.52 to 7.56 mg/L, TDN from 303 to 361 µg/L, TDP from 4.20 to 5.83 µg/L and SO_4^{2-} from 0.80 to 1.90 mg/L among limnocorrals. Water chemistry parameters did not differ significantly among treatments (Graves et al. 2019a). Mean measured aqueous TSe concentrations were 0.12 ± 0.03, 1.0 ± 0.10, and 8.9 ± 2.7 µg/L for the control, 1.0 and 10 µg/L treatments, respectively. Experimental groups are referred to by mean measured aqueous TSe concentrations (0.12, 1.0 and 8.9 µg/L) throughout the Results and Discussion.

3.4.2 Benthic macroinvertebrates

Invertebrate taxa collected from the 0.12 µg/L Se enclosures on d 77 were Aeshnidae, Gammaridae, Ceratopogonidae, Chironomidae, Hirudinea, Sphaeridae, and Planorbidae (Table 3.1). Taxa collected at the same time from the 1.0 µg/L Se treatment were Aeshnidae,

Table 3.1: Benthic macroinvertebrate taxa collected from Hester-Dendy samplers from 0.12, 1.0 and 8.9 µg Se/L experimental groups on d 77 of the exposure period (n=3 per group).

Experimental Group	0.12 µg/L	1.0 µg/L	8.9 µg/L
Taxa Collected at d 77	Aeshnidae	Aeshnidae	Chironomidae
	Ceratopogonidae	Ceratopogonidae	Gammaridae
	Chironomidae	Chironomidae	Hirudinea
	Gammaridae	Gammaridae	Polycentropodidae
	Hirudinea	Polycentropodidae	
	Planorbidae	Sphaeridae	
	Sphaeridae		

Gammaridae, Ceratopogoniidae, Chironomidae, Polycentropodidae, and Sphaeridae, and collected from the 8.9 µg/L Se treatment were Gammaridae, Chironomidae, Hirudinea, and Polycentropodidae (Table 3.1). The Shannon diversity index was significantly greater in the 0.12 µg/L Se group than in the 1.0 µg/L (glm and Tukey, p=0.021; Table 3.2) and the 8.9 µg/L (glm and Tukey, p=0.001; Table 3.2) Se treatments. Shannon diversity indices were not significantly different for the 1.0 and 8.9 µg/L Se treatments (glm, p=0.644; Table 3.2). The Simpson diversity index for benthic macroinvertebrates collected on d 77 was also significantly greater in the 0.12 µg/L Se group than in the 1.0 µg/L (glm and Tukey, p=0.023; Table 3.2) and the 8.9 µg/L (glm and Tukey, p=0.011; Table 3.2) Se treatments. Simpson diversity indices were not significantly different between the 1.0 µg/L and 8.9 µg/L Se treatments (glm, p=0.960; Table 3.2). Taxa richness was significantly greater in the 0.12 and 1.0 µg/L Se groups than the 8.9 µg/L Se treatment (glm and Tukey, $p<0.036$; Table 3.2), but there was no significant difference in taxa richness between the 0.12 and 1.0 µg/L Se groups (glm and Tukey, p=0.656; Table 3.2).

Chironomidae density and biomass appeared to be affected by selenite at the 8.9 µg/L Se treatment level (Figure 3.1). Significant differences in density and biomass of Chironomidae at the 8.9 µg/L Se treatment level over time were detected (glmm timextreatment; $p<0.001$; Figure 3.1). Pairwise contrasts showed that Chironomidae biomass and density were both significantly lesser in the 8.9 µg/L Se treatment compared to the 0.12 and 1.0 µg/L Se groups (pairwise contrasts; $p<0.05$; Figure 3.1), and that density and biomass were not significantly different in the 1.0 µg/L Se treatment relative to the 0.12 µg/L Se group (pairwise contrasts; $p>0.05$; Figure 3.1).

Gammaridae density and biomass appeared to be affected by selenite at the 1.0 and 8.9 µg/L Se treatment levels (Figure 3.2). Gammaridae density and biomass were significantly lesser in the 8.9 µg/L Se treatment compared with the 1 µg/L and 0.12 µg/L Se treatment over time (glmm timextreatment; $p<0.023$; Figure 3.2). Both density and biomass were significantly greater in the 1.0 µg/L Se treatment relative to the control (pairwise contrasts; $p<0.05$; Figure 3.2), and both density and biomass were significantly lesser in the 8.9 µg/L Se treatment relative to the 1.0 µg/L Se treatment and the 0.12 µg/L Se group (pairwise contrasts; $p<0.05$; Figure 3.2).

3.4.3 Fathead minnow

There were two fish mortalities observed during the exposure (both in the 8.9 µg/L Se treatment). An additional two fish from the 8.9 µg/L Se treatment limnocorrals and two fish from the 0.12 µg/L Se limnocorrals were never retrieved.

Table 3.2: Benthic macroinvertebrate diversity in 0.12, 1.0 and 8.9 µg/L treatments expressed as Shannon's Index, Simpson's Index, and taxa richness (at the family level) (mean ± SD; n=3) for invertebrates collected from Hester-Dendy samplers at d 77 of the exposure period.

Experimental Group	Shannon Index[1]	Simpson Index	Taxa Richness
0.12 µg/L	0.7 ± 0.1^{a}	0.4 ± 0.1^{a}	4.0 ± 1.7^{a}
1.0 µg/L	0.3 ± 0.1^{b}	0.1 ± 0.1^{b}	3.0 ± 1.0^{a}
8.9 µg/L	0.2 ± 0.3^{b}	0.1 ± 0.2^{b}	1.3 ± 0.6^{b}

[1]Different letters indicate statistically significant differences among treatments detected using generalized linear models

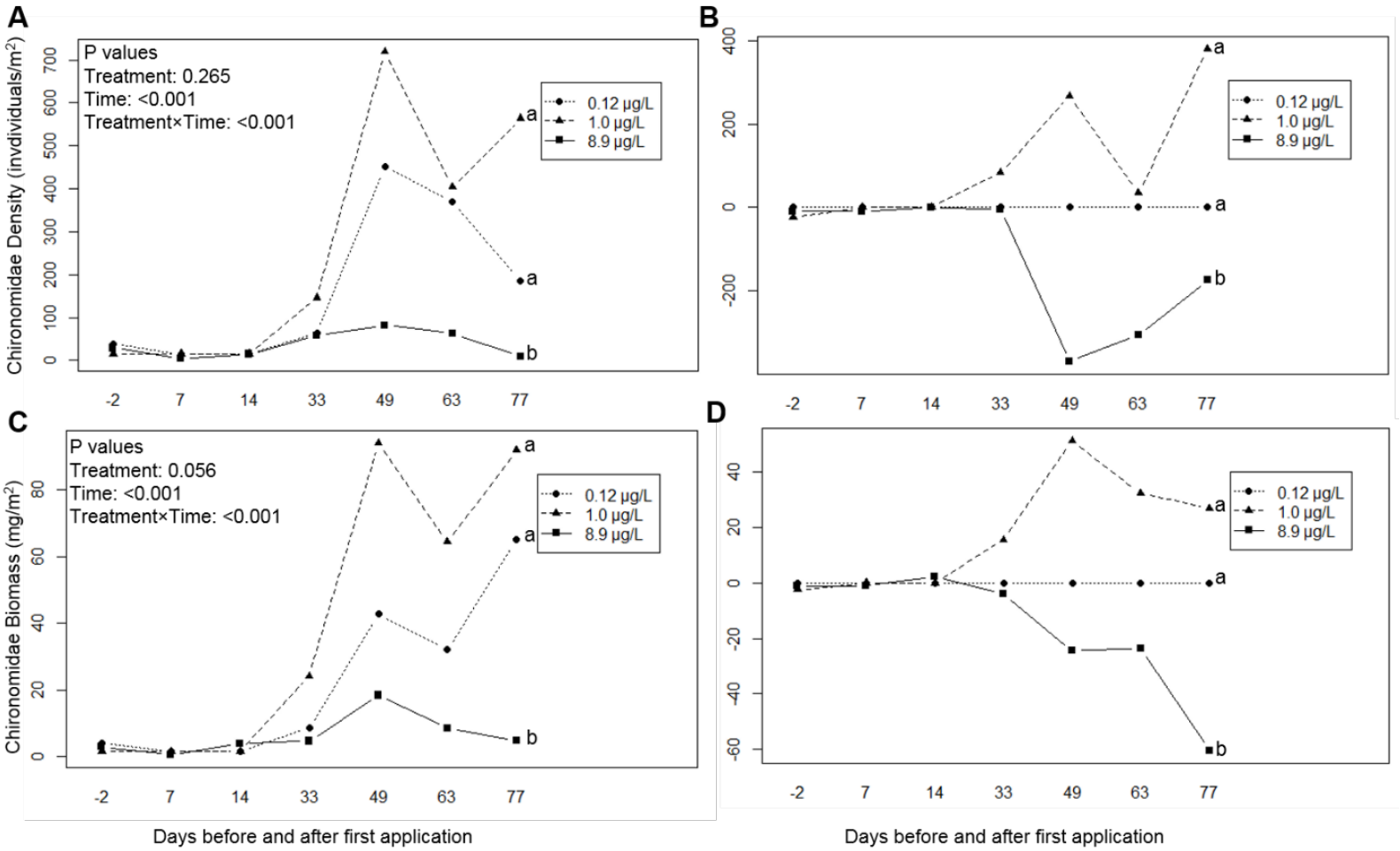

Figure 3.1: Mean absolute Chironomidae density and biomass (A,C) and differences in density and biomass relative to the controls (B,D) for 0.12, 1.0, and 8.9 µg/L Se groups (n=3) on d -2, 7, 14, 33, 49, 63, and 77. Different letters indicate significant differences among groups ($p<0.05$)

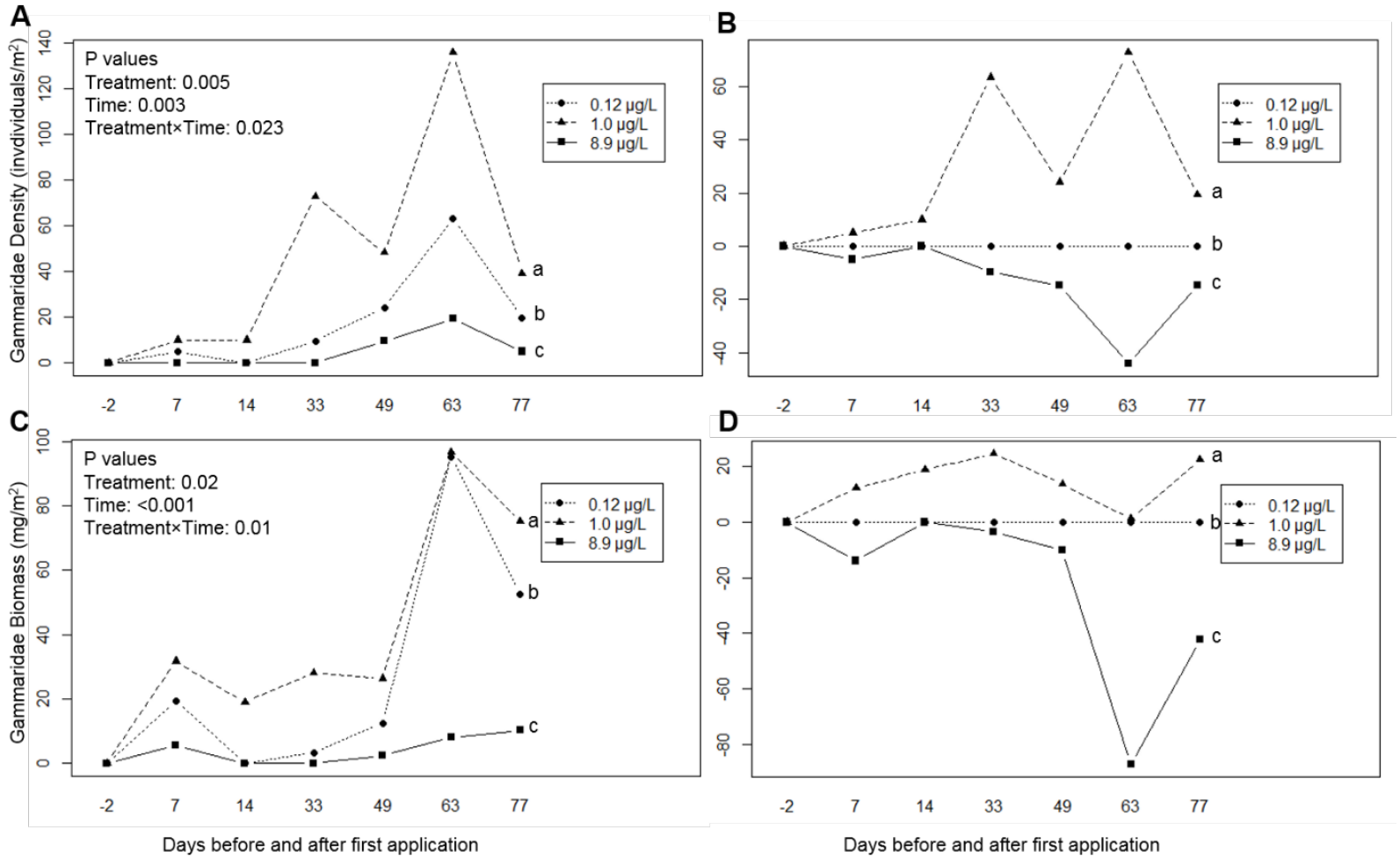

Figure 3.2: Mean absolute Gammaridae density and biomass (A,C) and differences in density and biomass relative to the controls (B,D) for 0.12, 1.0, and 8.9 µg/L Se groups (n=3) on d -2, 7, 14, 33, 49, 63, and 77. Different letters indicate significant differences among groups ($p<0.05$).

Fulton's condition factor for baseline female fathead minnows collected from Lake 114 proper on d 33 of the study was 1.12 ± 0.11 (n=5). On d 49 (16 d of exposure for fish), condition factor for fish in the 0.12 µg/L Se group was 1.50 ± 0.08 (n=3), which was significantly greater than the condition factor of fish in the 1.0 µg/L (0.96 ± 0.08; n=3, glm and Tukey, p=0.004; Figure 3.3A) and 8.9 µg/L (0.97 ± 0.05; n=3, glm and Tukey, p =0.009; Figure 3.3A) Se treatments. Condition factor of fish in the 1.0 µg/L Se treatment was not significantly different from condition factor of fish in the 8.9 µg/L Se treatment (glm and Tukey, p=0.959; Figure 3.3A). On d 77 (after 44 d of exposure for fish), condition factor was significantly greater in 0.12 µg/L (1.00 ± 0.06; n=7) and 1.0 µg/L (1.02 ± 0.09; n=9) Se groups compared to the 8.9 µg/L Se treatment (0.77 ± 0.09; n=7, glm and Tukey, p<0.001; Figure 3.3A). There was no difference in condition factor of fish from 0.12 µg/L or 1.0 µg/L Se groups (glm and Tukey, p=0.968; Figure 3.3A).

Gonadosomatic index for baseline female fathead minnow collected from Lake 114 proper on d 32 of the study was 7.01 ± 1.25%. The gonadosomatic index was significantly greater in the 0.12 µg/L (9.07 ± 6.91%) than in the 1.0 µg/L (2.98 ± 0.79%) and 8.9 µg/L (2.06 ± 0.44%) Se treatments on d 49 (after 16 d of exposure for fish; glm and Tukey, p<0.01; Figure 3.3B). There was no difference in GSI for the 1.0 and 8.9 µg/L Se treatments on d 49 (glm and Tukey, p=0.617; Figure 3.3B). On d 77 (after 44 d of exposure), there were no significant differences in GSI among the groups (glm, p>0.05; Figure 3.3B).

Liversomatic index for fish in the 0.12 µg/L Se treatment on d 49 (after 16 d of exposure) was 1.41 ± 0.11%, which was not significantly different from that of fish in the 1.0 µg/L (1.46 ± 0.49%) and 8.9 µg/L (1.14 ± 0.15%) Se treatments (glm, p=0.282; Figure 3.3C). On d 77, there was no difference in LSI among the groups (glm, p=0.327; Figure 3.3C).

3.4.4 Periphyton and phytoplankton

Periphyton chl *a* was significantly greater in the 8.9 µg/L Se treatment (0.67 ± 0.53 µg/cm^2) compared to the 0.12 µg/L (0.22 ± 0.01 µg/cm^2; glmm and Tukey, p<0.001; Figure 3.4A) and 1.0 µg/L (0.11 ± 0.07 µg/cm^2; glmm and Tukey, p<0.001; Figure 3.4A) Se treatments. Periphyton chl *a* was not significantly different between the 0.12 µg/L and 1.0 µg/L Se treatments (glmm, p=0.303; Figure 3.4A). Periphyton AFDM was also significantly greater in the 8.9 µg/L Se treatment (6.15 ± 5.32 g/m^2) compared to the 0.12 µg/L (1.74 ± 0.88 g/m^2; glmm and Tukey, p=0.008; Figure 3.4B) and 1.0 µg/L (0.64 ± 0.20 g/m^2; glmm and Tukey, p<0.001; Figure 3.4B) Se treatments.

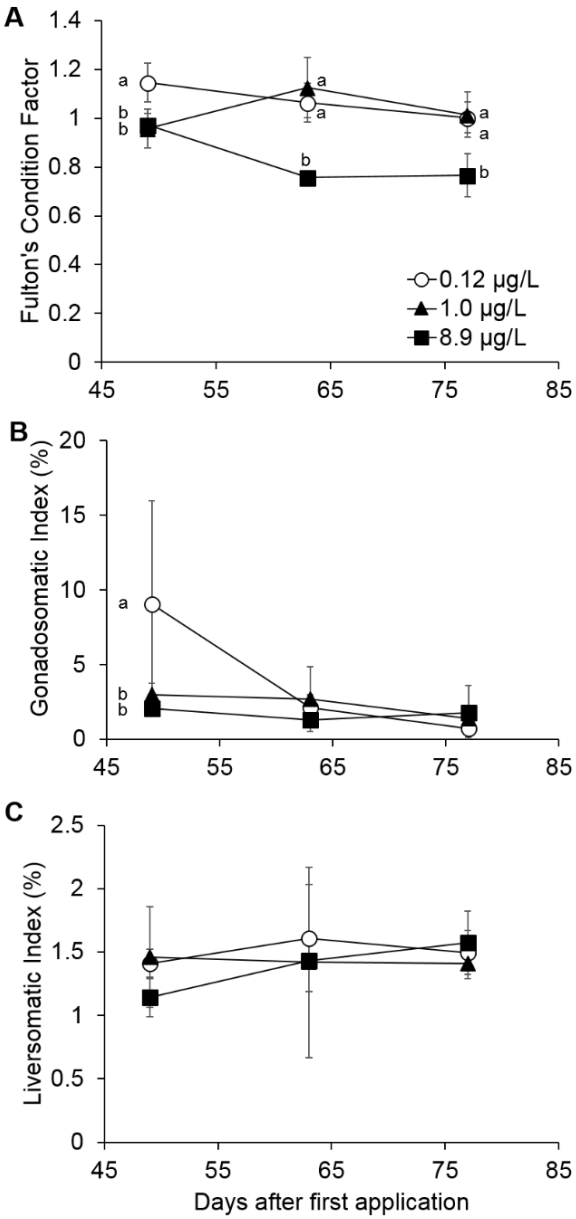

Figure 3.3: Fulton's condition factor (mean ± SD; A), Gonadosomatic Index (GSI; mean ± SD; B) and Liversomatic Index (LSI; mean ± SD; C) for female fathead minnows from 0.12 µg/L (open circles), 1.0 µg/L (black triangles), and 8.9 µg/L (black squares) Se treatments on d 49, 63 and 77 of the exposure period, representing 16, 30, and 44 d of exposure for fish (n=1-3 per treatment on d 49 and 63, n=7-9 per treatment on d 77). Different letters indicate statistically significant differences among groups within time points ($p<0.05$).

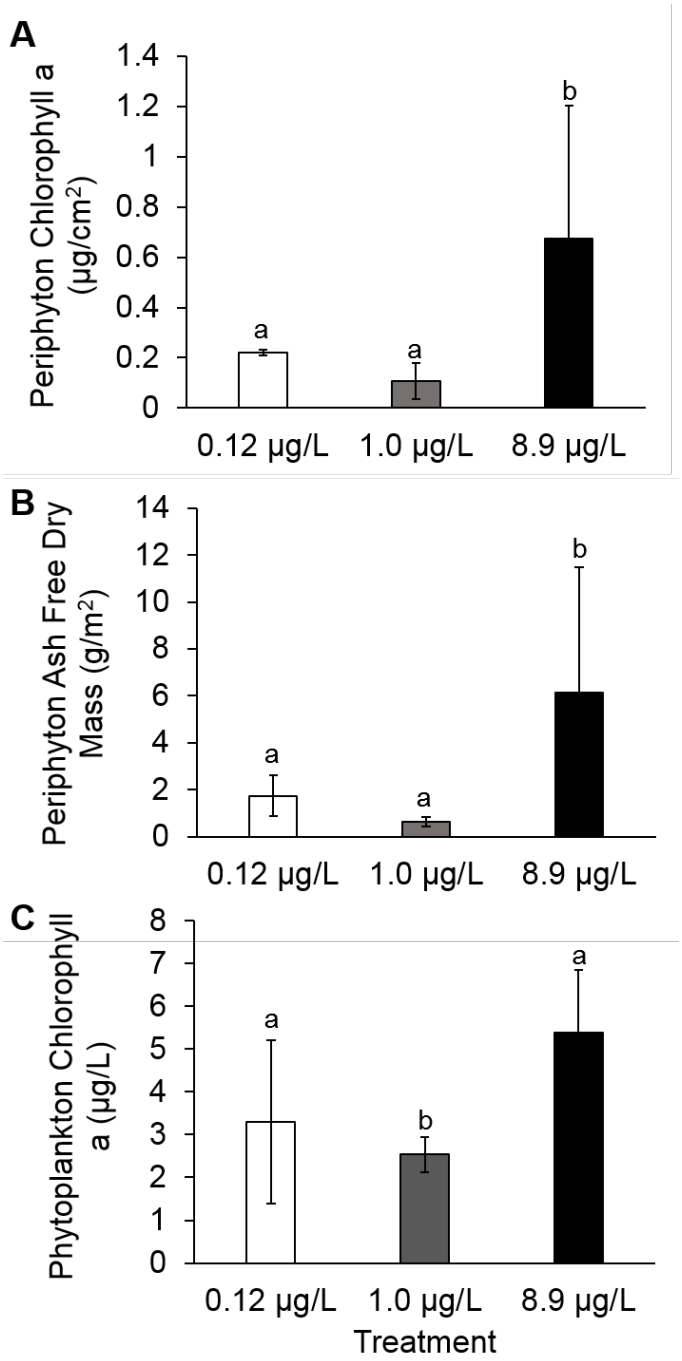

Figure 3.4: Periphyton chlorophyll *a* (mean ± SD; n=3; A) periphyton ash free dry mass (mean ± SD; n=3; B), and phytoplankton chlorophyll a (mean ± SD; n=3; C) on d 77 of the exposure period from 0.12, 1.0 µg/L and 8.9 µg/L Se experimental groups. Different letters indicate statistically significant differences among groups ($p<0.05$).

Phytoplankton chl *a* was not significantly different among treatments over time (glmm, p=0.270), but there was a significant effect of day on phytoplankton chl *a* (glm, p=0.034). There was no interaction between treatment and day (glm, p=0.281). At d 77, phytoplankton chl *a* in the 8.9 µg/L Se treatment (5.35 ± 1.47 µg/L) was significantly greater than chl *a* in the 1.0 µg/L Se treatment (2.54 ± 0.41 µg/L; glmm and tukey, p=0.010; Figure 3.4C), but it was not significantly different from chl *a* in the 0.12 µg/L Se treatment (3.30 ± 1.91 µg/L; glmm, p=0.136; Figure 3.4C).

Periphyton AFDM was significantly and inversely related to Chironomidae abundance (glm, p=0.015, pR^2=0.57; Figure 3.5A) and Chironomidae biomass (glm, p=0.021, pR^2=0.58; Figure 3.5B). Periphyton chl *a* followed a similar trend (glm, p=0.014, pR^2=0.57; Figure 3.5C, Figure 3.5D).

3.5 Discussion

The major findings of the present study were that exposure to 8.9 µg Se/L as selenite significantly reduced diversity, biomass and density of benthic macroinvertebrates as well as body condition of a resident fish species in a natural boreal aquatic system. Growth of periphyton was inversely associated with biomass and density of invertebrates, suggesting that periphyton growth was not directly affected by Se and that increased periphyton growth was associated with decreased grazing pressure by invertebrates. The present study demonstrates that invertebrates may be more sensitive to selenite than previously assumed, which implies that lessening food sources may present another stressor (in addition to teratogenicity) for fish in environments with elevated Se. In addition, shifts in diet for fish may influence Se trophic dynamics and alter bioaccumulation of Se, consequently influencing Se exposure.

Chironomidae and Gammaridae density and biomass decreased in the present study after exposure to aqueous selenite concentrations of 8.9 µg/L for 77 d, indicating that the survival and growth of individuals was impaired. These results are similar to a previous study wherein 714-L mesocosms were spiked with 10 µg/L selenite for 6 weeks, and larval and adult insect abundance and biomass were assessed (Henry and Wesner 2018). These authors reported that exposure to 10 µg/L Se reduced emergence of aquatic insects by 37% and significantly decreased abundance of benthic macroinvertebrate larvae after 42 d relative to controls, although no differences in periphyton or phytoplankton chl *a* were observed (Henry and Wesner 2018). These mesocosm studies suggest that toxicity to aquatic insect larvae and adults can occur at much lower aqueous selenite concentrations than previously thought, and that short-term, controlled waterborne selenite

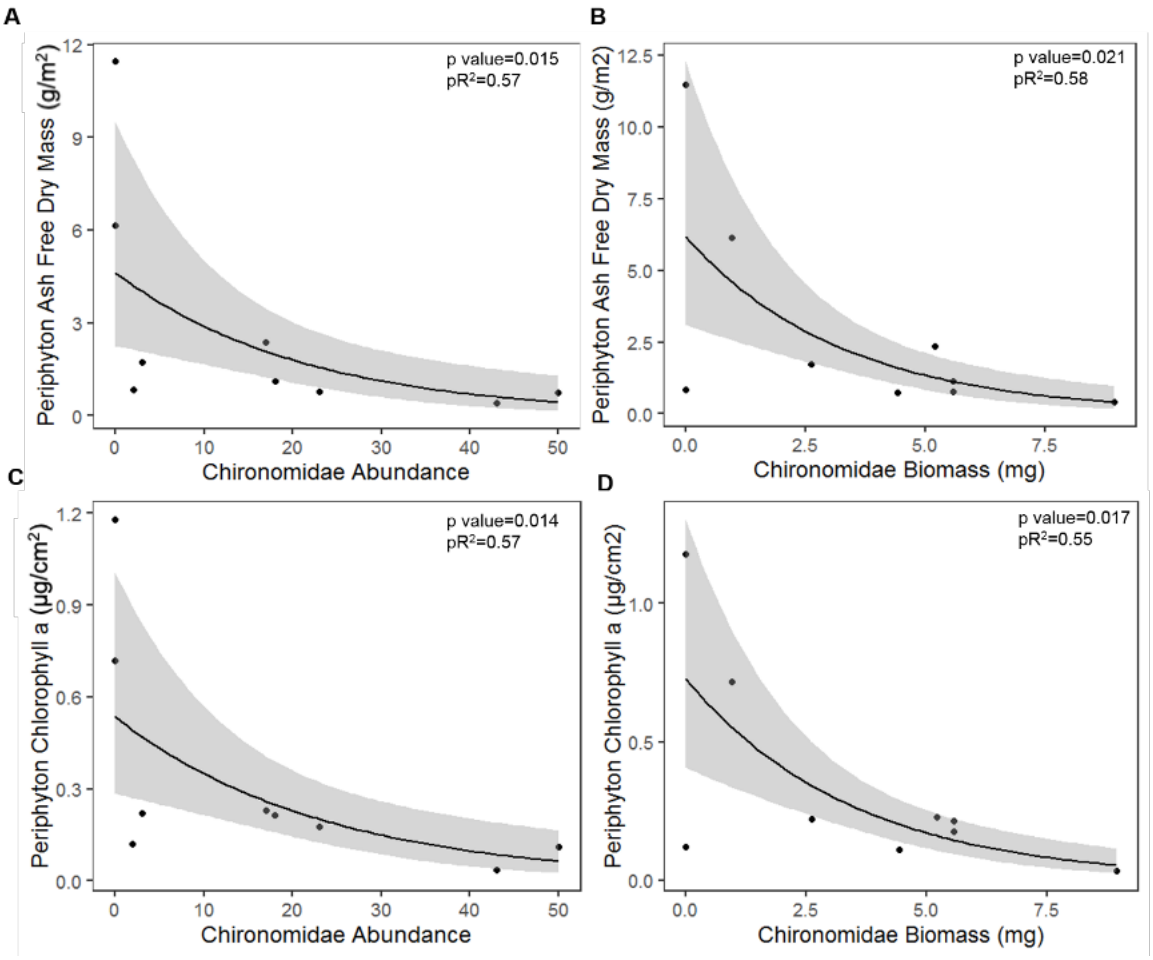

Figure 3.5: Relationship between periphyton ash free dry mass and Chironomidae abundance (A), ash free dry mass and Chironomidae biomass (B), chlorophyll *a* and Chironomidae abundance (C), and chlorophyll *a* and Chironomidae biomass (D) pooled from 0.12 µg/L, 1.0 µg/L, and 8.9 µg/L Se experimental groups on d 77 of the exposure (n=9). P values are for generalized linear models of the relationships. The black line represents the line of fit for the non-linear model and the shading represents the 95% confidence interval for the non-linear model. PR^2 is a pseudo-R^2 calculated using the null and residual deviance of the model.

exposures in the laboratory, which have produced 48-h LC_{50}s in the mg/L range for *Chironomus* and Amphipoda (*Hyalella azteca)* (Brasher and Ogle 1993; Call et al. 1983; Maier and Knight 1993; Pieterek and Pietrock 2012), are not adequate to assess Se toxicity to natural invertebrate populations. Further, although previously reported acute waterborne LC_{50}s for selenite were extremely high for aquatic invertebrates, body burdens were similar to those measured in the present study. For example, after 48 h exposure to selenite, the LC_{50} for *Chironomus decorus* was 48.2 mg/L with body burdens reaching 85 µg/g Se dry mass (dm) from the aqueous source alone (Maier and Knight 1993). In the present study, TSe concentrations in combined Chironomidae were 3.6 ± 1.7, 20 ± 8.5, and 153 ± 57 µg/g dm in the 0.12, 1.0, and 8.9 µg/L Se groups, respectively, and corresponding Gammaridae TSe concentrations were 1.6 ± 0.17, 8.4 ± 2.5, and 41 ± 9.8 µg/g dm, respectively (Graves et al. 2019a). Given that body burdens can be high even when aqueous Se concentrations are relatively low, and that Se bioaccumulation can be extremely variable and site specific, future risk assessments should include assessment of invertebrate body burdens in addition to aqueous Se levels to assess toxicity risks to invertebrates.

Although not always statistically significant, the general trends in Gammaridae and Chironomidae density and biomass were 1.0 µg/L > 0.12 µg/L > 8.9 µg/L. This may indicate a hormesis effect, where a small amount of added Se is beneficial to invertebrates, causing an increase in abundance or biomass, but a greater amount of added Se is detrimental. Alternatively, the greater density and biomass of these two taxa in the 1.0 µg/L may be an artefact of the significantly lesser macroinvertebrate diversity observed in the 1.0 µg/L treatment. Since diversity of invertebrates decreased in the 1.0 µg/L, there may have been less competition for Chironomidae and Gammaridae and therefore greater biomass and density were observed.

Benthic macroinvertebrate diversity indices significantly declined after exposure to 1.0 and 8.9 µg/L Se relative to control limnocorrals. In previously studied areas downstream of mining activities in the Canadian Rockies, declines in invertebrate diversity including Ephemeroptera family density and richness, Ephemeroptera, Plecoptera, Trichoptera (EPT) richness, and % Ephemeroptera were observed in streams with Se concentrations of approximately 100 µg/L Se, but Chironomidae density did not appear to be affected (Kuchapski and Rasmussen 2015). However, the mine-affected streams also had higher conductivity and alkalinity relative to reference sites, which are additional stressors and may potentially confound the observed toxicity results (Kuchapski and Rasmussen 2015). Declines in invertebrate diversity, biomass, and density

in the present study suggest that sensitivity of invertebrates to Se toxicity may be higher than previously thought. Specifically, our study suggests that lethal or sublethal effects may occasionally occur at currently allowable water Se concentrations (current Canadian Council of Ministers of the Environment federal water quality guideline for the protection of aquatic life= 1 µg/L; CCME 2003), and that further investigation into the effects of Se on invertebrate diversity in natural environments are warranted.

Reduced macroinvertebrate density and biomass in the 8.9 µg/L treatment corresponded to concurrent reductions in the overall health (measured as condition factor) of female fathead minnow in the 8.9 µg/L Se treatment by 23%. Condition factor of fish represents a metric of energy storage, and a fish that has greater mass at a given length is assumed to have better general health (Bagenal and Tesch 1978). A decrease in condition factor greater than 10% is considered an ecologically significant reduction for fish (Kilgour et al. 2005). Food availability is the most important factor influencing condition in most species (Russell et al. 1996; Krohn et al. 1997), but it can also be affected by environmental factors such as season and spawning time, characteristics of lakes such as temperature and total dissolved oxygen, and contaminant exposure (Imsland et al. 1995; Miller et al. 1995; Khallaf et al. 2003). Metabolic rate and lipid metabolism can increase in response to certain pollutants, and energy intake can decrease due to changes in feeding behaviour, leading to decreased condition factor (Little et al. 1985; Randall et al. 1996; Smolders et al. 2003). In the present study, it is difficult to determine whether a lack of food, contaminant exposure, or a combination of the two caused the decrease in condition factor of fathead minnows in the 8.9 µg/L treatment, although both factors are related to Se exposure. Indirectly, Se may have decreased food availability via toxicity to invertebrates as discussed above. As well, shifts in diet and available food may have altered the dietary Se exposure of fish. Directly, Se may have had sublethal effects on the metabolism or physiological function of the adult fathead minnows. Studies on the effects of Se on adult fish are generally lacking, and mostly focus on reproductive endpoints. However, sublethal effects such as decreased metabolic rate and aerobic scope were observed in adult zebrafish (*Danio rerio*) exposed to 10 µg/L aqueous selenite for 14 d, although no changes in condition factor were observed among the treatment groups (Masse et al. 2013). Mean muscle TSe of fathead minnow in the present study was 21 ± 3.0 µg/g dm in the 8.9 µg/L treatment after 44 d (Graves et al. 2019a), which is less than in previously studied juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to diets of 15.8 and 47.8 µg Se/g dm as selenomethionine for 60

d (mean muscle TSe concentrations = 15.8 and 74 µg Se/g dm, respectively) (Pettem et al. 2018). In contrast to the present study, no decreases in condition factor relative to controls were observed in the previously studied juvenile rainbow trout (Pettem et al. 2018). However, trout did exhibit elevated liver glycogen and triglyceride stores, indicating an effect of Se on energy homeostasis (Pettem et al. 2018). More investigation into the sublethal effects of Se on adult fathead minnow are necessary to determine whether sublethal toxicity could occur at the observed muscle Se levels in the present study.

Gonadosomatic index of female fathead minnow decreased in the 1.0 and 8.9 µg/L Se treatments after 16 d of exposure (d 49 of the overall exposure period), and in all treatments after 30 and 44 d of exposure (d 63 and 77 of the exposure period). This may be due to the timing of the experiment; fathead minnow are fractional (asynchronous) spawners, with a main spawning season from June through July when the water temperature is above 17.6˚C (Dobie et al. 1956; Smith 1977), though the spawning period can extend into August in some regions (Scott and Crossman 1973). This suggests that fathead minnow GSI in the current study should be highest at the d 33 to 49 time points (July 4th and July 20th) and decrease on d 63 and 77 (August 3rd and August 17th), as was observed. In addition, the lack of males in the enclosures may have caused oogenesis to slow, since males perform several courting behaviours including lateral nudging, body vibrations, swimming around the nest, and stimulating the urogenital region of the female to cause the eggs to be released (Ankley et al. 2000; Ross 2001; Wisenden et al. 2009). The observation that GSI decreased in the 1.0 and 8.9 µg/L Se treatments before the 0.12 µg/L Se group could indicate an effect of lower food availability (and consequently less energy stores for producing eggs), or a direct effect of Se on oogenesis. However, in previously studied adult female wild cutthroat trout (*Oncorhynchus clarki lewisi*) from western Canadian coal mining sites with egg Se concentrations greater than 86.3 µg Se/g dm, oocyte development was not irregular, and oocyte atresia was not correlated with ovary Se concentration, indicating that effects of Se on fish larvae likely occur after oogenesis (Rudolph et al. 2008).

Liversomatic index did not change after Se exposure, suggesting that there were no effects on energy storage in the liver of fish at these Se levels. The relationship between LSI and contaminants is variable; liver size can increase as a result of hypertrophy (Heath 1995), or decrease as a result of oxidative stress, lipid peroxidation and decreased lipid reserves (Drevnick et al. 2008). Variable responses of LSI to Se exposure have been observed previously as well;

sunfish populations (*Lepomis* sp.) from coal ash effluent-receiving streams in Ohio showed no significant differences in LSI related to Se (Lohner et al. 2001), whereas muscle Se concentrations were positively associated with LSI in adult rainbow trout and brook trout (*Salvelinus fontinalis*) collected from a region in the Rocky Mountains with active open pit coal mines (Miller 2006). A change in LSI after contaminant exposure can be observed due to its central role in detoxification, with pollutants changing the size or number of vacuoles, lysosomes and hepatic cells, and lipid or glycogen content of the liver (Heath 1995). A lack of change in LSI with increasing Se exposure could indicate that direct toxicity to fish did not occur in the present study, further suggesting that the observed change in condition factor was related to a loss of food and not direct toxicity. However, LSI is also influenced by nutritional status (Lee et al. 1983), and it is unknown why the potential change in nutritional status was not reflected in this metric as well.

The observed decreases in invertebrate density and biomass at the 8.9 µg/L Se treatment in the present study were correlated with corresponding increases in periphyton growth relative to the 0.12 µg/L and 1.0 µg/L Se treatments. Microscopic analysis, which was not performed in the present study, would have been needed to determine if there were treatment-related differences in the community structure of the periphyton. Although periphyton is composed of algae, bacteria, fungi, protists, and detritus, the toxicity of Se to most of these components, except for algae, have not been well studied. Algae has been shown to be relatively insensitive to selenite in the past. For instance, the growth of green alga (*Scenedesmus quadricauda*) was only inhibited at concentrations of 10 mg selenite/L, a concentration much greater than the concentrations used herein (Umysova et al. 2009). Therefore, the increased growth of periphyton in the 10 µg/L treatment was likely a result of decreased grazing pressure following the decline in macroinvertebrates. The responses of the food web observed herein provide good reason to incorporate several ecosystem components to assess toxicity of Se to aquatic organisms, and indicate that more field-based studies may be necessary to better determine the effects on Se on different aquatic ecosystems.

The results of the current study show that exposure to elevated Se (i.e. 8.9 µgSe/L as selenite) can lead to a "middle-out" trophic cascade wherein both top-down and bottom-up type effects are observed and are dependent on primary consumers (i.e. benthic macroinvertebrates). The proposed trophic cascade observed herein is as follows: Benthic macroinvertebrate density, biomass and diversity decreased with aqueous selenite exposure, causing a decrease in grazing

pressure of primary producers which led to a significant increase in the growth of periphyton and phytoplankton. This can be thought of as a top-down cascade wherein the consumer population controls the primary producer population. In addition to the response of primary producers, it appears that the decrease in macroinvertebrates as a food source led to a decrease in energy storage of fish (measured as condition factor). This effect can be thought of as a bottom-up cascade, wherein the primary consumer population controls the energy transferred to higher trophic levels. This middle-out response of the food web to Se exposure demonstrates that the ecotoxicological effects of Se extend beyond the effects on individual organisms and that exposure to Se can lead to significant structural and functional food web perturbations.

This experiment demonstrated that due to the efficient uptake and trophic transfer of selenite in aquatic food webs, benthic macroinvertebrates in shallow, mesotrophic boreal lakes such as the one studied herein could be at risk of toxicity from selenite at concentrations that are at the current Canadian federal water quality guideline for the protection of aquatic life (1 µg Se/L; CCME 2003), the United States Environmental Protection Agency water quality criterion (1.3 µg Se/L in lentic water; US EPA 2016) and the British Columbia Ministry of the Environment water quality guidelines (1 µg Se/L alert concentration, 2 µg Se/L guideline; BC MoE 2014). The results of the current study indicate that invertebrate Se body burdens are important to consider in future ecological risk assessments of Se as they may indicate risk to invertebrate organisms, as well as an indirect mode of toxicity for fish through loss of food. In addition, toxicity to benthic macroinvertebrates may cause shifts in food web structure and dietary sources of Se for higher trophic level consumers, like fish, and future assessment of the trophic dynamics of Se will need to account for shifts in diet as a result of food web toxicity.

CHAPTER 4: TROPHIC DYNAMICS OF SELENIUM IN A BOREAL LAKE FOOD WEB

Preface

This research chapter was designed as a follow-up to Chapter 2, where I further investigated the bioaccumulation and trophic dynamics of Se in a boreal lake ecosystem. The goal of this study was to characterize the relationships between Se exposure and accumulation along a gradient of exposure concentrations in a boreal lake. Using a regression design, I determined the uptake patterns for Se in algae, invertebrates, and fish, and provided equations to describe uptake at each trophic level. These equations may be used in the future to predict or estimate Se bioaccumulation in similar systems. Similar to Chapter 2, I observed high bioaccumulation of Se at the current CCME water quality guideline, highlighting that, depending on the conditions of the system, Se may bioaccumulate to levels of concern in fish and invertebrates. The results of this study also show that there are taxonomic differences in accumulation for both algae and invertebrates within a system, highlighting the need to consider taxonomic-specific uptake of Se in the design and implementation of future ecological risk assessments of Se.

This research is in preparation for publication with co-authors Karsten Liber (University of Saskatchewan), Vince Palace (International Institute for Sustainable Development – Experimental Lakes Area), Markus Hecker (University of Saskatchewan), Lorne Doig (University of Saskatchewan), and David Janz (University of Saskatchewan).

4.1 Abstract

Selenium (Se) is both an essential micronutrient and a contaminant of concern that is of particular interest in mining-influenced waterbodies in Canada. The objective of this research was to characterize the trophic dynamics of selenium along a gradient of exposure concentrations in a Canadian boreal lake ecosystem. From June 20 to August 22, 2018, six limnocorrals (littoral, ~3000 L enclosures) were spiked with mean measured concentrations of 0.4, 0.8, 1.6, 3.4, 5.6 and 7.9 µg Se/L as selenite, and three limnocorrals served as untreated controls (background aqueous Se = 0.08 µg/L). Total Se (TSe) concentrations in water, periphyton, phytoplankton, sediment, benthic macroinvertebrates, zooplankton and female finescale dace (*Phoxinus neogaeus*; added on day 21 of the experiment) were measured throughout and at the end of the experiment. Total Se bioaccumulation by organisms was generally non-linear. Greater uptake by phytoplankton than periphyton was observed. Taxonomic differences in accumulation of TSe by invertebrates (Heptageniidae = Chironomidae > zooplankton) were observed as well. Fish muscle and ovary TSe concentrations were predicted by diet TSe, but tissue bioaccumulation was more variable than that at lower trophic levels. This research provides field-derived kinetic models for the saturable uptake of Se by algae and invertebrates, and contributes to a better understanding of the dynamics of TSe bioaccumulation over a gradient of exposure concentrations in cold-water lentic systems.

4.2 Introduction

Selenium (Se) is a contaminant of concern, particularly in North America where mining activities, agricultural irrigation, and oil and gas extraction can stimulate the release of Se to aquatic systems (Lemly 2004; Yudovich and Ketris 2006; Young et al. 2010). Once released, Se is incorporated into aquatic food webs by bacteria, algae, and other primary producers (Riedel and Sanders 1996; Baines et al. 2004; Fournier et al. 2006). Assimilated Se is readily converted to organic forms (e.g. selenocysteine, selenomethionine), which are subsequently transferred to higher trophic level organisms via dietary pathways (Fisher and Reinfelder 1991; Riedel and Sanders 1996). High accumulation of Se in organisms is a concern mainly because of the teratogenic effects observed in fish and other egg-laying vertebrates (Lemly 1993; Maier and Knight 1994; Hamilton 2003). Relative to warm-water systems in parts of the United States and western Canada, the distribution and bioaccumulation of Se in Canadian, cold-water boreal lake food webs is relatively under-studied, despite these lakes comprising a significant portion of Canada's freshwater ecosystems and being in close proximity to several anthropogenic activities where Se exposure may be a concern.

The areas of highest concern for Se bioaccumulation are generally those in which greater proportions of reduced aqueous Se species (i.e. selenite and organo-Se) are present, due to their greater bioavailability and uptake potential relative to selenate (Besser et al. 1993; Conley et al. 2013; Markwart et al. 2019; Ponton et al. 2020). As a result, areas that biogeochemically favour reduction of Se and/or areas with slow-moving waters, high residence times, and greater recycling of Se such as lakes, reservoirs, and wetlands are of greatest concern for Se bioaccumulation and toxicity (Lemly 2002; Meseck and Cutter 2006; Presser and Luoma 2006). The proportions of selenite versus selenate in aquatic systems vary with source and receiving conditions, and this contributes to the high site-specificity of Se bioaccumulation. Selenate has been associated with agricultural drainage and mountaintop coal mining, while selenite has been associated with oil refinery and fly-ash effluents (Presser and Luoma 2010). In previously studied mine-influenced Canadian boreal lakes, proportions of total Se that were selenite ranged from <7% to 68% (Ponton and Hare 2013). In order to accurately model and predict Se bioaccumulation in organisms, particularly in organisms of top priority (i.e. fish and birds), there is a need to better understand the trophic dynamics of Se, and how bioaccumulation changes as aqueous exposure concentrations increase.

A previous limnocorral study conducted within a similar Canadian boreal lake showed that distribution coefficients and trophic transfer factors decreased with increasing Se exposure concentration, even between control (0.12 µg/L) and 1.0 µg/L levels (Graves et al. 2019a). To improve the prediction of Se bioaccumulation in boreal lakes, a better understanding of the relationships between exposure and uptake of Se for several different organisms under environmentally realistic conditions is necessary. Though some laboratory studies have characterized the uptake kinetics of different Se species into several algal taxa, the same has not been done under field conditions, and further, invertebrate Se uptake has not been assessed over a gradient of concentrations in a field setting.

The goal of the present study was to improve the current understanding of Se bioaccumulation and trophic transfer in Canadian boreal lakes using a field-based experimental approach. Selenium (as selenite) was added to limnocorrals and bioaccumulation in organisms was assessed along a gradient of Se concentrations ranging from below to above the current Canadian Council of Ministers of the Environment (CCME) Se water quality guideline for protection of aquatic life (1 µg/L; CCME 2003). While selenate and selenite are both typically present in aquatic systems, this study focused on selenite due to its greater bioavailability and relevance to lentic systems. The objectives of this study were to 1) measure Se bioaccumulation in all food web compartments (water, periphyton, phytoplankton, sediment, zooplankton, benthic macroinvertebrates and a small-bodied fish species) and determine how accumulation changes as aqueous Se concentration increases, 2) model these changes using saturable Michaelis-Menten uptake kinetics, 3) determine if there are differences in Se accumulation among different organisms exposed to the same Se concentrations, and 4) determine if stable isotope ratios can explain some of the variability in observed fish Se bioaccumulation.

4.3 Materials and Methods

4.3.1 Study area

The experiment was conducted within Lake 239 (coordinates: 49.6592, -93.7138) at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) in northwestern Ontario (Figure A.4.1). Lake 239 is a small lake with a surface area of 540,457 m^2, total volume of 6,169,186 m^3, and maximum depth of 31.5 m. The lake is classified as oligotrophic with mean total dissolved nitrogen (TDN) ranging from 233 to 275 µg/L and mean total dissolved

phosphorus (TDP) from 0.89 to 3.5 µg/L in the epilimnion of the lake from June to August in 2018 (IISD-ELA, unpublished data).

4.3.2 Experimental set-up and design

Nine limnocorrals (*in situ* circular enclosures; 1 m deep and 2 m in diameter) were deployed in a sheltered bay within Lake 239 in May 2018 (Figure A.4.1). The limnocorrals were sealed to the sediment with a continuous row of sandbags to enclose the natural sediment, water, and biota of the lake, and were also supplemented with artificial substrates to facilitate collection of benthic macroinvertebrates and periphyton throughout the experiment (described in Supporting Information 1). To estimate potential leakage throughout the experiment, sodium chloride (NaCl; Fisher Scientific, Ontario, Canada), was added to the limnocorrals to increase Na^+ concentrations to approximately two-fold higher than background levels (described in Supporting Information 2, Figure A.4.2). To start the experiment with similar zooplankton communities across all experimental groups, each limnocorral was seeded with zooplankton collected using a 150 µm horizontal tow net in a nearby area of the lake (described in Supporting Information 3). On day 21, five sexually mature female finescale dace (*Phoxinus neogaeus*) were added to each limnocorral. Finescale dace were chosen for the present study as they are a small-bodied fish present in the lake that could be supported by the biomass of food contained in 3,000 L limnocorrals, they are a widespread Cyprinidae species that represent a secondary or tertiary consumer (Scott and Crossman 1973), and they are closely related to the well-studied fathead minnow (*Pimephales promelas*). Only females were used in the present study because the most ecotoxicologically relevant route of Se exposure to fish is hypothesized to be maternal transfer via vitellogenin deposition to developing eggs (Janz et al. 2010). Finescale dace were collected from one of the lake's inflow streams (coordinates: 49.6609, -93.7133) using baited "gee" type minnow traps. Fish were placed in aerated coolers for transport and fish lengths were recorded prior to addition to the limnocorrals.

Six limnocorrals were randomly assigned as treatments and three limnocorrals were untreated controls with only background levels of Se. The experimental period was from June 20 to August 22, 2018 (a total of 63 d). Selenium was added as sodium selenite (Na_2SeO_3) to limnocorrals to reach nominal concentrations of 0.5, 1, 2, 4, 7, 10 µg Se/L. These concentrations were chosen to represent a range of typically observed water Se concentrations. The 0.5 and 1 µg/L treatments represent allowable levels under the current CCME guideline. The test

concentrations above the CCME guideline are relevant, particularly in Ontario, where the provincial water quality criterion for the protection of aquatic life is 100 µg/L (Ontario MOE, 1979) and concentrations up to 3 µg/L have recently been observed in mining-influenced boreal lakes in Ontario and Quebec (Ponton and Hare 2013).

Water samples from each limnocorral were collected on day 0, 3, 7, and then weekly throughout the experimental period to determine rates of dissipation and when to add selenium again to maintain nominal concentrations. Selenium was added to limnocorrals three times throughout the experimental period (on day 0, 28 and 53). The Se addition procedure followed methods described in Graves et al. 2019a. Briefly, a stock solution of Se was created by dissolving sodium selenite in ultrapure water. On the day of Se additions, the stock solution for each enclosure was added to 1 L of limnocorral water, transported to the field site in a cooler on ice, added to limnocorral water and mixed gently with a wooden paddle.

4.3.3 Sample collection

Water samples for total Se (TSe) analysis were collected from each limnocorral on day -2 (two days before the experiment start), 0 (the first day of the experiment), 3, 7, 14, 21, 28, 35, 42, 49, 56, and 63 using methods described in Graves et al. 2019a. *In situ* water parameters (temperature, dissolved oxygen, depth) were measured weekly using methods described in Graves et al. (2019a). Other water chemistry parameters (pH, dissolved organic carbon (DOC), TDN and TDP) were measured bi-weekly following the methods of Stainton et al. 1977. Periphyton, zooplankton, and benthic macroinvertebrates were collected for TSe analysis on day -2, 21, 35, 49, and 63 using methods described in Graves et al. 2019a. Briefly, surface water samples incorporating the top 0.75 m of the water column were collected using a depth-integrated sampler (constructed in-house), periphyton was scraped from clay tiles, zooplankton was collected using a 150-µm mesh tow net, and benthic macroinvertebrates were collected by retrieving submerged Hester-Dendy samplers set at the sediment-water interface. For benthic macroinvertebrates, the dominant taxa collected at all timepoints were Heptageniidae and Chironomidae, and thus, these taxa were analyzed for TSe concentrations. Phytoplankton was also collected for TSe analysis on day -2, 21, 35, 49, and 63 using the same collection method as water. Immediately upon return to the field laboratory, 1 L water samples were passed through 53 µm mesh and filtered onto 0.65 µm polyvinylidene fluoride (PVDF) filters (Sigma-Aldrich, Ontario, Canada). Filters were placed in acid-cleaned plastic petri dishes, wrapped in tin foil and frozen at -20ºC until further analysis.

The top 1 cm of sediment was collected on day -2, 21 and 63 for TSe and organic matter content analysis using a sediment corer constructed in-house as described in Graves et al. (2019a).

To assess baseline Se and stable isotope ratios for finescale dace upon their addition to the experiment, three females were from the east inflow stream of Lake 239 were euthanized, held on ice, transported to the lab and dissected. In the lab, fish were weighed and fork length measured, and liver and ovaries were excised and weighed. Ovaries, a portion of liver, and a sample of dorsal muscle were frozen at -20°C for later TSe analysis. Another portion of liver and muscle were collected and frozen for stable isotope analysis. On day 35 and 49, one fish from each limnocorral was collected and processed in the same manner as described above. On day 63, the remaining three fish from each limnocorral were retrieved and processed as above (morphometrics summarized in Table A.4.1). Of the 45 fish added to the limnocorrals, two were not retrieved (one from a control and one from the 7.9 µg/L treatment). All fish collection and handling procedures used in the present study were approved by the Animal Research Ethics Board at the University of Saskatchewan (protocol no. 20170046) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

4.3.4 Total selenium analysis

Total selenium in water, sediment, periphyton, phytoplankton, benthic macroinvertebrates, bulk zooplankton, and finescale dace tissues (ovary and muscle) were analysed using inductively coupled plasma - mass spectrometry (ICP-MS) at the University of Saskatchewan using previously validated in-house protocols as described in Graves et al. 2019a. For phytoplankton, the samples were digested off the PVDF filter and only the mass of phytoplankton on the filter was used to calculate the concentration of Se on a dry mass (dm) basis. Sediment was digested using a separate, previously validated in-house protocol at the University of Saskatchewan. Specifically, sediment was lyophilized, ground, and 0.1 g of material was digested in 5 mL of 69% ultrapure HNO_3 (Fisher Scientific, Ontario, Canada), 2 mL of 30% American Chemical Society (ACS) grade H_2O_2 (Fisher Scientific) and 1.5 mL of 48% ultrapure HF (Fisher Scientific). Then, 12 mL of 5% ACS grade boric acid (Fisher Scientific) was added to each sample to deactivate HF. Samples were then diluted to 2% HNO_3. In August 2019, surface water samples at five IISD-ELA lakes were collected to determine the speciation of Se in boreal lakes that vary in physico-chemical characteristics, and collection and analysis are described in Supporting Information 4.

Quality assurance/quality control measures included an instrumental standard, certified reference materials, instrumental and method blanks, and sample duplicates. Natural water 1640a (National Institute of Standards and Technology, Gaithersburg, Maryland) was run with all samples as the instrumental standard reference material with an analytical accuracy and precision of 99.1 ± 1.8% (n = 64). The certified reference material for biota (TORT-3, lobster hepatopancreas; National Research Council Canada, Ottawa, Ontario) had an analytical accuracy and precision of 97.8 ± 0.92 % (n = 32). Ultrapure water was also digested with samples as a method blank, with concentrations generally lower for all analyzed samples than the instrumental quantification limit, which ranged from 0.01 to 0.07 μg/L among runs.

4.3.5 Stable isotope analysis

Stable isotope analyses of carbon and nitrogen were conducted for periphyton, Ephemeroptera, and fish tissues (finescale dace liver and muscle) collected at day 63, using continuous flow – isotope ratio mass spectrometry (CF-IRMS). Samples were dried in an oven at 60°C and then ground to a powder using a glass stir rod or a small mortar and pestle. Ground tissue was then weighed into a tin capsule to obtain a target mass of 1.0 mg for animal tissue and 3.0 mg for algal tissue. Stable isotopes of carbon and nitrogen were measured at the Stable Isotopes in Nature Laboratory at the University of New Brunswick. Quality assurance/quality control measures for the analysis included ten check standards that had a mean analytical precision of ± 0.10 and ± 0.06 (standard deviation) for carbon and nitrogen, respectively. Stable isotopes of carbon and nitrogen are expressed as parts per thousand (‰) relative to the international standards PeeDee Belemnite for carbon and air for nitrogen. Carbon stable isotope ratios for fish liver were lipid corrected using a non-linear equation defined by Logan et al. 2008; equation 4.1.

$$\delta^{13}C + (3.093 \times \ln(C/N)) - 2.976 \qquad (4.1)$$

The difference in isotopic values between muscle and liver tissue reflects the differences in turnover time for the two tissues; previous studies have demonstrated that half-lives for carbon and nitrogen isotopes in fish muscle and liver are highly variable (ranging from 18 to 385 d and 6 to 277 d, respectively; Boecklen et al. 2011) but liver typically turns over faster than muscle (Perga and Gerdeaux 2005; Suzuki et al. 2005). Due to the slower turnover of muscle relative to liver tissue in the study herein (demonstrated by the lack of change in muscle $\delta^{13}C$ over the 42 d exposure period), the difference between liver and muscle $\delta^{13}C$ or $\delta^{15}N$ can be used as an indicator of different rates of feeding among limnocorrals. The change in $\delta^{15}N$ between muscle and liver

($\delta^{15}N_{muscle\text{-}liver}$) and the change in $\delta^{13}C$ between muscle and liver ($\delta^{13}C_{muscle\text{-}liver}$) were calculated and used as measures of tissue turnover.

4.3.6 Mass balance of Se

The total mass of selenium added to each treatment limnocorral was compared to the total mass of selenium measured in each compartment at day 63 using methods described in Graves et al. (2019a) and is described in detail in Supporting Information 5 and Tables A.4.2 and A.4.3.

4.3.7 Statistical analyses

All statistical analyses were performed using the RStudio software environment (R Studio Team 2018), associated base packages, and the "drc' package (Ritz et al. 2015). For all statistical analyses, alpha was set at 0.05.

4.3.7.1 Water chemistry, time to steady state, and Se differences among taxa

All TSe data were log-transformed prior to analysis to increase the linearity of the data. Linear regressions (LR) were performed to determine if there were any relationships between chemical and physical water parameters (pH, TDN, TDP, DOC, DO, temperature) and aqueous TSe concentration at the end of the experimental period. To determine when each compartment reached steady state, analysis of covariance (ANCOVA) was performed to determine if the relationship between periphyton, phytoplankton, or sediment TSe and aqueous TSe, between chironomid or heptageniid TSe and periphyton TSe, and between zooplankton TSe and phytoplankton TSe varied between day 21, 35, 49 and 63 (within each compartment and taxa only). If there were no significant differences in relationships among the timepoints, mean values for all timepoints were used to determine overall relationships between trophic levels.

Analysis of covariance was also used to determine the relationships between primary producer TSe and aqueous TSe, invertebrate TSe and primary producer TSe, with the categorical factor "taxa" included to determine if there were differences in slope or intercepts between taxa (i.e. periphyton versus phytoplankton or chironomid versus heptageniid versus zooplankton). Linear regression (LR) analysis was used to determine the relationship between fish tissue TSe and invertebrate TSe.

Relationships between fish tissue TSe and liver $\delta^{13}C$ or $\delta^{15}N$ were also assessed using linear regressions. Relationships between liver or muscle $\delta^{13}C$ and $\delta^{15}N$ and average diet TSe for fish and aqueous TSe concentration were assessed using linear regressions as well. Pearson's correlation analysis was used to assess the relationship between muscle and ovary TSe.

4.3.7.2 Distribution and trophic transfer at each trophic level

Distribution coefficients (k_d) for particulates (periphyton, phytoplankton, and sediment) were calculated using equation 4.2:

$$k_d = \text{particulate TSe/aqueous TSe} \quad (4.2)$$

where k_d is the distribution coefficient (L/kg dm), particulate TSe is the concentration in algae (µg/kg dm), and aqueous TSe is the concentration in water (µg/L).

Trophic transfer factors for consumers (invertebrates, fish) were calculated using equation 4.3:

$$TTF = \text{consumer TSe/diet TSe} \quad (4.3)$$

where TTF is the trophic transfer factor (unitless), consumer TSe is concentration in invertebrate or fish (µg/g dm), and diet TSe is the concentration in the consumer's diet (µg/g dm).

Fish TTFs were calculated for each of the main invertebrate taxa (zooplankton, Heptageniidae, Chironomidae). Linear regressions were performed to determine the relationship between TTFs and aqueous TSe concentration.

4.3.7.3 Modelling Se uptake kinetics

To characterize the uptake kinetics of algae and invertebrates over a gradient of Se concentrations, the fit of Michaelis-Menten (MM), power and linear models were compared. A modified version of Michaelis-Menten uptake models, similar to that used in Baines and Fisher (2001) were used to fit both periphyton and phytoplankton data using Equation 4.4:

$$C_a = C_{max} \times C_w/(K_M + C_w) \quad (4.4)$$

where C_a is the concentration in algae (µg/g dm), C_{max} is the maximum concentration in algae at saturation (µg/g dm), C_w is the concentration of Se in water (µg/L), and K_M is the concentration of Se in water at one half of the C_{max} (µg/L).

The saturable kinetics of invertebrates were fitted based on aqueous Se using equation 4.5:

$$C_i = C_{max} \times C_w/(K_M + C_w) \quad (4.5)$$

where C_i is the concentration in invertebrates (µg/g dm), C_{max} is the maximum concentration in invertebrates at saturation (µg/g dm), C_w is the concentration of Se in water (µg/L), and K_M is the concentration of Se in water at one half of the C_{max} (µg/L).

Invertebrates were also fitted based on algae Se using equation 4.6:

$$C_i = C_{max} \times C_a/(K_M + C_a) \quad (4.6)$$

where C_i is the concentration in invertebrates (µg/g dm), C_{max} is the maximum concentration in invertebrates at saturation (µg/g dm), C_a is the concentration of Se in algae (µg/g dm), and K_M is the concentration of Se in algae at one half of the C_{max} (µg/g dm).

When the Michaelis-Menten model was not significant for a particular compartment, the fit of linear and power (non-linear regression; NLR) models were compared. The fit was evaluated by first comparing the significance of the intercept and slope of each model, and then by comparing the R^2 values of each model. In each of these cases, the power model was a better fit to the data, and as such, linear model results are not presented herein.

Similar models could not be constructed for fish muscle or ovary, because the pattern of Se uptake over the gradient of concentrations appeared to indicate that fish had not reached steady state (see Results) and raw fish tissue TSe values did not significantly fit any of the tested models.

Two-compartment models for the uptake phase of Se in periphyton and phytoplankton were developed based on equation 4.7. Because time-dependent growth was not assessed, C_o and t were set to 0 and equation 4.7 was modified to replace uptake and efflux rate constants with the field derived k_ds as shown in equation 4.8. Because of the noted changes in distribution of Se, two k_ds were used for periphyton and three k_ds were used for phytoplankton.

$$C_a = C_o + C_w \times k_u / k_e (1\text{-}e^{\text{-ket}}) \quad (4.7)$$

$$C_a = C_o + C_w \times (k_d / 1000) \quad (4.8)$$

where C_a is the concentration in algae (µg/g dm), C_o is constant (set to 0), C_w is the concentration in water (µg/L), k_u is the uptake rate (L/g/d), k_e is the efflux rate (/d), t is time (d).

4.3.7.4 Stable isotope analysis and fish Se bioaccumulation

The biomass of zooplankton and Chironomidae was generally too low within the limnocorrals to analyze both TSe and stable isotopes; therefore, fish muscle and liver, periphyton, and benthic macroinvertebrate (Ephemeroptera) $\delta^{13}C$ and $\delta^{15}N$ from the present study were combined with bulk littoral zooplankton and Chironomidae isotope values from a previous limnocorral study in Lake 239 (Mailman 2008) to determine dietary sources of fish using isotope mixing models using the "MixSIAR" package in RStudio (Stock et al. 2018). When these data from two separate studies were combined, we assumed that the stable isotope values of similar taxa collected in the same area of the lake and the same season would not have changed considerably over time. The mean $\delta^{13}C$ and $\delta^{15}N$ of the sources, isotope trophic enrichment

functions, and diagnostics of the mixing models are presented in the Supporting Information (Table A.4.4 and A.4.5).

The resource use of fish (proportion benthic food source) was then analyzed for relationships with aqueous TSe and average fish diet TSe using beta regression (BR) in the "betareg" package in R (Cribari-Neto and Zeileis 2010). $\delta^{15}N_{muscle\text{-}liver}$ and $\delta^{13}C_{muscle\text{-}liver}$ (described above) were related to log average diet TSe using linear regression to determine if there were differences in tissue turnover rates according to Se exposure.

4.4 Results and Discussion

4.4.1 Water chemistry

Mean dissolved aqueous TSe concentrations throughout the experimental period (calculated using area under the curve; Figure A.4.3) were 0.08, 0.08, and 0.09 µg/L for the three control limnocorrals. Mean dissolved aqueous TSe concentrations for limnocorrals with Se added were 0.4, 0.8, 1.6, 3.4, 5.6, and 7.9 µg/L. Baseline Se concentrations and Se speciation in surface water from Lake 239 proper and four other lakes at IISD-ELA are presented in Table A.4.6. Total aqueous Se ranged from 0.08 to 0.15 µg/L, and the proportion of TSe as selenite in surface water of the lakes ranged from 52 to 79% (54 ± 26% in Lake 239; Table A.4.6). The only other species of Se detected in lake water was selenate, which comprised 21 to 48% of TSe among lakes, indicating that selenite is often the dominant form of Se in these boreal lakes. Physical and chemical characteristics of water collected from limnocorrals were not related to aqueous TSe concentration at the end of the experimental period (LR, p=0.224 to 0.846). The temperature of water in the limnocorrals ranged from 20 to 25°C, pH ranged from 6.8 to 7.8, and DOC ranged from 6.90 to 9.98 mg/L throughout the experimental period (Table A.4.7, Figure A.4.4). Nutrient concentrations ranged from 242 to 435 µg/L for TDN and 1.64 to 6.52 µg/L for TDP throughout the experimental period (Table A.4.7, Figure A.4.4).

4.4.2 Algae and sediment selenium

Periphyton and phytoplankton TSe concentrations were not significantly different among day 21, 35, 49, or 63 (ANCOVA, p=0.98; Figure A.4.5), indicating that primary producers had reached a steady state before day 21. Mean values for all timepoints combined (day 21, 35, 49, 63) were thus used to describe primary producer Se bioaccumulation. Mean algae Se and k_ds for all timepoints combined are shown in Tables A.4.8 and A.4.9. Periphyton and phytoplankton TSe were significantly and positively related to aqueous TSe concentrations (ANCOVA, p<0.001,

R^2=0.98, Figure 4.1), but there was a difference in slopes of the relationships between the two types of primary producers (ANCOVA, p=0.033). Periphyton TSe concentrations ranged from 0.82 µg/g dm in control limnocorrals to 92.7 µg/g dm in the highest treatment, while phytoplankton ranged from 0.96 µg/g dm in control limnocorrals to 116 µg/g dm in the highest treatment (Figure 4.1), and high bioaccumulation was observed near the CCME water quality guideline (12 ± 0.7 and 17 ± 6.6 µg/g dm in periphyton and phytoplankton, respectively; Table A.4.8).

Periphyton, but not phytoplankton, could be fitted with Michaelis-Menten uptake kinetics (MM, p=0.007, Figure 4.2, Table A.4.10). Although the phytoplankton TSe data did not fit a statistically significant Michaelis-Menten model, a power equation fit the phytoplankton TSe better than a linear equation (NLR, p<0.001, Figure 4.2, Table A.4.10), indicating that some saturation was observed and that perhaps the phytoplankton taxa have a higher saturation point that was not reached in the present study. The saturation of Se uptake by algae could also be seen when looking at the k_ds for algae. Distribution coefficients ranged from 6,600 to 42,000 for phytoplankton and from 6,000 to 22,000 for periphyton across all experimental groups and experimental time points. These k_ds are similar to those calculated for particulates in other Canadian boreal lakes in Quebec and Ontario, which ranged from 9,136 to 42,530 (Ponton et al. 2018). Periphyton k_ds appeared to show a distinct decrease between Se treatments of 1.6 and 3.4 µg/L treatments, and phytoplankton k_ds were highest for 0.08-0.09 µg/L, moderate for 0.4-1.6 µg/L treatments, and lowest for the 3.4-7.9 µg/L treatments (Table A.4.9). Because of these changes in distribution over relevant levels of Se, models used to estimate enrichment of Se from water to algae need to consider that exposure concentration can influence the rate of uptake. Actual algal Se versus algal Se predicted using two-compartment models are presented in Figure A.4.6. Based on the observation that k_ds decrease at environmentally relevant levels of Se, it may be more useful to use the equations from the log-log relationships between trophic levels (Figure 4.1) or the saturated curves (Figure 4.2) to predict Se accumulation in organisms, rather than two-compartment models which require using different k_ds for different exposure levels.

The uptake curves modelled herein may be used to illustrate the maximum Se levels expected at different aqueous Se levels within boreal lakes. The bioaccumulation observed in the present study is considered to be “maximum” because we added Se as 100% selenite, the more bioavailable form of the two oxyanions generally present in water. It is anticipated that a mixture of selenite and selenate would result in lower overall bioaccumulation of Se.

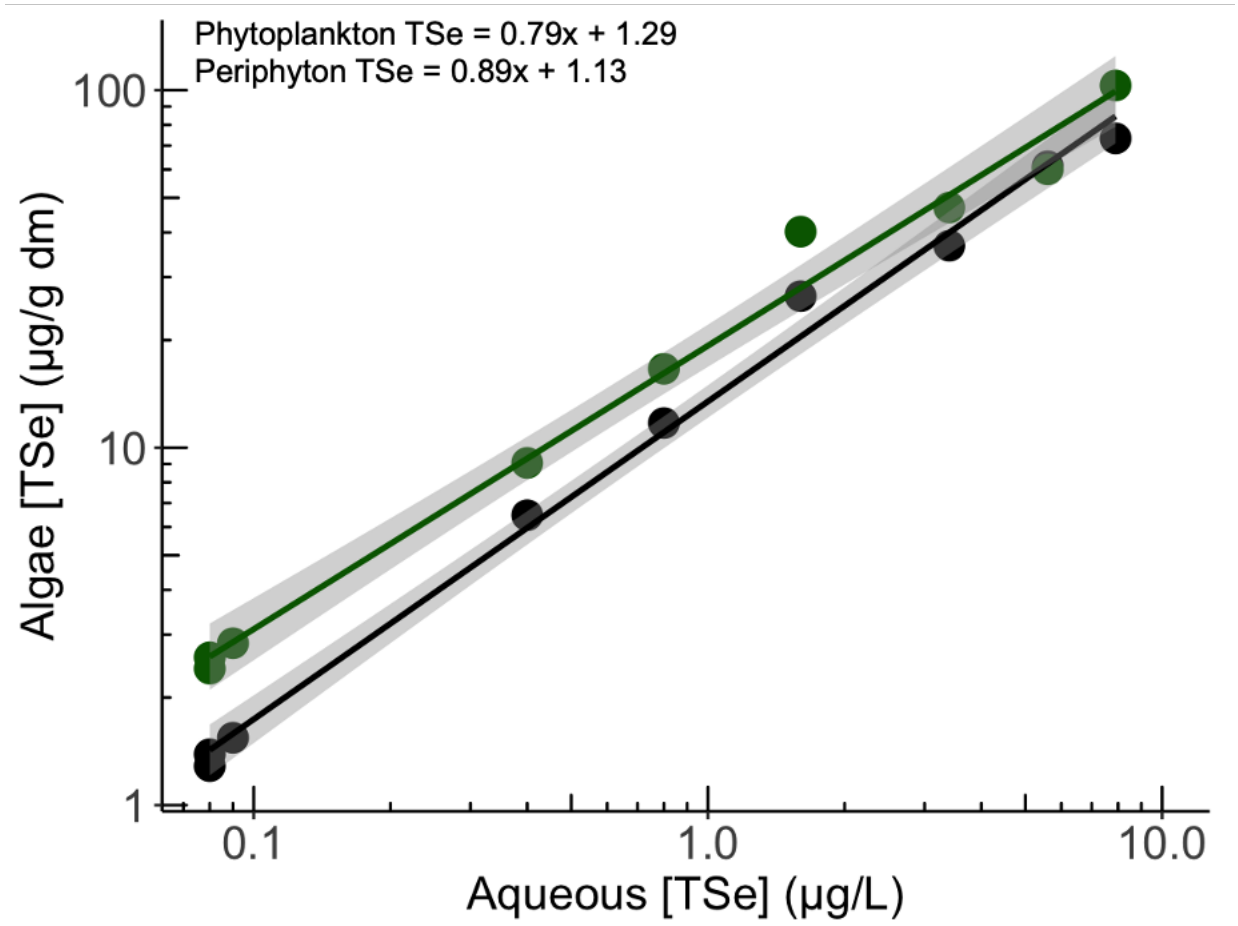

Figure 4.1: Relationship between log primary producer TSe (periphyton, green circles; phytoplankton, black circles) and log aqueous (dissolved) TSe analyzed using analysis of covariance. Intercepts and slopes were significantly different for phytoplankton and periphyton and are therefore represented using separate equations and lines of fit. Data points represent means from day 21, 35, 49, and 63 for each experimental group, solid lines represent line of fit for each compartment and shaded areas represent 95% confidence intervals for the lines of best fit.

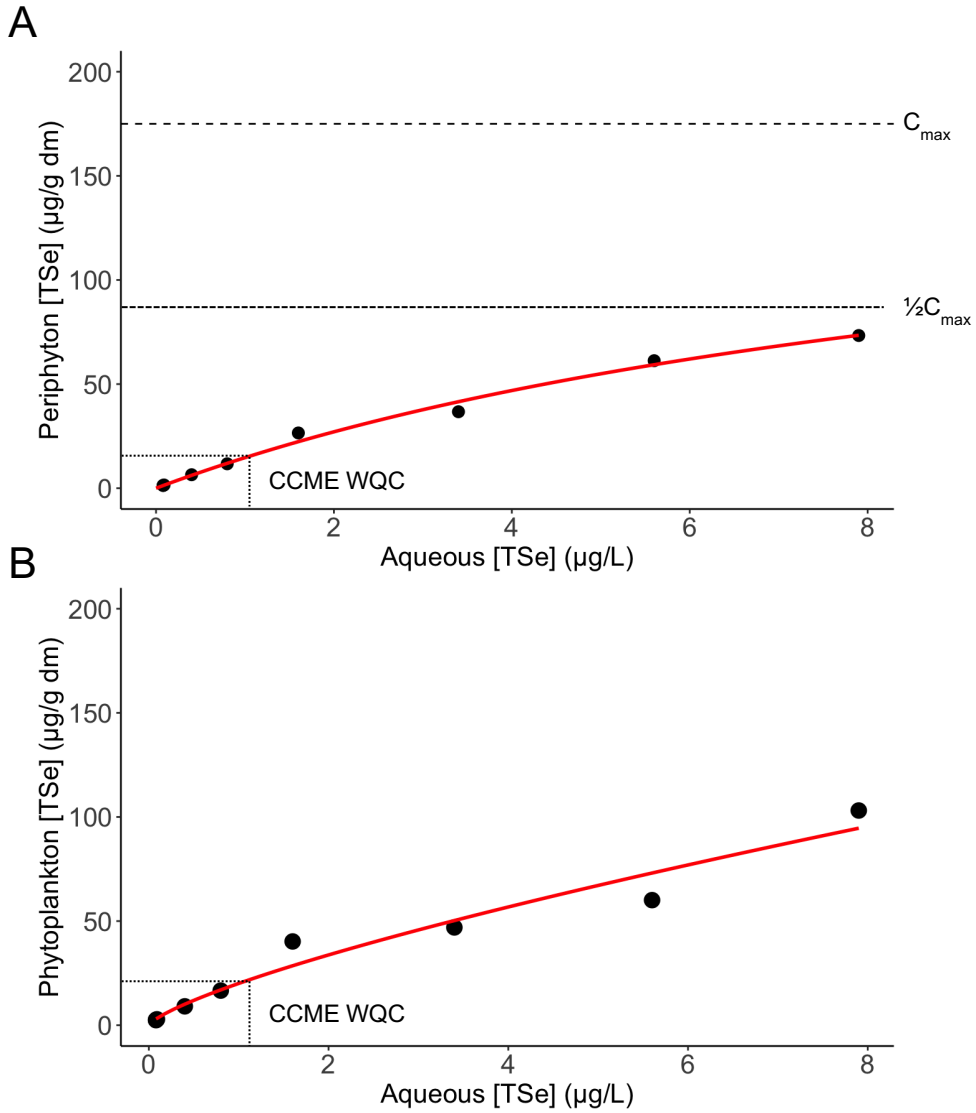

Figure 4.2: Relationships between periphyton (A) or phytoplankton (B) TSe and aqueous (dissolved) TSe. Periphyton was fitted with a Michaelis-Menten uptake curve (red line), where C_{max} is the modelled maximum concentration of Se. K_M was beyond the tested concentrations and is therefore not shown in the figure. For phytoplankton, the Michaelis-Menten curve was not statistically significant and the data best fit a power equation (red line). Dashed vertical lines represents the CCME water quality criterion (1 µg/L).

The difference in uptake capacity between different algal taxonomic groups has been observed previously in the laboratory, with accumulation of Se across different phytoplankton species differing up to four orders of magnitude following exposure to 0.03 µg/L selenite (Baines and Fisher 2001). Investigations into differences in Se accumulation among taxa from field-collected samples are fewer, but in periphyton collected from a mine-affected stream in West Virginia, Se concentrations were 18-fold greater in diatom/sediment fractions compared to filamentous algae fractions (Arnold et al. 2017). In the present study, it is possible that the dominant algal taxa present in periphyton samples reach saturation at a lower concentration of water Se than phytoplankton. This is supported by the higher uptake seen in phytoplankton. Though algal species identification was not conducted in the present study, taxonomic data previously collected from IISD-ELA showed that diatoms are dominant in epilithic periphyton in Lake 239, with *Achnanthes minntissima* generally being the most abundant species (Stockner and Armstrong, 1971). The phytoplankton communities were composed of relatively smaller proportions of diatoms, and were typically dominated by Chrysophyta and Dinophyta taxa (IISD-ELA, unpublished data). It is also possible that the difference in accumulation between periphyton and phytoplankton observed herein may be related to surface area, as phytoplankton, living in the water column, may have more interaction and therefore greater uptake of Se than periphyton attached to substrates.

The top 1 cm of sediment in each limnocorral was composed largely of organic matter (OM; 71 to 91%; Table A.4.11), and sediment TSe in this section on day -2 was 2.97, 2.05 and 1.73 µg/g dm in three representative cores collected near the limnocorrals. The relationship between sediment TSe and aqueous TSe did not differ significantly between days 21 and 63 (ANCOVA, $p=0.463$; Figure A.4.7). Sediment TSe concentrations ranged from 1.96 µg/g dm in a 0.08 µg/L limnocorral to 8.65 µg/g dm in the 7.9 µg/L limnocorral throughout the experimental period, and sediment TSe was significantly and positively related to aqueous TSe concentration (LR, $p<0.001$, $R^2=0.77$). The OM-corrected distribution coefficients for sediment ranged from 15,560 to 33,803 L/kg dm OM in the 0.08-0.09 µg/L group, and from 643 to 4,914 L/kg dm OM in six Se-treated limnocorrals. In general, k_ds were very similar among all treatment limnocorrals, and were less than k_ds from the 0.08-0.09 µg/L group. In this oligotrophic lake, sediment TSe concentrations were low relative to primary producer TSe. In addition, dissipation of added Se from water to other compartments was slower and less compartmentalization of Se to sediment

was observed relative to a previously studied mesotrophic lake over a comparable time period (Graves et al. 2019a). These differences highlight that lake-specific characteristics, such as nutrient status, microorganism activity, productivity, and biogeochemistry, can influence Se distribution. Although TSe concentrations were lower for sediment than primary producers, the total mass of Se in sediment within the limnocorrals was much larger than for phytoplankton or periphyton (Table A.4.2), suggesting that sediment is an important sink for Se. It is likely that steady state concentrations in sediment are reached at a much slower rate than in biotic components of a system, and that a steady state for Se in sediment was not reached within this 63-d experimental period. At water TSe concentrations of < 0.1 (in a reference lake), 0.7 and 2.7 µg/L (in two mining-influenced lakes) in previously studied boreal lakes in northern Saskatchewan, Canada, sediment Se concentrations were 5.7, 25.6 and 62.2 µg/g dm, respectively (35). These concentrations were 1.4, 7.5 and 9.4-fold greater than those measured at comparable aqueous TSe concentrations in the present study. The greater amounts of Se observed in sediment in other Canadian boreal lakes may be due to the settling of dead or organic materials over several decades that have accumulated greater amounts of Se, or perhaps these lakes have different underlying geologic sources affecting the compartmentalization of Se. Due to the relatively short duration of the current experiment, there may not have been time for selenium to accumulate from dead organisms to a comparable extent.

4.4.3 Invertebrate selenium

The relationships between zooplankton, Chironomidae, or Heptageniidae TSe concentrations and their presumed diet (phytoplankton or periphyton) were not significantly different for each taxon among day 21, 35, 49 and 63 (ANCOVA, p=0.324 to 0.862, Figure A.4.8), suggesting that steady state concentrations were reached before 21 d of Se exposure. Mean invertebrate Se and TTFs for each limnocorral are presented in Tables A.4.8 and A.4.9. Invertebrate TSe concentrations for all taxa were significantly and positively correlated to their presumed diet TSe (ANCOVA, $p<0.001$, $R^2=0.93$, Figure 4.3). There was no difference in slope between any of the taxa (ANCOVA, p=0.765), but zooplankton TSe concentrations were significantly lesser than Chironomidae and Heptageniidae at a given diet TSe concentration (ANCOVA, $p<0.001$; Figure 4.3). Heptageniidae TSe concentrations ranged from 2.05 to 91.0 µg/g dm, Chironomidae from 1.54 to 93.4 µg/g dm, and bulk zooplankton from 1.16 µg/g to 62.8 µg/g dm across the experimental groups.

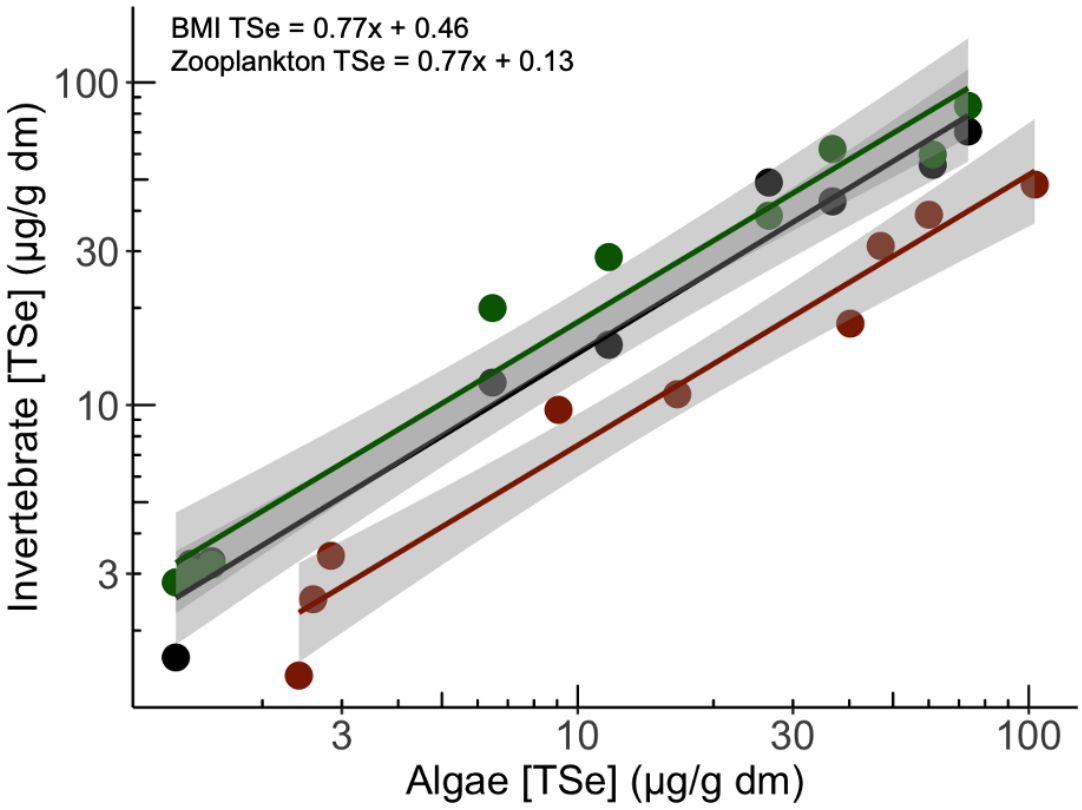

Figure 4.3: Relationship between log invertebrate TSe (heptageniid, green circles; chironomid, black circles; zooplankton, red circles) and log diet TSe (periphyton for benthic macroinvertebrates (BMI) and phytoplankton for zooplankton) using analysis of covariance. Intercepts were significantly different for benthic macroinvertebrates (chironomid and heptageniid) and zooplankton, and are therefore represented using separate equations and lines of fit. Data points represent means from day 21, 35, 49, and 63 for each experimental group, solid lines represent line of fit for each compartment and shaded areas represent 95% confidence intervals for the lines of best fit.

Despite having a food source that was significantly higher in TSe, zooplankton accumulated the least TSe of the invertebrates evaluated. Zooplankton tend to have greater lipid content than benthic macroinvertebrates (Kattner et al. 2007) and this may have resulted in lower accumulation of the typically protein-incorporated Se. In addition, differences in Se uptake and depuration kinetics have been documented for zooplankton and benthic invertebrates. Marine copepod assimilation efficiencies for Se ranged from 0.50 to 0.97 and calculated TTFs for Se ranged from 1.35 to 3.1 (Fisher and Reinfelder 1991; Schlekat et al. 2002), while freshwater bulk zooplankton had a TTF of 1.5 compared to a TTF of 2.7 for Ephemeroptera and Chironomidae species (Saiki et al. 1993; Casey 2005). Efflux rates may contribute to this difference as well, since the efflux rate constant (k_e) for the marine copepods mentioned above ranged from 0.13 to 0.16 (Fisher and Reinfelder 1991; Schlekat et al. 2002), compared to a k_e of 0.03 for a mayfly (*Centroptilum triangulifer*; Riedel and Cole 2001). It is difficult to separate the ecological (habitat, diet) versus physiological (assimilation, excretion) possibilities for the differences in Se uptake for field-collected invertebrates. It is interesting that Chironomidae and Heptageniidae, which are generally classified as collector-gatherers (Merritt and Cummins 1984), show similar patterns of Se accumulation, while zooplankton, which may feed selectively or non-selectively on phytoplankton/particulate matter or other zooplankton in the water column (Wetzel 2001) show a significant difference in accumulation.

The difference in TSe accumulated among invertebrate taxa indicate that individual fish living in the same system but differing in diet could have up to a 2-fold difference in whole body TSe concentration. This has implications for the risk assessment of Se; often invertebrate samples for the analysis of Se are collected in bulk and are pooled into coarse categories or functional feeding groups. The pooled taxa can represent several different species, despite the evidence here and in previous studies that different species can differ greatly in accumulation of Se, even within the same habitat (Presser and Luoma 2010; Graves et al. 2019a). Given that invertebrate Se can vary with habitat and species, investigators should use caution when pooling invertebrate taxa as it may give a diluted measure of Se accumulation and alter conclusions about exposure risks for the higher trophic levels. Additionally, at 1 µg/L (the current CCME guideline), invertebrates accumulated up to 25 µg Se/g dm (Figure 4.3). This is well above the interim guideline of British Columbia of 4 µg/g dm for fish diet (BC MOE 2014), highlighting that invertebrates can be an important vector for Se transfer to fish, and that invertebrates themselves may also be at

ecotoxicological risk from high Se accumulation (Graves et al. 2019b), even at relatively low aqueous Se exposure concentrations (in that study, decreased diversity of BMIs was observed at an aqueous Se level of 1 µg/L). Again, the level of bioaccumulation observed in invertebrates likely represents a "maximum" concentration that could be observed at a given aqueous Se concentration, due to the high proportion of the more bioavailability oxyanion selenite.

Similar to accumulation in algae, the log-linear relationship between invertebrate TSe and diet TSe with a slope less than 1 indicated a plateau of Se accumulation at higher exposure concentrations. Chironomids and heptageniids were fitted with statistically significant Michaelis-Menten uptake curves (MM, p=0.002 to 0.05, Figure 4.4, Table A.4.10), but zooplankton could not be fitted to a Michaelis-Menten curve (MM, p=0.126, Figure 4.4, Table A.4.10). Still, a power equation fit the data better than a linear equation (NLR, p<0.001), suggesting some saturation occurred over the test concentrations. Though the level of saturation (C_{max}) calculated for chironomids and heptageniids were well above what would occur at the current CCME guideline (43-52 µg/g dm), TTFs tended to decrease around 0.8 – 1.6 µg/L treatment levels, indicating again that saturation of uptake curves is relevant at current allowable Se levels (Table A.4.9). Trophic transfer factors decreased with increasing aqueous Se concentrations (ANCOVA, p=0.016, R^2=0.63, Figure A.4.9). There was no difference in the slope of the relationship for the three taxa investigated (ANCOVA, p=0.268), but the two benthic macroinvertebrates (Heptageniidae and Chironomidae) had a significantly greater intercept than zooplankton (ANCOVA, p<0.001). The decreasing TTFs with increasing exposure concentrations have been observed previously (Conley et al. 2013, Graves et al. 2019) and again, this non-linear accumulation of Se in invertebrates is important to account for if Se trophic transfer is to be accurately modelled.

All invertebrates were fitted to Michaelis-Menten uptake curves with aqueous Se as the substrate (Figure A.4.10, Table A.4.10). Although it is assumed that invertebrates would assimilate greater amounts of Se from their diet than from water, previous research showed that aqueous Se uptake was estimated to be 20-40% of internal Se for *Daphnia magna* (Roditi and Fisher 1999; Tsui and Wang 2007). Further, this model is useful for visualizing Se uptake in invertebrates as a function of water Se. The K_Ms for all invertebrates ranged from approximately 2-5 µg/L for zooplankton, chironomids, and heptageniids, suggesting that rates of Se uptake do change within environmentally relevant concentration ranges of aqueous Se (Figure A.4.10).

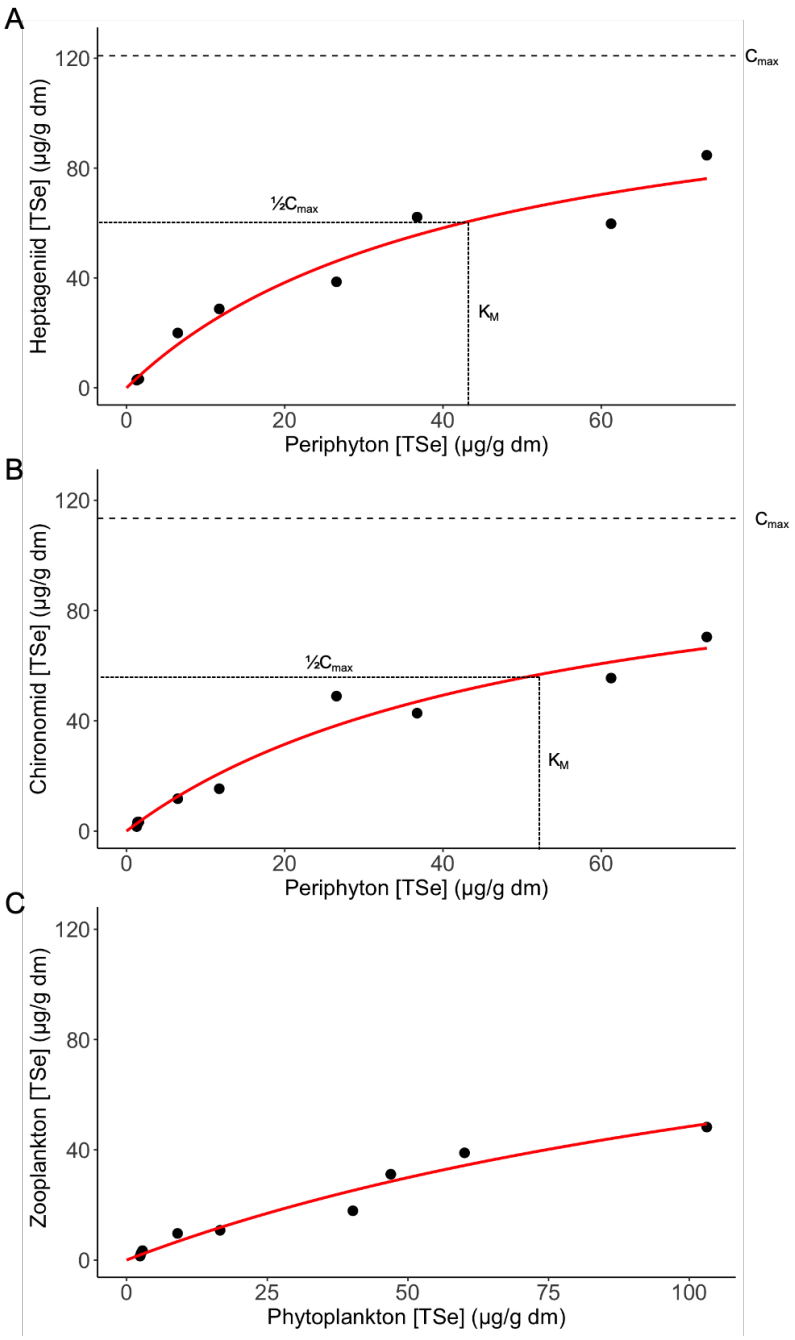

Figure 4.4: Michaelis-Menten uptake curves showing the relationship between invertebrate TSe bioaccumulation and algae TSe concentration, where C_{max}= modelled maximum concentration and K_M = concentration of aqueous Se at ½C_{max}. The heptageniid (A) and chironomid (B) Michaelis-Menten curves were statistically significant. The zooplankton (C) curve was not, and is instead fit with a power equation.

In the present study, we were not able to assess feeding rates of invertebrates in each limnocorral. It is possible that some of the saturation in Se bioaccumulation observed for invertebrates is due to food aversion. Though to our knowledge, no studies have assessed Se-laden food aversion in invertebrates, avoidance of foods high in Se have reported previously for multiple fish species (*Lepomis macrochirus* and *Salmo gairdneri*) and mallards (*Anas platyrhynchos*) at Se levels of 10 – 20 µg/g dm (Hilton et al. 1980; Finley 1985; Heinz and Sanderson 1990).

4.4.4 Fish selenium

After 42 d of exposure to Se, log transformed fish ovary and muscle tissue TSe were both positively related to log average diet TSe (LR, $p<0.001$-0.026, $R^2=0.46$-0.80, Figure 4.5). Ovary TSe concentrations were approximately two-fold greater than muscle TSe, and the tissue Se concentrations were positively correlated ($p=0.016$, $R^2=0.72$). Mean fish tissue TSe concentrations and TTFs are presented in Tables A.4.8 and A.4.9. Fish muscle TSe did not exceed the US EPA tissue criterion (11.3 µg/g dm) in any of the limnocorrals, but at an aqueous TSe level of 0.8 µg/L (closest to the 1 µg/L CCME guideline), fish muscle accumulated 8-10 µg Se/g dm, approximately double the interim guideline for fish muscle set by the BC MoE (4 µg/g; BC MoE 2014; Figure 4.6). Ovary TSe exceeded US EPA tissue criterion (15.1 µg/g dm) in the 7.9 µg/L treatment. Fish ovaries accumulated 12-15 µg/g dm Se in the 0.8 µg/L treatment, which is above the comparable BC MoE guideline (11 µg/g dm; Figure 4.6).

The fish tissue TSe concentrations observed herein at exposure concentrations near the CCME water quality guideline are slightly higher than previously observed in northern pike (*Esox lucius*) collected downstream of mining operations of in northern Saskatchewan, which showed no signs of deformities at egg Se concentrations of 8.02 µg/g dm (Muscatello and Janz 2009). In another mesocosm study in northern Saskatchewan wherein uranium milling effluent was diluted to 25% and Se concentrations in water were 10 µg/L, reduced hatching success and larval survival were observed in fathead minnow with ovary Se concentrations of approximately 15-20 µg/g dm (Driessnack et al. 2011). These previous studies suggest that the level of bioaccumulation observed at the CCME water quality guideline herein can be of concern for fish survival. Fish TTFs based on muscle tissue TSe using zooplankton, chironomid and heptageniid separately as diet items all showed similar inverse relationships with aqueous TSe concentrations (ANCOVA, $p<0.01$, $R^2=0.62$-0.74, Figure A.4.11). Trophic transfer factors based on different diet items (chironomid, heptageniid, or zooplankton) were not significantly different among

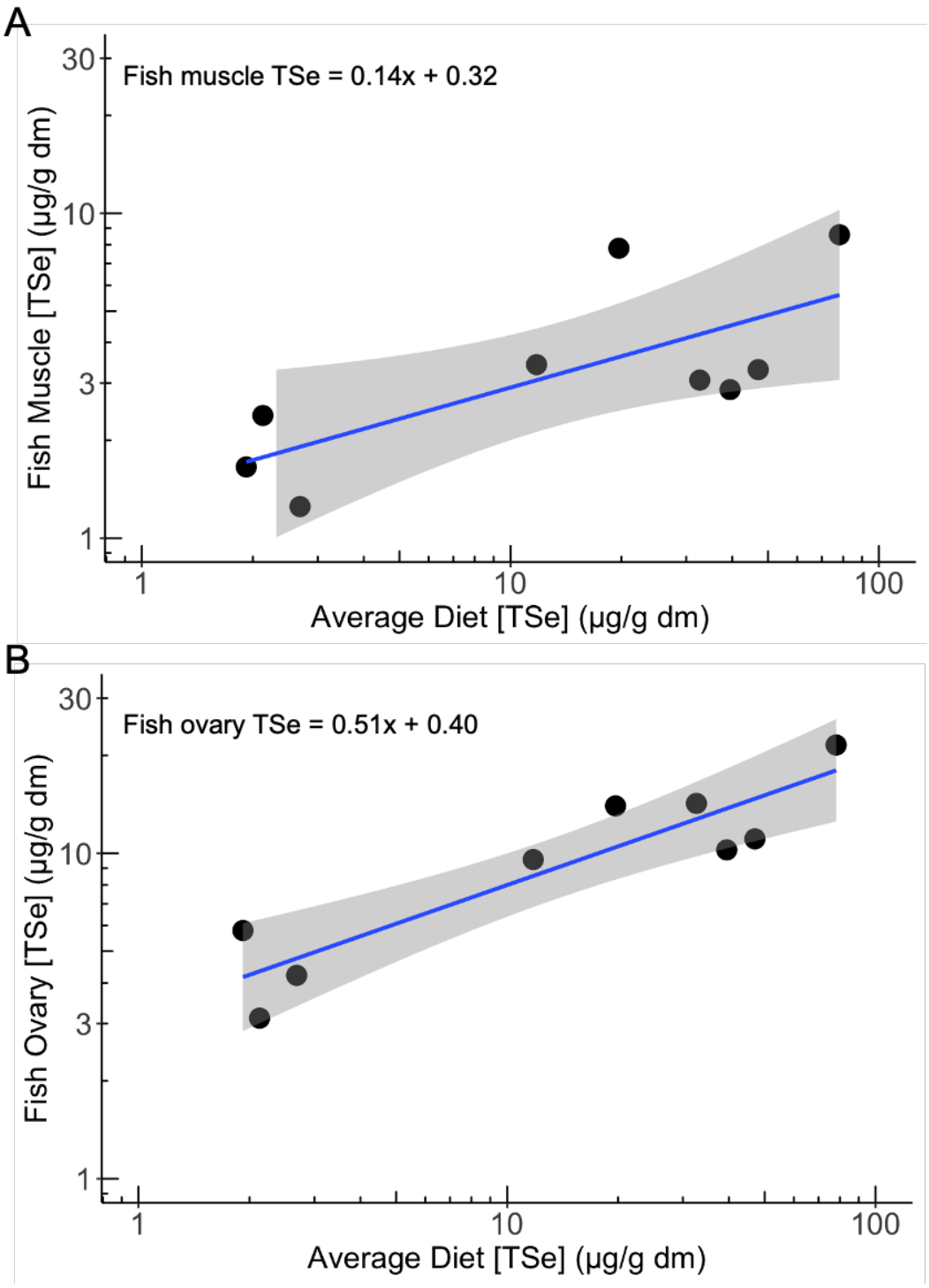

Figure 4.5: Relationships between log muscle (A) or ovary (B) TSe and log diet TSe (mean of heptageniid, chironomid, and zooplankton TSe) from d 63 of the experimental period (42 d of exposure for fish; n=2-3 per limnocorral). Solid line represents line of fit and shaded area represents 95% confidence interval for the line of best fit.

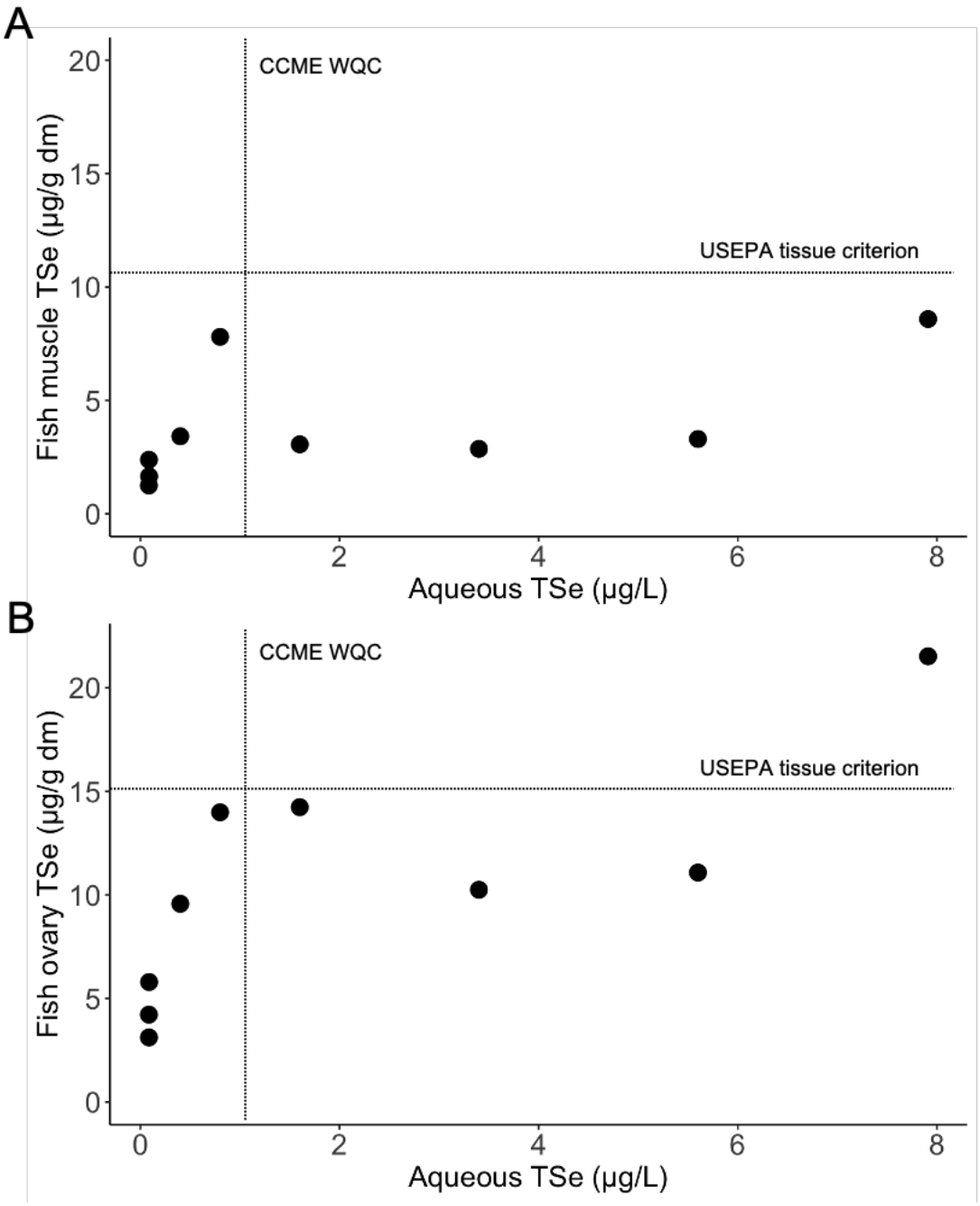

Figure 4.6: Fish muscle (A) and ovary (B) TSe as a function of dissolved aqueous TSe. Vertical dashed lines indicate the CCME water quality criterion (WQC; 1 µg/L), and horizontal lines indicating the US EPA tissue criterion for muscle (11.3 µg/g dm) and ovary (15.1 µg/g dm).

taxa (ANCOVA, p=0.41 to 0.80), and ranged from 0.5 to 1.4 in the 0.08-0.09 µg/L group, decreasing to a value as low as 0.03 in the 7.9 µg/L group. Based on the bioaccumulation of Se in fish muscle and ovary tissue over the 42 d experimental period (Figure A.4.12), no plateau in Se concentration was observed in the Se-treated limnocorrals. Therefore, these low TTFs could be because fish did not reach steady state over the 42 d experimental period. Indeed, most previously studied wild fish have TTFs near 1.0 (Presser and Luoma 2010); however, the inverse relationship between Se exposure concentration and TTF has been observed previously (DeForest et al. 2016; Graves et al. 2019a) and is similar to the decreases in k_ds and TTFs observed for the other biota (algae and invertebrates) in the present study. Thus, the decreasing trend may indicate that there is saturation of selenium uptake transporters, or an increase in the excretion of Se with greater exposure concentrations. Previous research has shown that efflux rates of Se increase with increasing dietary Se loading (Hilton et al. 1982). It is possible that food avoidance could contribute to a very slow time to steady state for fish. As discussed above, avoidance of foods high in Se have reported previously for fish at Se levels of 10 – 20 µg/g dm (Hilton et al. 1980; Finley 1985; Heinz and Sanderson 1990). Further, in this low productivity lake, biomass available for consumption by fish was likely low. Direct toxicity to fish seems unlikely to have affected Se uptake, as levels of up to 10 µg/g dm in fish muscle tissue are not generally associated with toxicity in adult fish, and no difference in condition factor was observed between treatments (Table A.4.1).

4.4.5 Stable isotope ratios and food web structure

The finescale dace used in the present study had muscle and liver $\delta^{13}C$ values of -30 to -28‰ and $\delta^{15}N$ values of 7.1 to 8.3‰ at the beginning of the experiment. Fish muscle $\delta^{13}C$ for all treatments after 42 d of exposure differed little from the original values, ranging from -29 to -27‰, and $\delta^{15}N$ values also did not change substantially, ranging from 6.1 to 8.4‰ (Figure 4.7). However, fish liver $\delta^{13}C$ values ranged from -27 to -21‰ and $\delta^{15}N$ ranged from 5.5 to 7.7‰ after 42 d in the limnocorrals (Figure 4.7), indicating the fish diets in the limnocorrals were isotopically distinct from diets in the in-flow stream that they were collected from. Neither ovary nor muscle tissue TSe concentrations were predicted by fish muscle or liver $\delta^{13}C$ or $\delta^{15}N$ isotope signatures after 42 d of exposure to Se (LR, p=0.426 to 0.920).

Although log transformed fish tissue TSe was positively and linearly related to log diet TSe, there was a greater amount of unexplained variability in fish tissue TSe relative to the lower trophic levels. Upon visual inspection of the data, the bioaccumulation of Se in fish appeared to

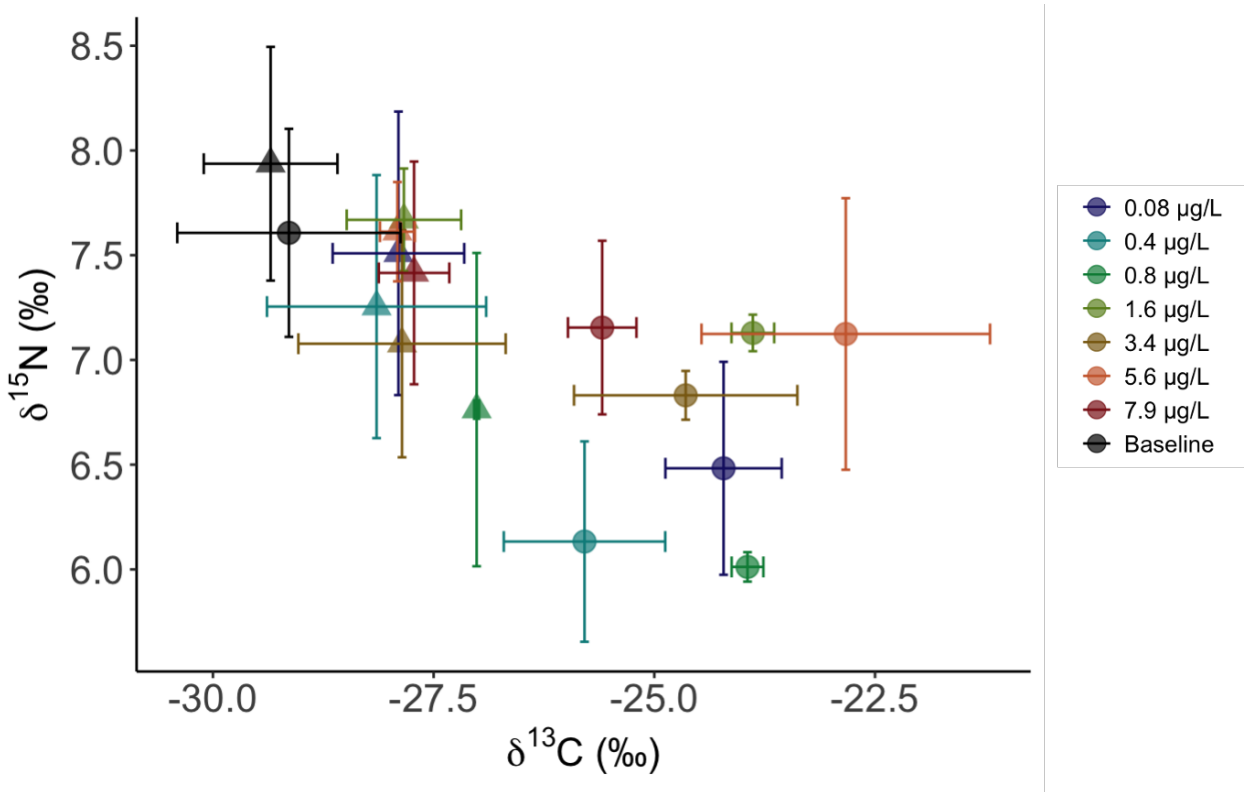

Figure 4.7: Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) shown as mean ± SD for fish muscle (triangles; black is baseline and colours in legend are after 42 d of exposure to a gradient of Se) and liver (circles; black is baseline and colours in legend are after 42 d of exposure) from Lake 239 limnocorrals.

exhibit a polynomial-type trend over the tested aqueous exposure concentrations, which may have been caused by possible changes in feeding rates or changes in diet items. Stable isotope mixing models revealed that fish were feeding on different proportions of benthic versus pelagic organisms among limnocorrals, with the percentage of benthic resource use ranging from 42 ± 16% in the 0.8 µg/L treatment to 88 ± 9% in the 5.6 µg/L treatment (Figure A.4.13). The percent benthic resource use was positively related to aqueous Se concentration (BR, p=0.020, pR^2=0.47) and fish diet Se concentration (BR, p=0.014, pR^2=0.51). $\delta^{15}N$ tissue turnover ($\delta^{15}N_{muscle\text{-}liver}$) was negatively related to log invertebrate TSe (a proxy for fish diet TSe exposure; LR, p=0.008, R^2=0.24, Figure A.4.14), indicating that there was a difference in isotopic turnover rates with increasing TSe treatment. This might indicate that the resource use differences detected with the mixing models are actually a reflection of differences in tissue turnover rates.

The stable isotope data presented herein suggest that either food availability was low, and turnover of isotopes was slower in fish exposed to higher TSe, or perhaps the diet of fish shifted as TSe exposure increased. Both low food availability and a shift in diet could be due to the direct toxicity of TSe to certain invertebrate taxa, as has been observed previously (Graves et al. 2019b). Shifting the diet from one invertebrate taxon to another could affect the accumulation of TSe in fish due to differences in TSe accumulation among invertebrates, and this change in feeding dynamics (and diet TSe exposure) may in part explain the greater variability in the relationship between fish muscle and diet TSe. It is important to note that after 42 d of exposure the liver tissues of fish may still have not reached equilibrium with their diet, and that the stable isotope values presented herein represent the accumulated diet that includes some fraction of the original diet from the source stream. Nonetheless, the differences in fish tissue $\delta^{13}C$ and $\delta^{15}N$ among treatments did help to identify a change in diet among fish that may be confounding the bioaccumulation pattern of TSe in fish. The difference in tissue turnover or resource use as Se exposure concentrations increased provides evidence that there was either a shift in the diet of fish and/or a difference in food availability of fish among treatments. This illustrates that food web dynamics of exposed organisms can affect Se bioaccumulation patterns and demonstrates the importance of studying bioaccumulation at the ecosystem level.

CHAPTER 5: RESPONSE OF CRUSTACEOUS ZOOPLANKTON AND BENTHIC MACROINVERTEBRATE COMMUNITIES TO SELENIUM ADDITIONS

Preface

This chapter was designed as a follow-up to Chapter 3. I was interested in the observed effects of Se on benthic macroinvertebrates, which have been relatively under-studied in the past compared to oviparous vertebrates. This study occurred as part of the experiment described in Chapter 4, and the goal was to determine changes in both crustaceous zooplankton and benthic macroinvertebrate community composition, biomass, and density following exposure to Se. The major findings were that both zooplankton and benthic macroinvertebrate community composition changed as a function of Se treatment, with zooplankton being affected at lower exposure levels than benthic macroinvertebrates. Biomass and density of sensitive taxa decreased over the tested Se concentrations. This research shows that some invertebrate taxa are more sensitive to Se than previously thought, and that effects on some taxa can occur at concentrations similar to those that impact fish and birds.

This chapter is in preparation for publication with co-authors Karsten Liber (University of Saskatchewan), Vince Palace (International Institute for Sustainable Development – Experimental Lakes Area), Markus Hecker (University of Saskatchewan), Lorne Doig (University of Saskatchewan), and David Janz (University of Saskatchewan).

5.1 Abstract

Selenium (Se) is a contaminant of concern in Canada mainly due to its teratogenic effects on egg-laying vertebrates in aquatic ecosystems, but few studies have assessed the effects of Se on invertebrates in a field setting. The objective of this experiment was to assess potential ecosystem-level impacts of Se additions on a boreal lake food web. From June to August, 2018, Se (as selenite) was added to six limnocorrals in Lake 239 at the Experimental Lakes Area to achieve mean measured concentrations of 0.4, 0.8, 1.6, 3.4, 5.6 and 7.9 µg Se/L, and three limnocorrals were left untreated as controls (background aqueous Se = 0.08 µg/L). Periphyton, phytoplankton, invertebrates (zooplankton and benthos), and a small-bodied fish (finescale dace; *Phoxinus neogaeus*) were monitored for 63 d. Zooplankton communities shifted according to Se exposure, with Cladocera biomass and density decreasing with increasing Se treatment. Cumulative abundance and biomass of Heptageniidae decreased with increasing treatment of Se throughout the experimental period. This study demonstrated that Se can have impacts on aquatic invertebrates at environmentally relevant exposure levels, and that future ecological risk assessments should consider the impacts of Se on both vertebrates and invertebrates.

5.2 Introduction

Selenium (Se) is a naturally occurring trace element that can be mobilized from the earth's crust and soils during anthropogenic activities such as mining for coal and metal ores, oil extraction and refining, and agricultural irrigation (Lemly 1985; Nriagu 1989; Yudovich and Ketris 2006). This mobilization can lead to elevated inputs of Se in aquatic systems, and increased uptake by organisms within the aquatic environment. Organisms at the base of the food web, such as microalgae and bacteria, assimilate inorganic Se (i.e. selenite; SeIV and selenate; SeVI) from water and convert it to organic forms (i.e. selenomethionine; SeMet and selenocysteine; SeCys), with consumers being exposed to Se mainly through their diet and mainly to organic Se (Wallenberg et al. 2010; Young et al. 2010).

Ecological risk assessments of Se have generally focused on the adverse effects of Se on oviparous vertebrates. Indeed, the most well-documented impacts of elevated Se in aquatic ecosystems are teratogenicity in egg-laying vertebrates such as fish and birds (Lemly 1993; Hamilton 2003; Janz et al. 2010). This is likely a result of maternal transfer of Se to developing ovaries, followed by increased oxidative stress as Se is metabolized within the embryo (Palace et al. 2004; Janz et al. 2010). In the most extreme cases, Se exposure has caused population declines and reproductive failure of fish and bird populations (Lemly 1993; Hamilton 2003). The US EPA has established fish muscle and ovary tissue Se criteria for the protection of aquatic life (11.3 and 15.1 µg/g dm, respectively), which are considered more protective of ecosystems than water Se guidelines due to the high variability in Se bioaccumulation among systems (US EPA 2016). However, little information is available regarding the potential ecological impacts of elevated Se on other organisms, such as benthic macroinvertebrates and zooplankton, and these fish tissue guidelines may not be protective of all invertebrate taxa inhabiting an aquatic system. Further, there is a need for organism-based tissue Se guidelines for the protection of aquatic life in fishless waters.

Our understanding of how excess Se impacts non-vertebrate organisms in aquatic systems remains primitive relative to that of fish and birds. Invertebrate Se toxicity studies to date have been mostly laboratory-based and largely focused on the toxicity of waterborne Se to some representative invertebrate taxa such as Chironomidae and *Daphnia magna*. These studies have concluded that waterborne Se toxicity to invertebrates occurs in the mg/L range (Ingersoll 1990; Maier and Knight 1993). However, direct uptake of waterborne Se does not likely represent the

most realistic route of exposure for consumers, and more recently, experiments have been conducted that incorporate multiple trophic levels to increase the realistic nature of Se exposures. Benthic macroinvertebrate (BMI) emergence and larvae abundance decreased following 42 d of exposure to 10 µg Se/L in 714-L mesocosms (Henry and Wesner 2018). In 3000-L limnocorrals spiked with an average of 8.9 µg Se/L, BMI diversity, biomass, and density decreased relative to controls after a 77-d experimental period (Graves et al. 2019b). In addition, deBruyn and Chapman (2007) demonstrated that internal Se effect concentrations (ECs) for invertebrates ranged from 1.0 to 60 µg/g dm and lethal concentrations (LC) range from 8.3 to 125 µg/g dm. These studies provide some evidence that invertebrates can be adversely affected at more environmentally relevant levels of Se, but there are many knowledge gaps regarding the ecosystem-level impacts of elevated Se levels.

The goal of this study was to determine the aquatic food web response to excess Se, added as selenite, in a boreal lake ecosystem using in-lake enclosures (limnocorrals). Selenium was added to limnocorrals to represent a gradient of concentrations from background (0.08 – 0.09 µg/L) to 7.9 µg/L Se. The objectives of this research were to 1) determine if there were changes in community composition, density and biomass of benthic macroinvertebrates and crustaceous zooplankton due to Se exposure, 2) determine if there were changes in primary production as a result of Se exposure, and 3) determine if fish health was adversely affected by Se exposure.

5.3 Materials and Methods

5.3.1 Study site and experimental design

This experiment was conducted at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) in Lake 239 (coordinates: 49.6592, -93.7138). The experimental design and set-up are described in detail in Graves et al. (2020). Briefly, in May 2018 nine limnocorrals (circular enclosures, ~3000 L) were placed in a shallow bay of Lake 239. Selenium was added (as sodium selenite; Na_2SeO_3) to six limnocorrals to obtain nominal concentrations of 0.5, 1.0, 2.0, 4.0, 7.0 and 10 µg/L. The procedure for addition of Se is described in detail in Graves et al. (2019a). The experiment took place from June 20 to August 22 (63 d) and mean measured concentrations in each limnocorral were 0.08, 0.08, 0.09, 0.4, 0.8, 1.6, 3.4, 5.6, and 7.9 µg/L. Enclosures are referred to by mean measured concentration throughout the Results and Discussion.

On day 21 of the experiment, 50 female finescale dace (*Phoxinus neogaeus*) were collected from one of the lake's inflow streams (coordinates: 49.6609, -93.7133), measured, and five fish were added to each limnocorral, so that fish were exposed for a total of 42 d. An additional five fish were collected from the east inflow to Lake 239 to determine baseline muscle and ovary tissue Se. Finescale dace were used to represent a third trophic level consumer, and they were chosen because they are a small-bodied fish present in the lake that could be supported by the biomass in the ~3000L enclosures. To increase the similarity of plankton samples in the limnocorrals, zooplankton amendments were made to the limnocorrals as described in Graves et al. (2020).

5.3.2 Sample collection

The sampling events and methods for the collection of water (for total Se and water chemistry analyses), periphyton, phytoplankton, benthic invertebrates, zooplankton and fish (for total Se analyses) are described in Graves et al. (2020). Water chemistry analyses included weekly measurements of temperature and dissolved oxygen (DO), and bi-weekly measurements of pH, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP). In addition to collecting biota throughout the experimental period for total Se (TSe) analysis, water and biota were also collected and processed as described herein. A depth-integrating sampler constructed in-house that incorporated the top 0.75 m of the water column was used to collect 1 L surface water samples for water chemistry and phytoplankton chlorophyll *a* (chl *a*) analysis. In the field laboratory, 200 mL of the water sample was filtered through 0.45 µm GF/C filter, placed in a plastic petri dish, wrapped in foil, and stored at -20°C until further processing. Periphyton was scraped from a known area of clay tiles into a pre-cleaned falcon tube and topped up with reverse osmosis water to 50 mL. Subsamples (10 mL) of the collected periphyton slurry were taken to measure chl *a* following methods described in Graves et al. (2019b). Quantitative benthic macroinvertebrate samples were collected by removing Hester-Dendy samplers from each enclosure on day -2, 21, 35, 49 and 63. Hester-Dendy samplers were disassembled and gently rubbed to dislodge attached periphyton and invertebrates. The slurry was collected in 1 L mason jars and transferred to the field laboratory. Quantitative zooplankton samples were collected in duplicate from each limnocorral on day -2, 7, 21, 49, and 63 using a 10-L Schindler-Patalas trap. Zooplankton were filtered through 53 µm mesh into 100 mL containers and transported to the field laboratory on ice. Immediately following collection, samples were preserved in 5% sugar buffered formalin. Fish were re-captured from each enclosure using baited

"gee" type minnow traps suspended in the water column. One fish was collected from each enclosure on day 35 and 49, and the remaining three fish were collected on day 63. Fish were euthanized and transported back to the field laboratory on ice.

5.3.3 Sample processing and analyses

Periphyton and phytoplankton chl *a* were measured using a Trilogy Fluorometer (Turner Designs, San Jose, California) using the method described in Graves et al. (2019b). Benthic macroinvertebrates were picked from the collected slurry, identified to Family using the identification manual by Merritt and Cummins (1984), counted, lyophilized and weighed dry. Crustaceous zooplankton were identified to species where possible using the identification manuals by Witty (2004) and Balcer et al. (1984). A zooplankton counting chamber was used to enumerate and identify zooplankton taxa. Copepod adults were identified to species, while juveniles (copepodids and nauplii) were counted but not identified to species. For adult Copepods, females and males were identified when possible and the presence of egg sacs was recorded to determine the proportion of reproductively mature females. Biomass of each zooplankton taxon was estimated using average biomass values obtained from IISD-ELA (unpublished data). As a univariate measure of invertebrate community composition, Shannon's diversity index was calculated using equation 5.1 (Shannon and Weaver 1949).

Shannon's diversity index (H) = $-\Sigma p_i \ln p_i$ (5.1)

where p_i is the proportion of individuals found in taxa *i.*

In the field laboratory, fish were weighed, fork length measured, and ovary, liver and muscle tissues were excised. Five female finescale dace (collected from the east inflow) were sacrificed on day 21 to obtain baseline muscle and ovary TSe concentrations as well as measurements of Fulton's condition factor (K), liversomatic index (LSI) and gonadosomatic index (GSI; equations 5.2, 5.3, and 5.4). All fish used in this study were approved by the Animal Research Ethics Board at the University of Saskatchewan (protocol # 20170046) and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

$K = 100 \times (\text{body mass (g)}/\text{fork length (mm)}^3)$ (5.2)

$LSI = 100 \times (\text{liver mass (g)}/\text{body mass(g)})$ (5.3)

$GSI = 100 \times (\text{ovary mass (g)}/\text{body mass(g)})$ (5.4)

5.3.4 Statistical analyses

All statistical analyses were conducted using the RStudio software environment and associated base packages (RStudio Team, 2015) or the *vegan* package (Oksanen et al. 2019). Alpha was set at 0.05 for all statistical tests. Principal response curves (PRC) were used to determine if there were differences in community composition of crustaceous zooplankton or benthic macroinvertebrates in the Se-treated limnocorrals relative to the controls over time (Van den Brink and Ter Braak 1999). All count data were ln(2x+1) transformed prior to analyses (Van den Brink and Ter Braak 1999). In these analyses, the canonical coefficient represents the treatment + treatment:time interaction. Species weights >0.5 were considered to have a high affinity for the principal response curve. To determine the statistical significance of Se treatment in PRC analyses, permutation tests were conducted to determine the period of influence of treatment. Dunnett contrasts between each Se treatment and the controls were conducted for day 63 to determine which level of Se was significantly different from controls.

Principal component analysis (PCA) was used to determine the variance in invertebrate community composition explained by environmental variables at three main time points (baseline; day -2), before fish additions (day 21), and the end of the experiment (day 63). The log-transformed variables Se, DO, DOC, chl *a*, pH, TDN, TDP and fish resource use (day 63 only; calculated in Graves et al. 2020) were used as predictors of community composition.

To visualize the changes in density of the zooplankton taxa that responded most strongly to Se (*Bosmina longirostris*, *Diaphanosoma birgei*, *Tropocyclops extensus*, *Mesocyclops edax*, cyclopoid copepodites, and cyclopoid nauplii), the density of each of these taxa was plotted over time for each limnocorral. To determine the response of the main taxonomic groups at each experimental time point, linear regression analyses were used to determine the relationship between the log-transformed biomass of Cladocera, adult Copepoda, copepod nauplii or copepodids and log aqueous Se concentration within each time point.

For BMIs, cumulative summed biomass of all taxa, as well as cumulative biomass of single taxa that were most abundant throughout the experimental period (Chironomidae and Heptageniidae), was calculated to determine if there were differences in slopes of the relationship between biomass of BMIs and Se treatment over time. Linear mixed models (LMM) were used to determine if there was an interaction between time and treatment, with the term "enclosure" included as a random factor to account for the repeated sampling of each limnocorral over time.

The response of BMI biomass was also assessed at each time point using linear regression analysis to determine the relationship between log transformed BMI biomass and log aqueous Se concentration.

Linear regression analysis was used to determine the relationship between both periphyton and phytoplankton chl *a* and log aqueous Se concentration at each time point, and the relationships between BMI or zooplankton biomass and periphyton or phytoplankton chl *a* within each time point. The relationships between K, LSI, and GSI and fish tissue Se concentrations on day 63 of the experimental period were also assessed using linear regression analysis.

5.4 Results

5.4.1 Zooplankton community composition, density, and biomass

Zooplankton species composition was similar among the limnocorrals prior to Se additions, with 11-16 species identified within each limnocorral on day -2. Zooplankton taxa collected at the beginning and end of the study in control limnocorrals are listed in Table 5.1. Densities of the zooplankton taxa collected were generally greatest on day -2 and decreased throughout the experimental period (Table 5.1).

While species composition between limnocorrals was similar, there were some differences in total abundance of individual species in the baseline samples that influenced the starting PRC values seen in Figure 5.1. Most notably, *Bosmina longirostris* density was much greater in the 5.6 µg/L treatment (444 individuals/L) and the 0.09 µg/L control (33 individuals/L) limnocorrals on day -2, relative to the other limnocorrals where *Bosmina* density ranged from 0.35 to 6.1 individuals/L. Despite some differences in the starting densities of certain taxa, the differences in community composition of baseline zooplankton samples were not statistically significant (PRC, $p>0.103$).

Treatment explained 33% of the variance in zooplankton community composition among limnocorrals over time, and time explained 44% of the variance in community composition (PRC, Figure 5.1). The first axis of the PRC, shown in Figure 5.1, explained 39% of the total treatment variance and the second axis explained 23% of the variance. Selenium treatment had a significant influence on zooplankton community composition on days 21, 49, and 63 ($p=0.001$ to 0.005). At the end of the experimental period, community composition in limnocorrals with Se concentrations of 0.8 to 7.9 µg/L were significantly different from the controls ($p<0.019$), and only the 0.4 µg/L treatment was not significantly different from controls ($p=0.089$).

Table 5.1: Average density (individuals/L) of zooplankton taxa from three control limnocorrals on days -2 and 63 of the experimental period.

Species Name	Average density day -2	Average density day 63
Acroperus harpae	0.03	0.18
Bosmina longirostris	14.1	9.28
Chydorus sp.	0.06	0.61
Copepod nauplii	5.60	4.90
Copepodid	2.30	2.35
Diacyclops bicuspidatus	0.13	0.08
Diaphanosoma birgei	3.10	3.11
Daphnia galeata mendotae	0.11	0
Diaptomus minutus	0.98	0
Epischura lacustris	0.08	0
Holopedium gibberum	0.81	0
Mesocyclops edax	3.92	0.41
Ophryoxus gracilis	0	0.01
Polyphemus pediculus	4.41	0.01
Sida crystallina	3.72	0
Scapheloberis kingii	0.02	0
Tropocyclops extensus	11.4	3.24

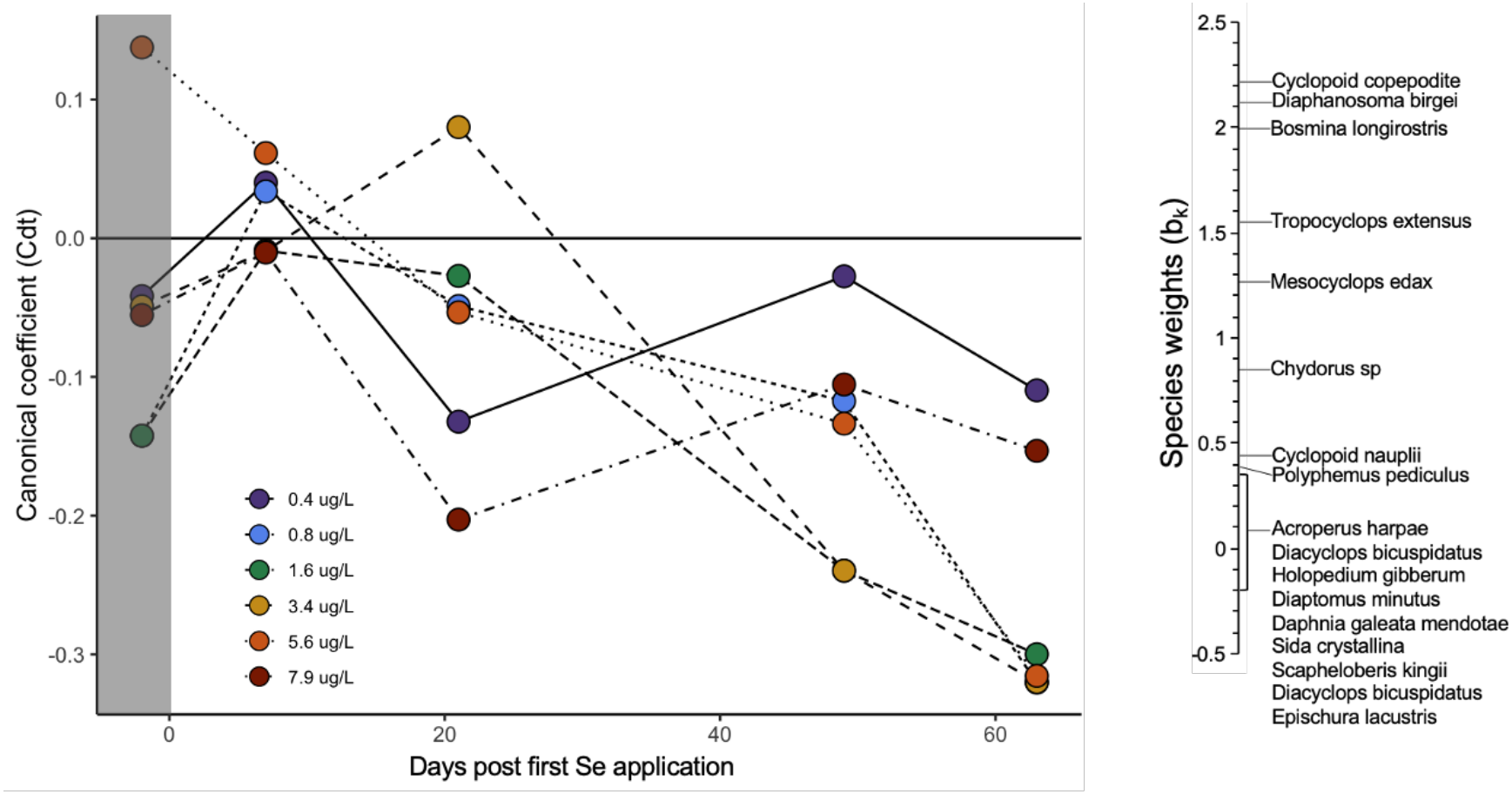

Figure 5.1: Principal response curves indicating the effect of selenium additions on zooplankton communities over a 63-day experimental period. Treatment effects (canonical coefficients) are compared to the control communities (set to 0). Greater species weights indicate a greater response by that taxa. Grey shading indicates the time before Se additions.

Based on the species weights calculated from PRC analysis, taxonomic groups that showed the greatest response to Se treatment were cyclopoid copepodids, *Bosmina longirostris*, *Diaphanosoma birgei*, *Mesocyclops edax*, *Tropocyclops extensus*, and *Chydorus* sp (Figure 5.1). Clustering of species based on their species scores from the first two axes of PRC (Figure 5.2) indicated that *Bosmina* and *Diaphanosoma* (cladocerans) showed similar responses to Se additions, while *Mesocyclops* and *Tropocyclops* (cyclopoids) also showed similar responses to Se treatment (Figure 5.2). *Bosmina* and *Diaphanosoma* showed clear sensitivity to Se treatments, demonstrated by positive species weights >0.5 on both the first and second PRC axes, while the response of cyclopoids was less clear, showing positive species weights on the first axis but negative weights on the second axis (Figure 5.2).

Principal component analysis at three main time points identified environmental variables that influenced zooplankton community composition. On day -2, PCA axis scores were correlated with both DOC and chl *a* (PCA, p=0.041 to 0.49, R^2=0.60, Figure 5.3A) and helped explain some of the differences in baseline zooplankton communities (visualized in Figure 5.1). For instance, the 5.6 µg/L and 0.09 µg/L treatment groups had greater chl *a* and greater amounts of *Bosmina longirostris* than all other treatments. On days 21 and 63, Se was correlated with zooplankton axis scores (PCA, p=0.02 to 0.046, R^2=0.56 to 0.73, Figure 5.3B,C), indicating a relationship between Se treatment and differences in zooplankton community composition among experimental groups at both time points. Shannon's diversity index, a univariate measure of community composition and diversity, also decreased with increasing aqueous Se concentration on days 49 and 63 (LR, p=0.017 to 0.023, R^2=0.48 to 0.52, Figure 5.4).

The density of the most abundant cladocerans (*Bosmina longirostris* and *Diaphanosoma birgei*) was more affected by Se exposure over time than Copepod density (Figure 5.5). The total biomass of cladocerans decreased significantly with increasing aqueous Se concentration on days 49 and 63 of the experimental period (LR, p=0.001 to 0.006, R^2=0.63 to 0.80, Figure 5.6). No relationship between biomass and aqueous Se concentration at any of the time points were observed for adult copepods, copepodids, or nauplii (LR, p=0.059 to 0.892, Figure 5.6). However, copepod nauplii density was greatest in the 7.9 µg/L Se treatment at the end of the experimental period (Figure 5.6). Although the total number of nauplii and copepodids did not differ between treatments, the proportion of copepods in the nauplii stage increased with increasing aqueous Se

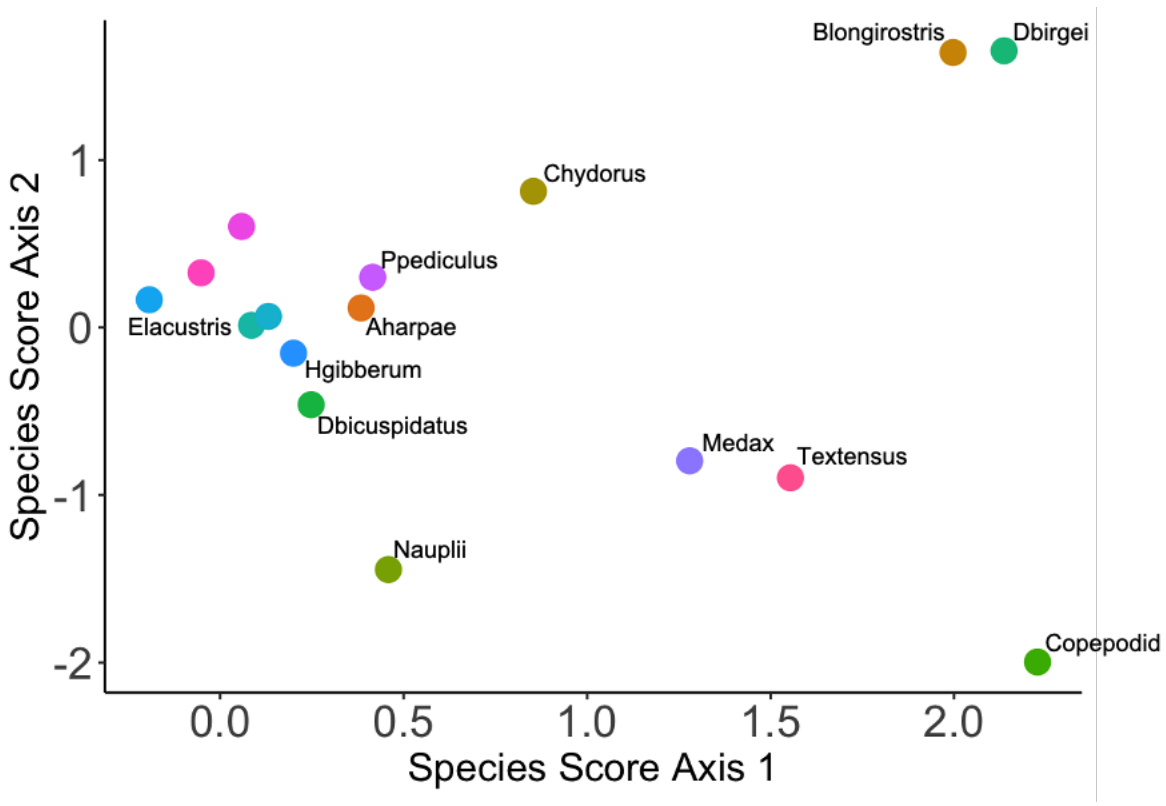

Figure 5.2: Zooplankton species scores for the first two axes from principal response curves (Figure 5.1) of community composition over time in limnocorrals with aqueous Se concentrations ranging from 0.08 to 7.9 µg/L. Only taxa with weights >0.1 are labelled. Greater species weights indicate a greater response of the species to the Se treatment. Positive weights indicate a decrease and negative weights indicate an increase in abundance.

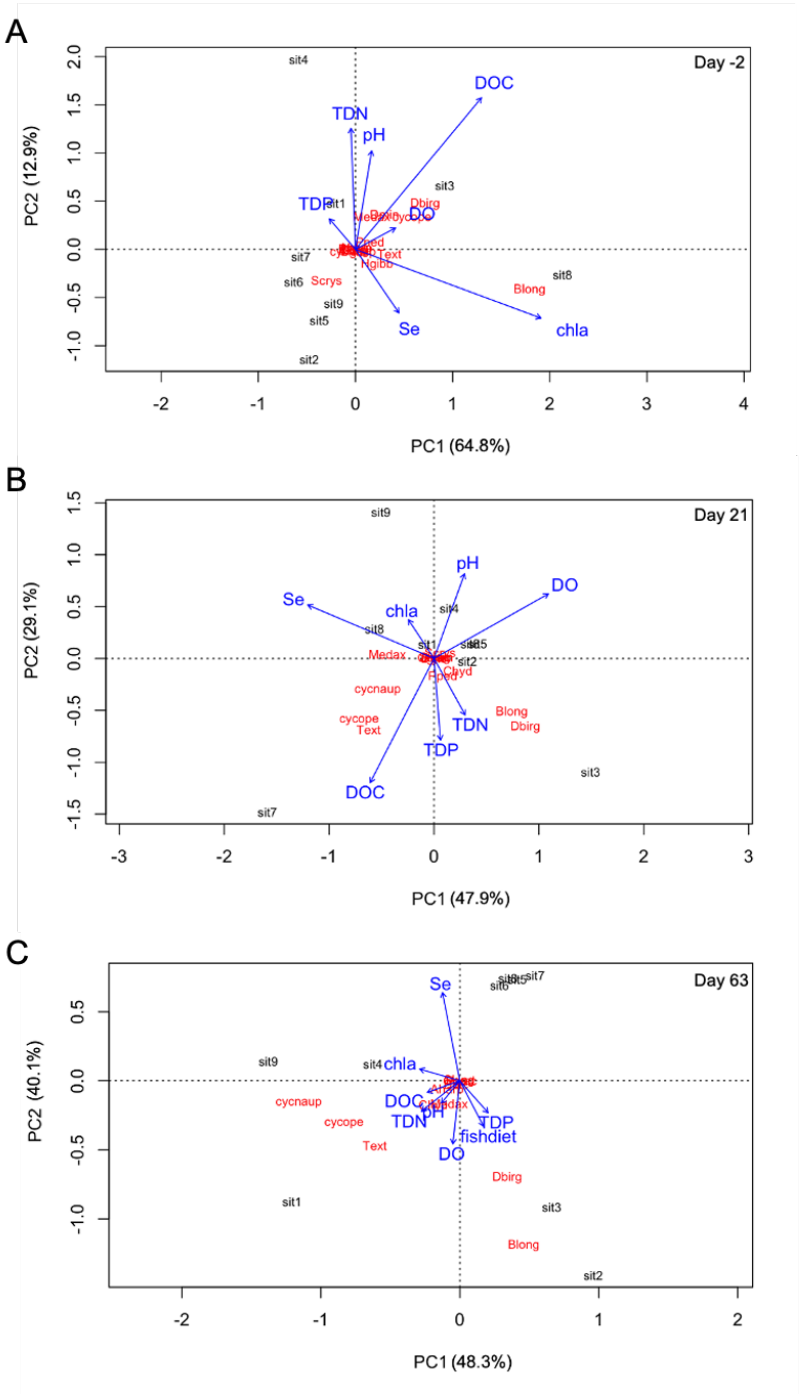

Figure 5.3: Triplots of zooplankton community composition on days -2 (A), 21 (B), and 63 (C) of the experimental period. Limnocorrals are labelled sit1 – sit9 in order of increasing Se concentration from 0.08 to 7.9 µg/L Se, and environmental variables are shown in blue text with arrows indicating the strength of the predictor variable. Zooplankton species are shown in red text. Species names that overlap show similar responses to treatment. Zooplankton species codes are listed in Table A.5.1.

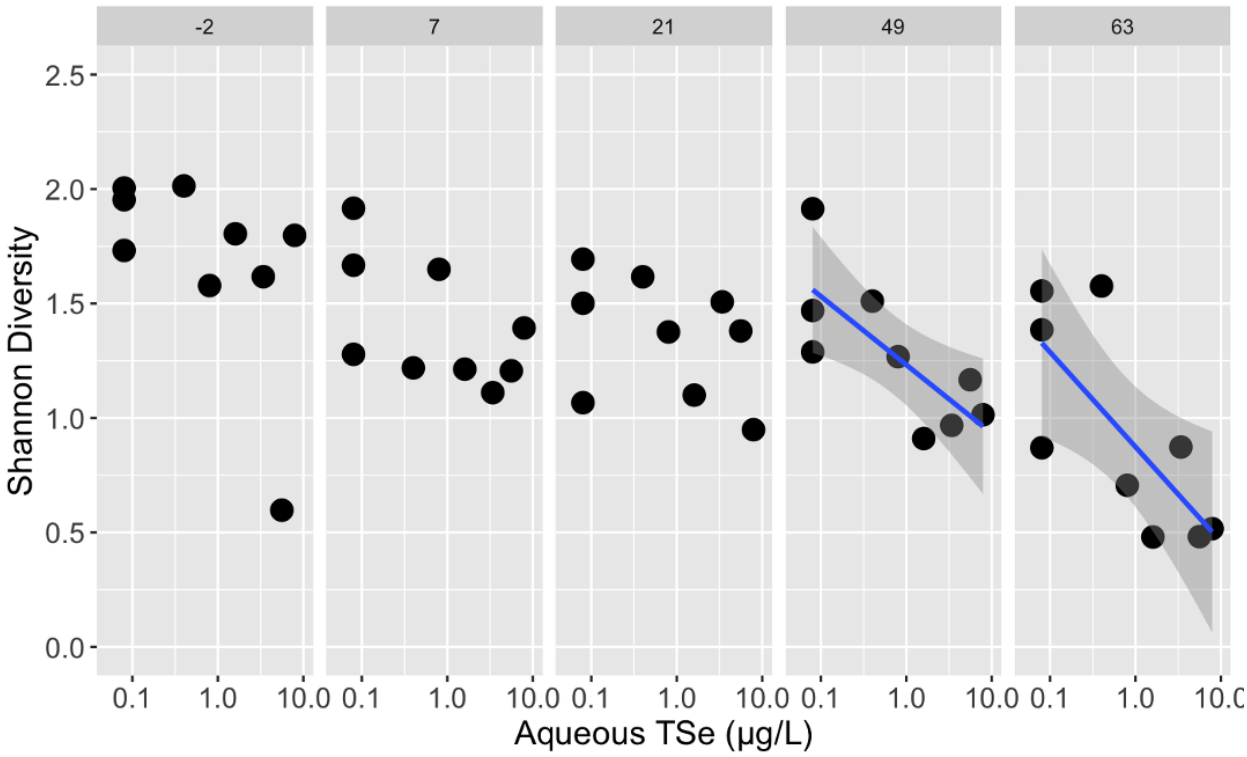

Figure 5.4: Linear regression analysis of Shannon diversity index for zooplankton communities and log aqueous Se concentration in each limnocorral at each time point (day -2, 7, 21, 49, and 63) during the experimental period. Blue lines indicate statistically significant relationships ($p<0.05$) and shaded areas indicate 95% confidence intervals of the lines.

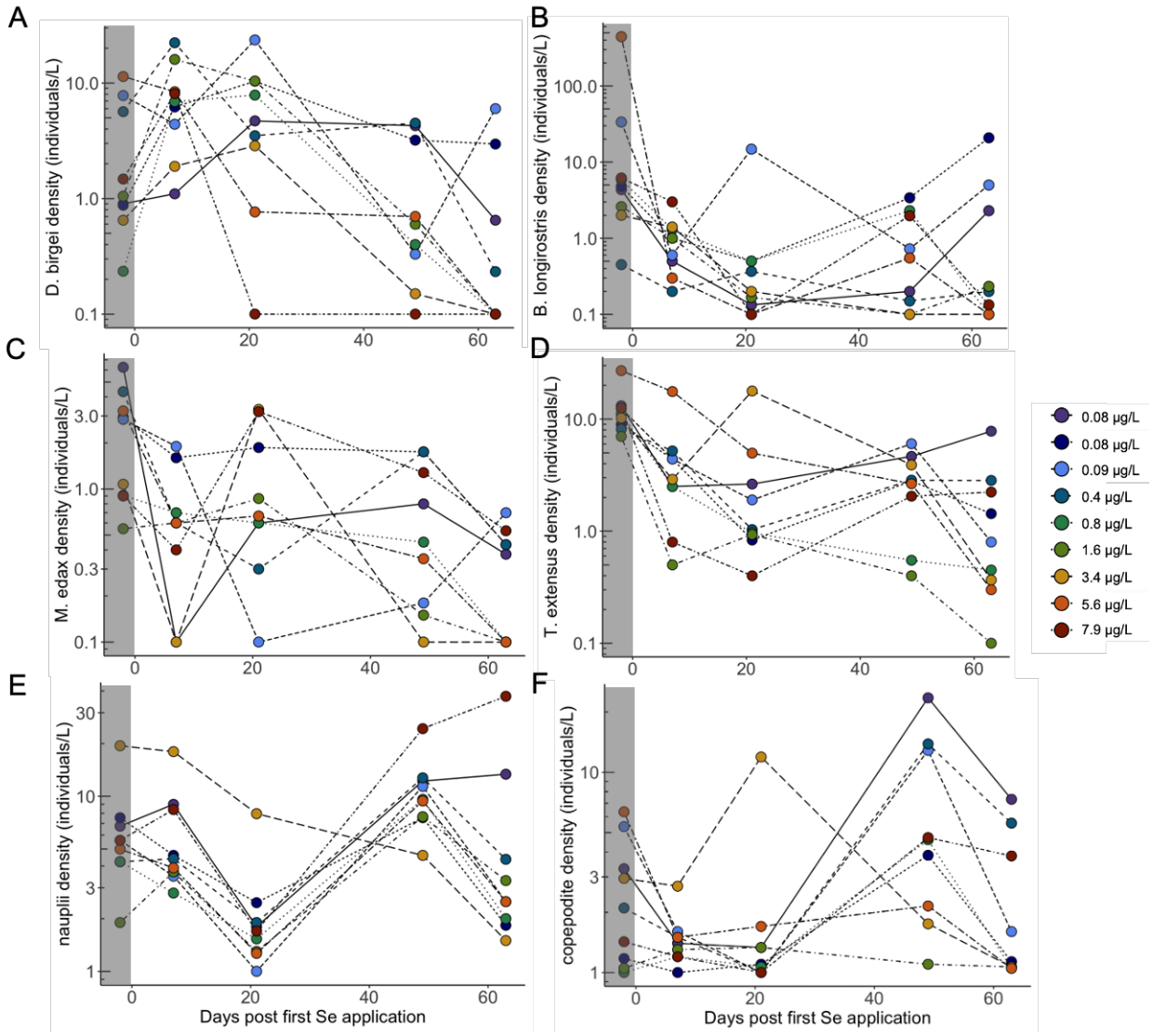

Figure 5.5: Density of *Diaphanosoma birgei* (A), *Bosmina longirostris* (B), *Mesocyclops edax* (C), *Tropocyclops extensus* (D), copepod nauplii (E), and copepodids over time during exposure to aqueous Se concentrations ranging from 0.08 to 7.9 µg/L. All data are presented on logarithmic scales. Grey shading indicates the time before Se additions.

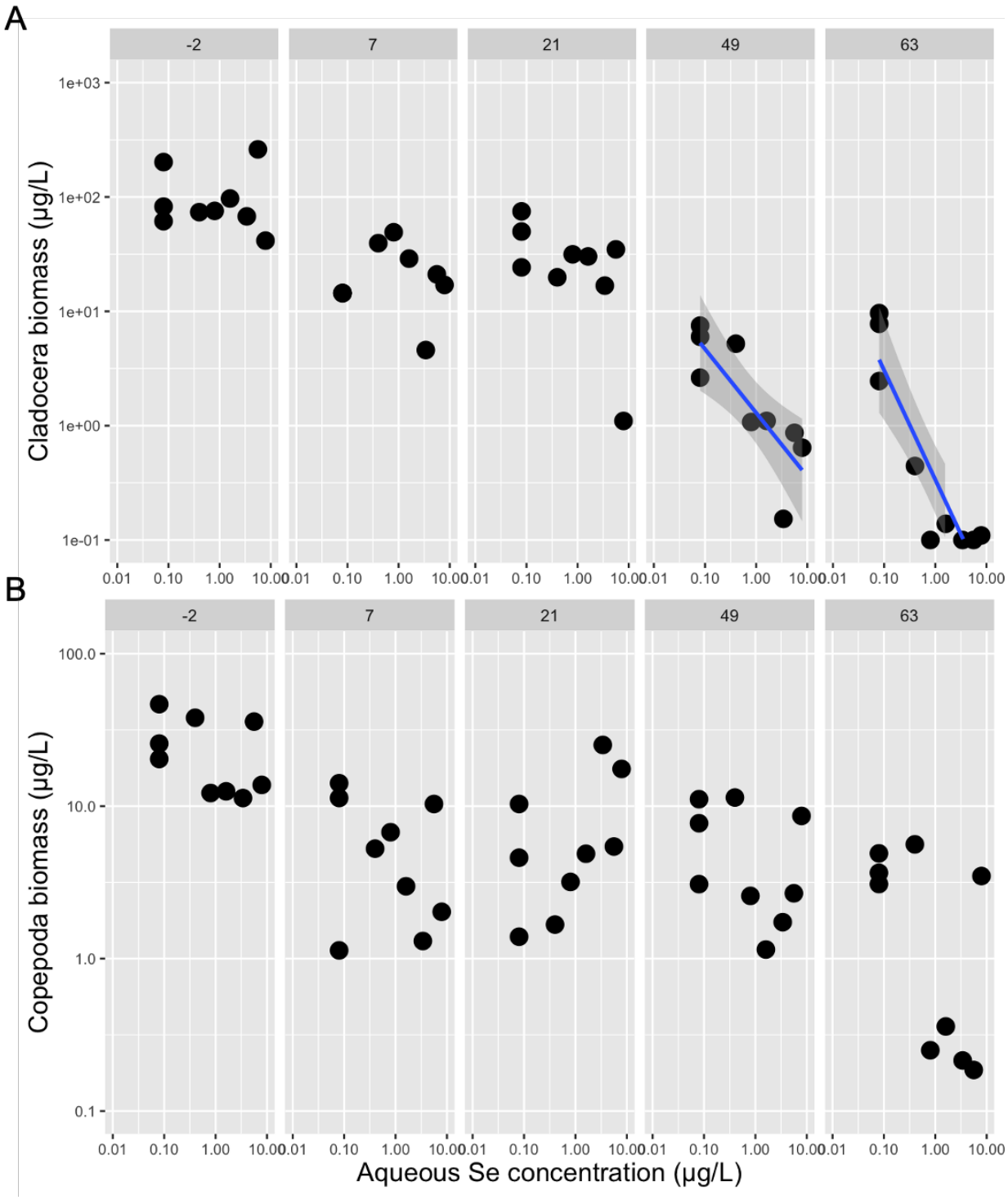

Figure 5.6: Linear regression analysis of log transformed Cladocera (A) and Copepoda (B) biomass and log aqueous Se concentration in each limnocorral at each time point during the experimental period (days -2, 7, 21, 49, 63). Blue lines indicate statistically significant relationships ($p<0.05$) and shaded areas indicate 95% confidence intervals of the lines.

concentration on d 63 (LR, p=0.003, R^2=0.69), but no changes in the proportion of copepodites was observed (LR, p=0.694). The number of *Mesocyclops edax* and *Tropocyclops extensus* females with egg sacs also did not change with increasing Se at any of the experimental time points (LR, p=0.057 to 0.622).

5.4.2 Benthic macroinvertebrates community composition, density, and biomass

Benthic macroinvertebrate taxa collected throughout the experimental period were Aeshnidae, Caenidae, Ceratopogoniidae, Chironomidae, Corduliidae, Dytiscidae, Gammaridae, Heptageniidae, Leeches, Hydrachnidae, Notonectidae, Perlidae, Phryganeidae, Planorbidae, Polycentropodidae, and Caenogrionidae. Most invertebrates were present in low abundance, and Chironomidae and Heptageniidae were the most commonly collected BMIs throughout the experimental period. The BMI communities in limnocorrals were similar both in taxonomic composition and in density prior to Se additions (Figure 5.7).

Selenium treatment explained 37% of the variance in benthic macroinvertebrate community composition over time, while time explained 52% of the variance. The first PRC axis explained 35% of the variance in treatment, while the second axis explained 21%. Selenium treatment did not have a significant influence on community composition scores on days -2, 21, and 35 (p=0.131 to 0.636, Figure 5.7), but treatment did have a significant effect on days 49 and 63 (p=0.024 to 0.048, Figure 5.7). At the end of the experimental period, the BMI communities in the 5.6 and 7.9 µg/L limnocorrals were significantly different from the controls (p=0.016 to 0.020, Figure 5.7). Only Heptageniidae and Gammaridae had weights >0.5 on the first axis. Based on the clustering of species scores for the first two PRC axes (Figure 5.8), four taxa responded to Se treatment, and the changes in community composition between treatments were likely due to the responses of the two most abundant taxa collected throughout the experiment (Heptageniidae and Chironomidae).

On day -2, none of the measured environmental variables were correlated with the axis scores for BMI community composition in the limnocorrals (PCA, Figure 5.9A). On day 21, DO explained some of the variance in BMI community composition among the limnocorrals (PCA, p=0.025, Figure 5.9B) and on day 63, both Se and chl *a* were correlated with BMI community composition axis scores (PCA, p=0.003 to 0.009, Figure 5.9C).

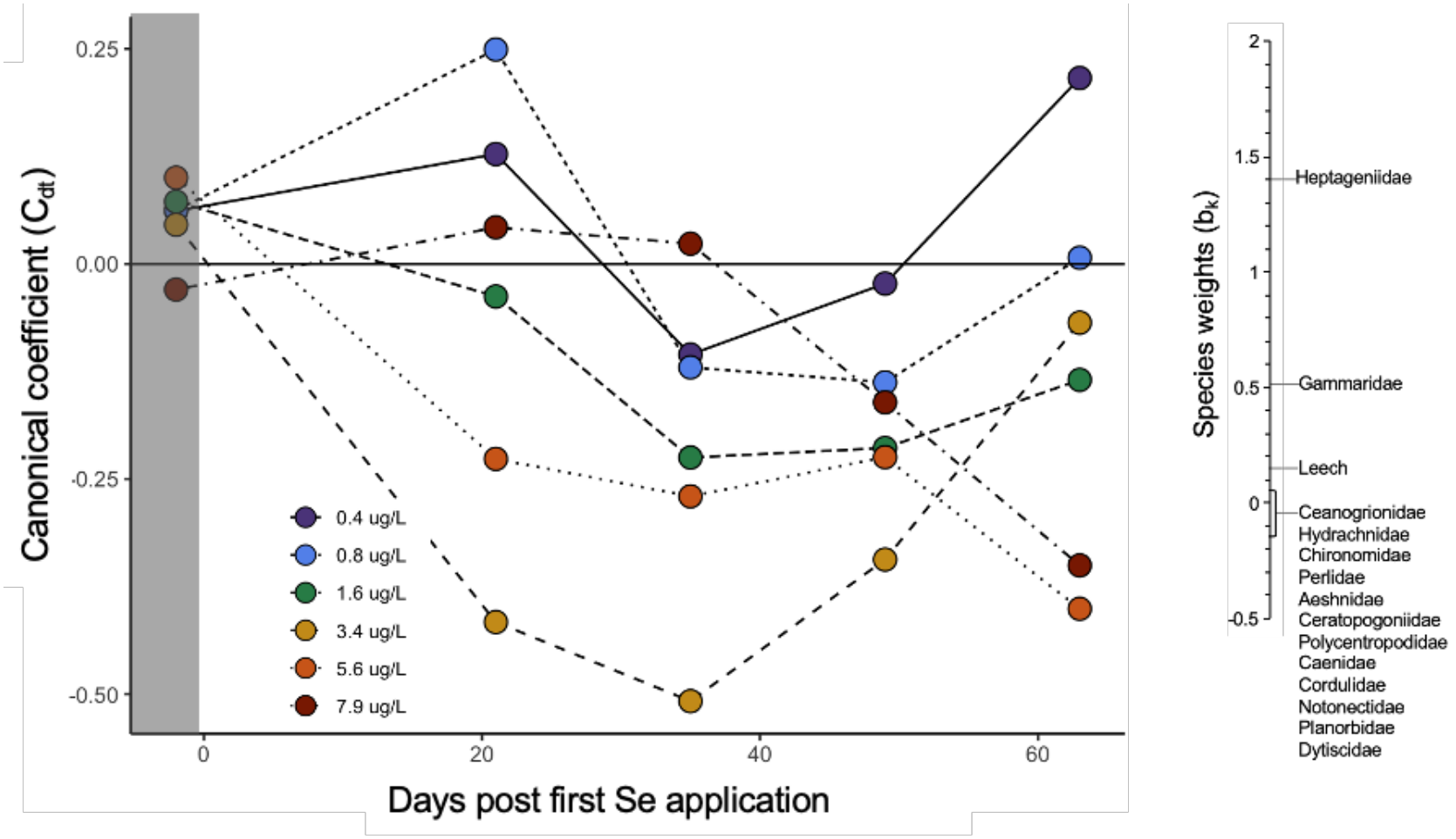

Figure 5.7: Principal response curves indicating the effect of selenium additions on benthic macroinvertebrate communities over a 63-day experimental period. Treatment effects (canonical coefficients) are compared to the control communities (set to 0). Greater species weights indicate a greater response by that taxa. Grey shading indicates the time before Se additions.

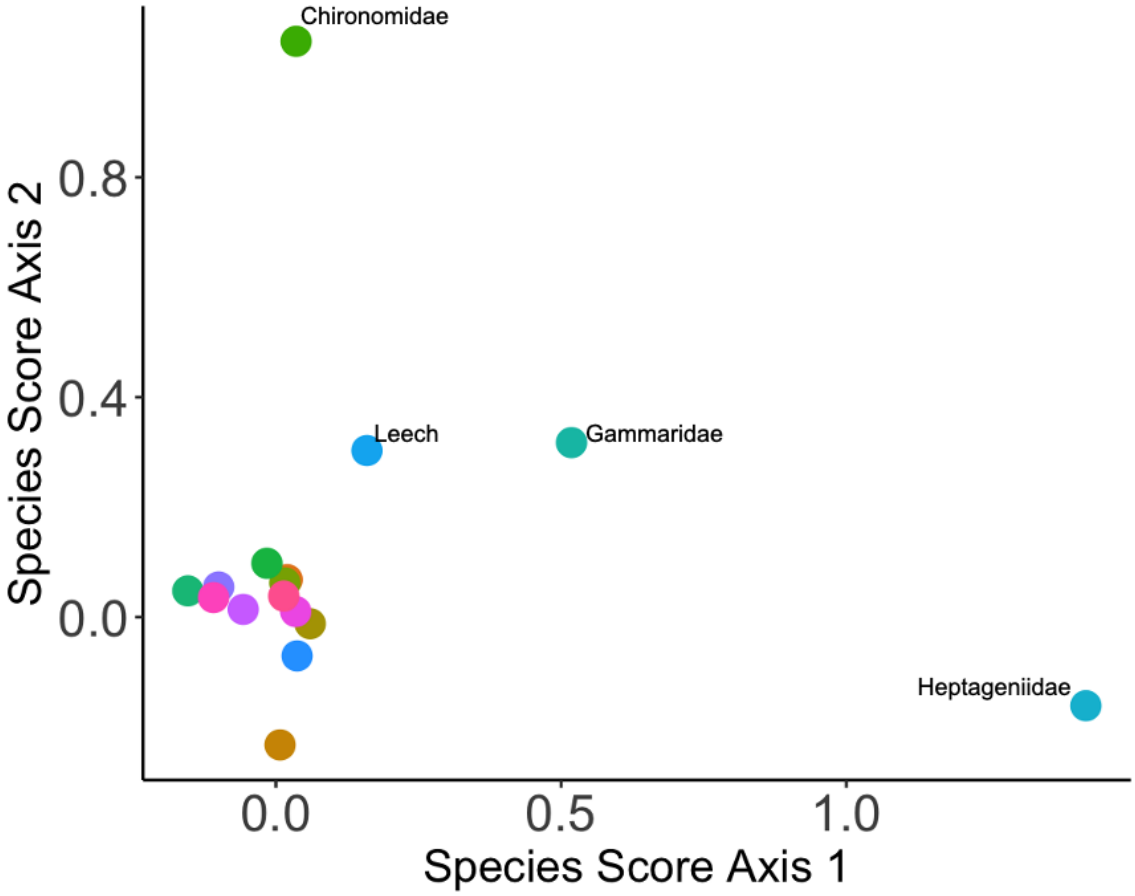

Figure 5.8: Benthic macroinvertebrate species scores for the first two axes from principal response curves (Figure 5.7) of community composition over time in limnocorrals with aqueous Se concentrations ranging from 0.08 to 7.9 µg/L. Only taxa with weights >0.1 are labelled. Greater species weights indicate a greater response of the species to the Se treatment. Positive weights indicate a decrease and negative weights indicate an increase in abundance.

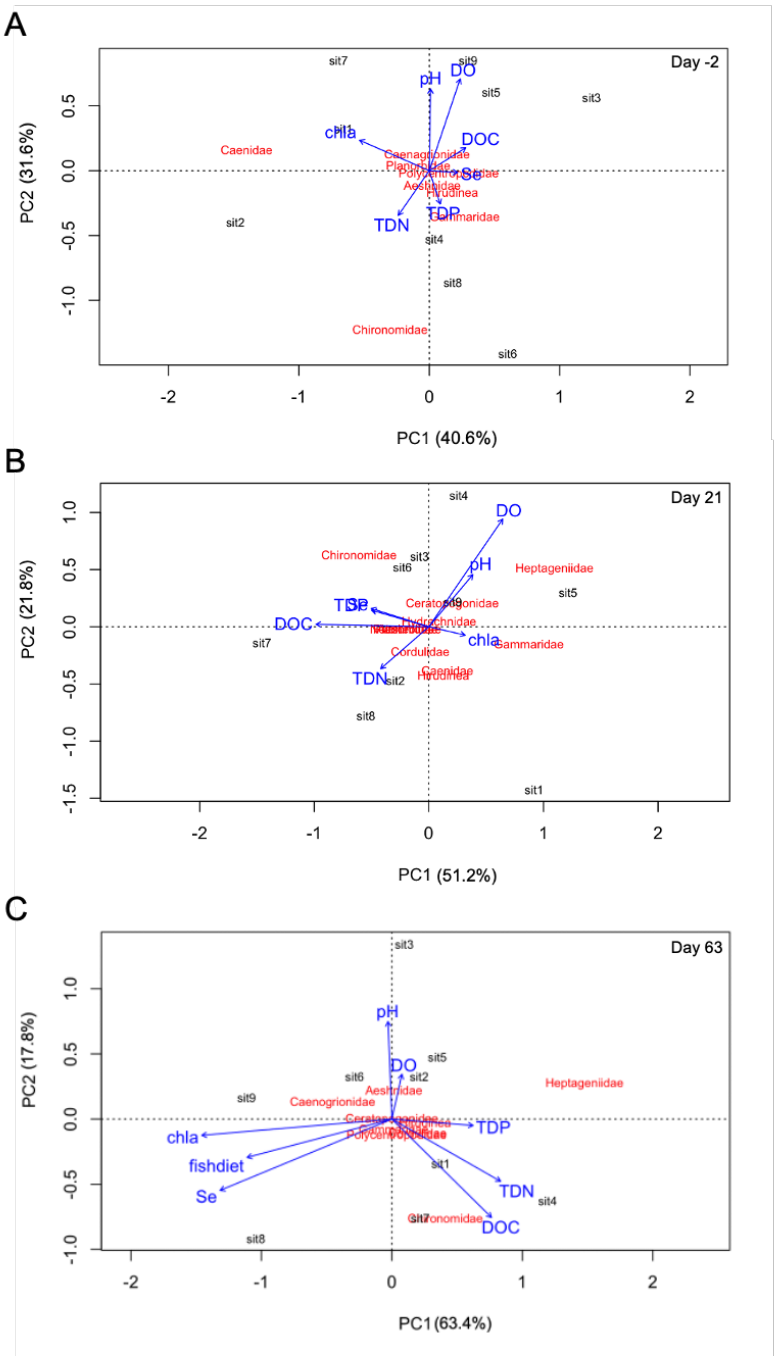

Figure 5.9: Triplots of benthic macroinvertebrate community composition on days -2 (A), 21 (B), and 63 (C) of the experimental period. Limnocorrals are labelled sit1 – sit9 in order of increasing Se concentration from 0.08 to 7.9 µg/L Se and environmental variables are shown in blue text with arrows indicating the strength of the predictor variable. Benthic macroinvertebrate taxa are shown in red text. Taxa names that overlap in the figure show similar responses to treatment. All taxa included in analysis are listed in Table A.5.2.

Shannon diversity index for BMIs was not correlated with aqueous Se concentration on days -2, 21, 49 or 63 (LR, p>0.128), but was significantly and negatively related to aqueous Se on day 35 (LR, p=0.003, R^2=0.71).

Cumulative BMI biomass decreased with increasing aqueous Se throughout the experimental period (LMM, p=0.019). This decrease was driven by the individual response of Heptageniidae biomass, which was negatively related to aqueous Se concentration over time (LMM, p=0.005, Figure 5.10). In contrast, no effects of Se exposure on cumulative Chironomidae biomass over time were detected (LMM, p=0.170, Figure 5.10). Within individual time points, BMI biomass and density decreased with increasing aqueous Se concentration on day 49 (LR, p=0.005 to 0.031, R^2=0.44 to 0.66, Figure 5.11). On day 63, invertebrate biomass and density tended to be lesser in the higher Se treatments, but the relationships with aqueous Se were not statistically significant (LR, p=0.073 to 0.096, Figure 5.11). However, both Heptageniidae and Chironomidae biomass were significantly and negatively related to aqueous Se at this time point (LR, p=0.011 to 0.040, R^2=0.40 to 0.57).

5.4.3 Primary production

Periphyton chl *a* was a significant predictor of benthic macroinvertebrate community composition on day 63 (PCA, p=0.003, Figure 5.9) and was significantly and positively related to aqueous Se concentration at that time point (LR, p=0.049, R^2=0.37, Figure 5.12). Benthic macroinvertebrate biomass was negatively related to periphyton chl *a* on day 63 (LR, p=0.016, R^2=0.53), but not on days -2, 21, 35, or 49 (LR, p= 0.167 to 0.938).

Phytoplankton chl *a* varied among limnocorrals on day -2, and was correlated with zooplankton community composition axis scores at that time point (Figure 5.3A). Phytoplankton chl *a* was also significantly and positively related to aqueous Se concentration on d 49 (LR, p=0.042, R^2=0.39, Figure 5.12). Although not statistically significant, phytoplankton chl *a* tended to be greater in the higher Se treatments than in the controls at the end of the experiment (LR, p=0.117, Figure 5.12). Phytoplankton chl *a* was related to total zooplankton biomass on d 21 (LR, p=0.009, R^2=0.60), but not on days -2,7, 49, or 63 (LR, p=0.287 to 0.955).

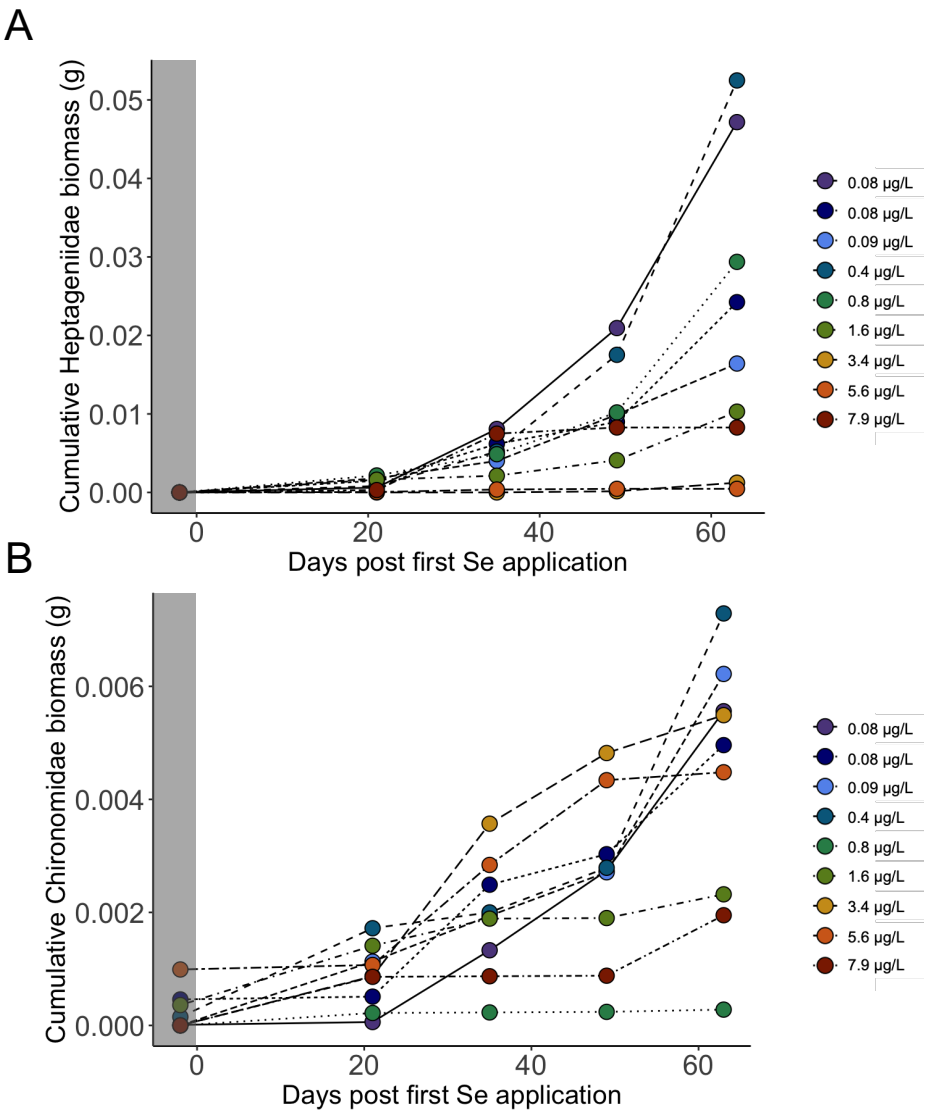

Figure 5.10: Cumulative Heptageniidae (A) and Chironomidae (B) biomass collected from Hester-Dendy samplers in limnocorrals with mean aqueous Se concentrations ranging from 0.08 to 7.9 µg/L during a 63-day experimental period. Grey shading indicates the time before Se additions.

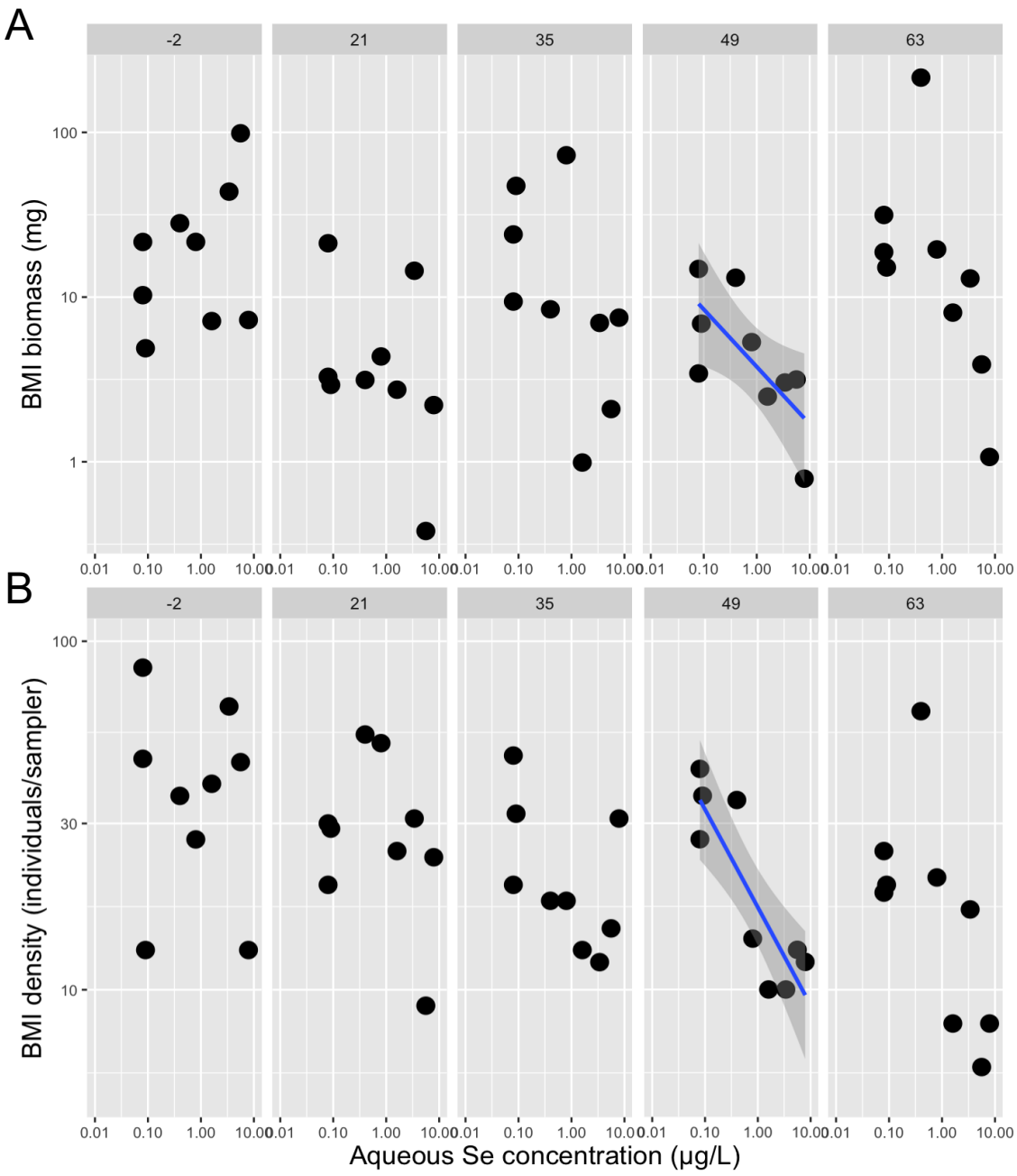

Figure 5.11: Linear regression analysis of log transformed benthic macroinvertebrate (BMI) biomass (A) and density (B) versus log aqueous Se concentrations on days -2, 21, 35, 49 and 63 of the experimental period. Blue lines indicate statistically significant relationships ($p<0.05$) and shaded areas indicate 95% confidence intervals of the lines.

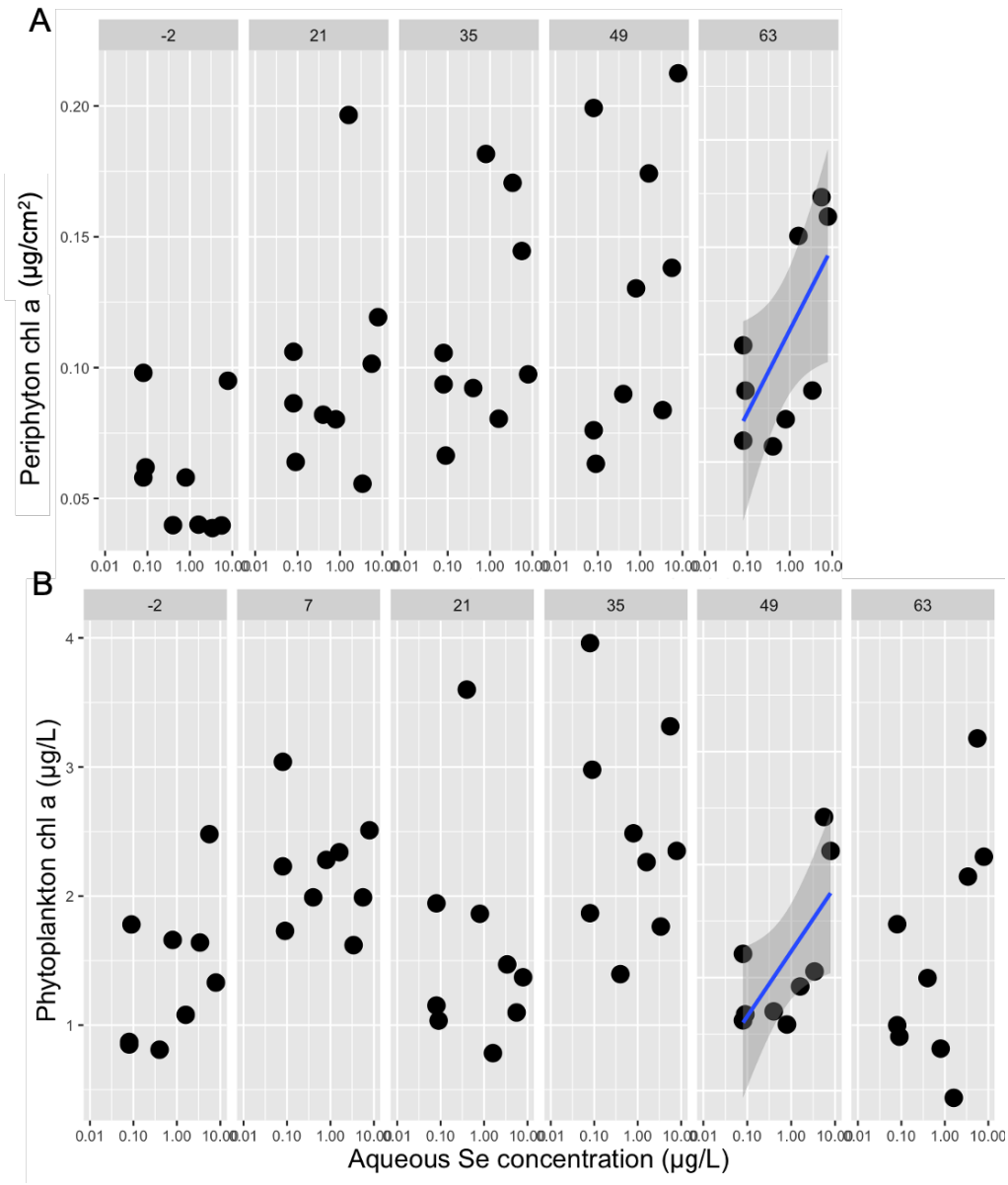

Figure 5.12: Linear regression analysis of periphyton (A) and phytoplankton (B) chlorophyll *a* versus log aqueous Se concentration on days -21, 7, 21, 35, 49, and 63 of the experimental period. Blue lines indicate statistically significant relationships ($p<0.05$) and shaded areas indicate 95% confidence intervals of the lines.

5.4.4 Fish health indicators

After 42 days of exposure to Se, finescale dace condition factor ranged from 0.78 to 1.01, GSI ranged from 0.48 to 2.33% and LSI ranged from 0.65 to 2.02% among Se treatments (Figure 5.13). None of these morphometric endpoints were significantly related to fish muscle Se (LR, p=0.507 to 0.859, Figure 5.13) or fish ovary Se (LR, p=0.685 to 0.971, Figure 5.13).

5.5 Discussion

The major findings of the present study were that adverse impacts on zooplankton and benthic macroinvertebrate community composition, biomass and density were related to Se additions ranging from 0.4 to 7.9 µg/L. For zooplankton, changes in community composition were detected at an aqueous Se concentrations of 0.8 µg/L, with cladocerans being the most affected group of taxa. Benthic macroinvertebrate community composition was affected at 5.6 µg Se/L, and the biomass and density of BMI decreased as a result of Se additions, with Heptageniidae being the most affected taxa.

Crustaceous zooplankton abundance decreased throughout the experimental period in all experimental groups, although densities of individuals at the end of the 63-day experiment were generally within the ranges observed for the lake at a similar sampling time (IISD-ELA, unpublished data). Taxonomic diversity (i.e. total number of species observed) generally decreased over time in all limnocorrals as well, likely due to some organisms being more tolerant of enclosures than others. In particular, in the present study, no Calanoida species were observed past day 7 of the experimental period and all copepods identified after this time point were Cyclopoids. This is likely due to Calanoids being almost entirely planktonic, while several Cyclopoid species are littoral (Wetzel 2001). Despite these observed enclosure effects on diversity and density of organisms, the community composition and diversity index of zooplankton, and the biomass of cladocerans, decreased with increasing Se treatment, suggesting effects of Se exposure on the survival of some zooplankton taxa. In contrast, copepod density and biomass were not different among Se treatments, suggesting that these two orders of crustaceous zooplankton differ in sensitivity or tolerance to Se.

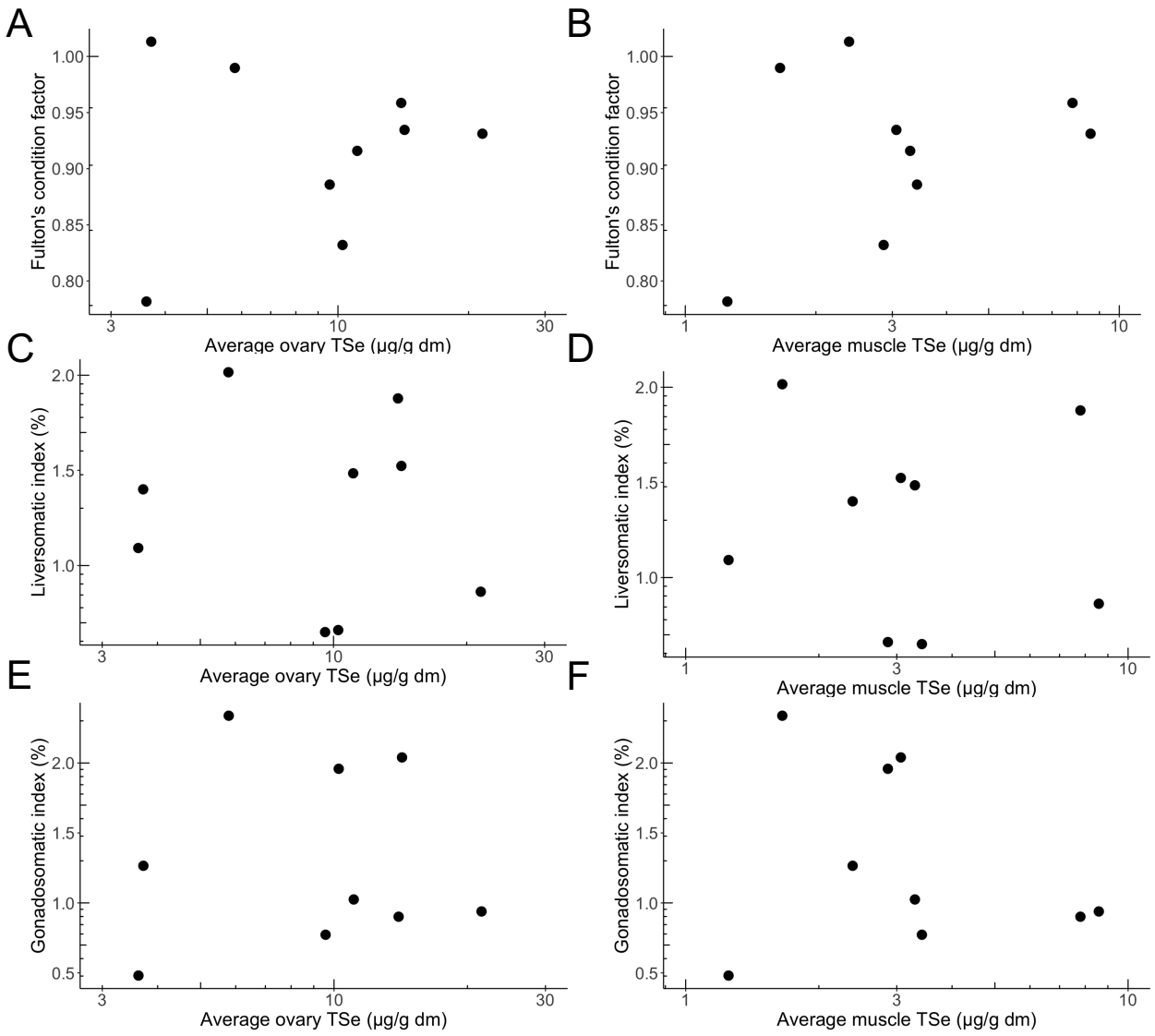

Figure 5.13: Linear regression analysis of the relationships between female finescale dace (*Phoxinus neogaeous*) morphometric endpoints and ovary or muscle TSe concentrations after 42 d of exposure in limnocorrals ranging in aqueous Se concentration from 0.08 to 7.9 µg/L.

The lowest aqueous Se concentration that significantly affected zooplankton community composition was 0.8 µg/L, when mean measured Se concentrations were 11 µg/g dry mass (dm) in bulk zooplankton samples (reported in Graves et al. 2020). This internal concentration is similar to a growth EC_{24} for *Daphnia magna* (15 µg/g dm) and lower than a reproductive EC_{50} measured for *Daphnia magna* (32 µg/g dm; Ingersoll et al. 1990). Differences in the methods of exposure and internal speciation of Se may help to explain the range in toxicity values among studies, since Ingersoll et al. (1990) conducted waterborne Se exposures at higher aqueous Se concentrations (in the mg/L range) with mixtures of selenite and selenate. In contrast, zooplankton in the present study were exposed to relatively lower aqueous Se concentrations and likely assimilated a greater proportion of organo-Se from their diet (Young et al. 2010).

While the toxicity of waterborne Se has been assessed in the laboratory using a representative zooplankton species (*Daphnia magna*), no studies for copepods or other cladoceran species could be found. Few field-based assessments of the potential impacts of Se on zooplankton exist, even though differences in effect concentrations between lab and field studies may be expected due to additional factors such as food availability/quality, predation, and the presence of other stressors. In Belews Lake, North Carolina, aqueous Se concentrations up to 10 µg Se/L were responsible for the elimination of most fish species (Cumbie and Van Horn 1978). Zooplankton diversity (measured as Shannon's diversity index) in the lake did not change following excess Se exposure, but the density of several cladoceran (and some copepod) species declined (Marcogliese et al. 1992). However, the authors attributed the changes in zooplankton community structure to alterations in fish community structure, rather than direct toxicity to invertebrates (Marcogliese et al. 1992). In the present study, effects of Se on zooplankton community composition were observed both before the addition of fish (at day 21) and when equal numbers of the same species of fish were added to each limnocorral. This suggests that the observed changes in zooplankton community composition in Se treated limnocorrals were not a result of fish predation. An enclosure study conducted in Clay Lake, Ontario, Canada with one-time additions of 1, 10, and 100 µg selenite/L observed very little accumulation of Se in invertebrates, and consequently, there was no evidence of acute or chronic effects of Se on zooplankton communities (Turner and Rudd 1983; Saiki et al. 1985). The range of results observed in the few field-based studies conducted to date indicate that there is a need for more environmentally relevant studies to assess the effects of Se

on zooplankton communities under realistic conditions (i.e. wild zooplankton exposed to primarily dietary Se).

The decrease in biomass and density of Cladocera suggests that perhaps species of this suborder are good indicators of Se pollution in lentic systems. With regards to mining-influenced areas, *Bosmina longirostris* declined in response to increases in several metals and metalloids (including Se) in Ross Lake, Manitoba, Canada, following the onset of mining and metallurgical activity (Doig et al. 2015). *Bosmina* densities also declined in response to uranium mining in northern Saskatchewan (Melville et al. 1995). The difference in response of cladocerans and copepods to Se may be related to physiological differences in the ability of organisms to excrete excess Se or to store the element in inert forms, as this does contribute to differences in sensitivity to other essential elements such as copper (Brix et al. 2001). Further, the specific mechanism of toxicity for Se in invertebrates is unknown, but these suborders do have distinctly different life history characteristics and reproductive strategies that may influence their susceptibility to Se toxicity. For instance, cladocerans are capable of asexual or sexual reproduction, while copepods reproduce sexually (Wetzel 2001). The lifespans of these two taxonomic groups can differ as well. *Bosmina* have a relatively short lifespan of up to 20 d, reaching sexual maturation at approximately 5 d (Hanazato and Yasuno 1987). For some *Mesocyclops* sp., the average lifespan is 60 d (Phong et al. 2008). In this case, we would have captured several cladoceran lifecycles throughout the present experiment but only one cyclopoid life cycle. Since cyclopoids typically reproduce in Spring (Wetzel 2001), it is possible that cyclopoid egg production in the present study occurred prior to Se exposure and that maternal transfer of Se to juvenile cyclopoids was minimal. Though little is known about the mechanism of Se toxicity to invertebrates, oviparous vertebrates are affected by maternal transfer of Se followed by metabolism and production of reactive oxygen species in developing young (Palace et al. 2004; Janz et al. 2010). Further research is necessary to determine if Se causes reproductive toxicity to zooplankton, and studies that consider the lifespan and life history characteristics of different zooplankton species are recommended.

The negative response of zooplankton to Se exposure may have implications for higher trophic level organisms. Zooplankton are primary and secondary consumers that represent an important food source for predatory zooplankton and planktivorous fish. Shifts in diet measured using stable isotopes of carbon and nitrogen were observed for finescale dace and are presented in Graves et al. (2020). In higher Se treatments, fish fed on greater proportions of benthic

macroinvertebrates and less zooplankton relative to control fish diets. In addition to the implications of lower food availability or less nutritious diet items for fish and other consumers, this shift in diet has implications for Se exposure and trophic transfer as well. The shift in diet from one taxon to another in an ecosystem could result in increased Se exposure.

Benthic macroinvertebrates overall appeared to be less sensitive to Se toxicity than zooplankton, with only the 5.6 and 7.9 µg/L Se treatments impacting community composition, while concentrations as low as 0.8 µg/L impacted zooplankton community composition. At 5.6 µg/L, internal concentrations of Se in Chironomidae and Heptageniidae were 54 and 76 µg/g dm, respectively (Graves et al. 2020). Similarly, the internal concentration as a result of waterborne Se exposure that was lethal to 50% of *Chironomus decorus* (LC_{50}) was 63 µg/g dm (Maier and Knight 1993). Both physiological differences in Se regulation and differences in life history characteristics may influence the differences in sensitivity observed between zooplankton and BMIs.

Heptageniidae appeared to be more sensitive to Se than Chironomidae. The general difference in sensitivity between these benthic macroinvertebrate taxa has been well-documented, as these organisms are often used as indicators of pollution (Rosenberg and Resh 1993). Ephemeroptera are typically regarded as sensitive species, intolerant of various pollution types including contaminants, decreased oxygen, and acidification (reviewed in Jacobus et al. 2019). Conversely, Chironomidae are known to tolerate degraded ecosystems (Cairns and Pratt 1993). Similar to the results herein, Ephemeroptera density, predator density and scraper richness decreased in streams in central Appalachia affected by surface coal-mining where stream-water Se concentrations ranged from 0.7 to 15.7 µg/L across sites (Drover et al. 2020). In the same Se-influenced streams, Chironomidae were ubiquitous and did not appear to be impacted by Se (Drover et al. 2020). Again, the mechanism of Se toxicity to BMIs is unknown, but Conley et al. (2009) fed *Centroptilum triangulifer*, a mayfly in the Family Baetidae, Se-enriched periphyton and observed decreased fecundity (Conley et al. 2009), suggesting that perhaps Se causes reproductive toxicity in invertebrates as well.

At the end of the present study, primary production increased approximately 2-3 fold in the highest Se treatment (7.9 µg/L) relative to the controls, suggesting that there was no inhibition of algal growth by Se, and rather, that algal growth may have increased indirectly as a result of Se exposure. This increase was observed previously (Graves et al. 2019b) and could be a result of decreased grazing pressure by invertebrates. In a previous study (Graves et al. 2019b) and the

study herein, invertebrate biomass decreased in the higher Se treatments at the end of the experimental period, suggesting that perhaps there were fewer invertebrates present to consume algae at higher Se levels. While the increased growth of algae may indicate no adverse effects on primary production, identification of algal species and comparison of communities among treatments is necessary to more definitively state whether Se additions had any effect on these organisms.

Given that embryos and developing fish are the most sensitive life stages to Se toxicity, overall health effects on adult fish were not anticipated. In a previous study, fathead minnow (*Pimephales promelas*) condition factor did decrease after exposure to 8.9 µg/L for 44 d, and in that study it was hypothesized that the decrease in condition was an indirect result of decreased food availability (Graves et al. 2019b). Though no changes to fish health indices were observed herein, there was some evidence of a similar effect in the current study. Stable isotope values of carbon (C) and nitrogen (N) presented in Graves et al. (2020) showed dietary shifts corresponding to increased Se exposure; greater proportions of BMIs and lesser proportions of zooplankton were consumed by fish in the higher treatments relative to controls. Differences in the turnover rate of stable isotopes were observed among treatments as well, indicating either differences in diet or differences in feeding rates among treatment groups (Graves et al. 2020). Changes in food abundance or food quality could influence fish population health and represent another indirect mechanism of toxicity related to Se exposure. In the future, adequate risk assessments of Se may need to consider these indirect modes of toxicity for aquatic ecosystems.

Overall, this study found that, due to high bioaccumulation of Se, both zooplankton and BMI community composition in a boreal forest lake can be impacted by selenite additions at concentrations of 0.8 to 7.9 µg/L. Relative to the ample literature regarding the toxic effects of Se on fish, little is known about its effects on invertebrates. As such, more research is needed to better understand the mechanism of toxicity to invertebrates, and risk assessments of Se may need to consider the impacts of Se on non-vertebrate organisms as well.

CHAPTER 6: PREDICTING SELENIUM BIOACCUMULATION IN AQUATIC SYSTEMS USING EMPIRICAL MESOCOSM DATA

Preface

In this chapter, I compared Se bioaccumulation within each compartment between two studied lakes, and between these boreal lakes and other previously studied systems across Canada and the United States. Selenium bioaccumulation is recognized as being highly site-specific, therefore, identifying differences in Se accumulation among lakes will improve the prediction of Se bioaccumulation in aquatic systems similar to those studied herein. First, bioaccumulation between the two study lakes was compared at each trophic level. Then, the data were used to determine equations describing relationships between Se bioaccumulation at each trophic level, from algae through to fish. These equations were used to compare data from the current research to other studies across the United States and Canada. The similarity in Se bioaccumulation between the two limnocorral studies varied by compartment, and the equations for some compartments were able to accurately predict literature Se values, while others were not. Several reasons for the variability in Se bioaccumulation and predictability are discussed. These trophic transfer equations are meant to improve upon the current biodynamic modelling approach to Se prediction, by acknowledging the changes in distribution coefficients and trophic transfer factors that occur as Se exposure concentrations increase. These equations may be useful for estimating Se bioaccumulation in lentic systems of concern, particularly in boreal lakes where bioaccumulation patterns are expected to be most similar.

6.1 Abstract

Global concern for selenium as a contaminant in aquatic ecosystems has increased due primarily to its adverse effects on egg-laying vertebrates. The bioaccumulation of Se in aquatic systems is highly site- and species-specific, creating uncertainty in the prediction of Se exposure within a specific system of interest. The objective of this study was to use two years of Se bioaccumulation data for algae, invertebrates, and fish collected using *in situ* limnocorral studies to develop models for the prediction of Se bioaccumulation in lentic systems. Models were tested on out-of-model data gathered from the literature, and accuracy was assessed using linear regressions of observed versus predicted values. Models for phytoplankton, sediment and some benthic macroinvertebrates (BMIs) were able to accurately predict literature Se values, while periphyton, most BMI, zooplankton, and fish models were less predictive. In general, these models will be useful when applied to similar systems, i.e. lentic systems with high proportions of reduced Se species. More information on bioaccumulation in different taxa and inclusion of other relevant endpoints (i.e. water chemistry characteristics, sample characteristics, dietary preferences) would improve the applicability of these models.

6.2 Introduction

Selenium (Se) is a naturally occurring element, but disruption of the earth's crust through anthropogenic activities such as mining for coal and metal ores, oil and gas extraction, and agricultural irrigation can lead to excess Se release to aquatic systems (Lemly 1985; Nriagu 1989; Yudovich and Ketris 2006). Due to the essentiality of Se for most organisms, the element is efficiently assimilated by organisms at the base of the food web (i.e. algae, bacteria) and is then transferred to each subsequent trophic level mainly through food consumption (Fan et al. 2002; Stewart et al. 2010). While small amounts of Se are necessary for proper physiological function of most organisms, excess bioaccumulation of Se is a concern for the health of aquatic organisms (Skorupa et al. 1998; Young et al. 2010).

The bioaccumulation of Se in organisms is highly site- and species-specific, such that it is generally not possible to predict Se bioaccumulation in aquatic organisms using water Se concentrations alone. The assimilation of Se by primary producers is particularly variable, with distribution coefficients (k_ds) ranging by four orders of magnitude across species and systems (reviewed in Presser and Luoma 2010; Ponton et al. 2020). The variability among consumers is lesser, but there have been notable differences in the bioaccumulation of Se for different taxa inhabiting the same system (Stewart et al. 2004; Presser and Luoma 2010). Research to date has suggested that the speciation of Se, physico-chemical characteristics of a receiving system, and both the physiological and ecological traits of an organism can all influence Se bioaccumulation (Stewart et al. 2010; Presser and Luoma 2010; Ponton et al. 2020). In terms of Se speciation, reduced species (e.g., selenite and organo-Se) tend to accumulate more readily and to a higher extent than selenate. Further, lentic or slow-moving waters generally have higher residence times and more reducing conditions, which provide greater biogeochemical cycling potential for Se and the production of reduced Se species (Hillwalker et al. 2006; Simmons and Wallschlager 2011). In the laboratory, investigations of Se uptake by several species using monocultures reveal orders of magnitude differences in uptake potential (Baines and Fisher 2001).

Predictive modelling of Se distribution and trophic transfer in aquatic systems offers several benefits, namely decreasing the need to collect live organisms, the cost of measuring Se in samples, and increasing our ability to predict Se levels of concern on a site-by-site basis. Currently, a preferred approach for characterizing selenium bioaccumulation and trophic transfer in aquatic systems is biodynamic modelling, as described by Presser and Luoma (2010). Distribution

coefficients (k_ds) are calculated for a system based on the amount of Se in particulates (phytoplankton, periphyton, or sediment) divided by the amount of Se in water (Presser and Luoma 2010). Trophic transfer factors (TTFs) are calculated using the concentration of Se in a consumer divided by the concentration of Se in their diet. While this approach may be useful when assessing Se bioaccumulation in a system with pre-existing data, it assumes only one k_d or TTF per organism. More recent research has indicated that these factors are dynamic and change with source Se concentration (DeForest et al. 2017; Graves et al. 2019a). As such, considering these distribution and trophic transfer factors as continuous variables may be a useful amendment to the current modeling approach.

The goals of the current study were to compare the bioaccumulation of Se in organisms within limnocorral studies (Graves et al. 2019a; Graves et al. 2020) to bioaccumulation in other systems across the United States and Canada, and to improve the prediction of Se bioaccumulation in organisms in order to advance the ecological risk assessment of selenium. Specifically, the objectives were to 1) compare Se bioaccumulation in two mesocosm studies conducted in Canadian boreal lakes, 2) use the mesocosm data to develop equations relating source Se concentrations to primary producers (algae) and primary and secondary consumers (invertebrates and small-bodied fish) and 3) test the predictive capability of developed models using existing Se data for lentic systems across the United States and Canada.

6.3 Materials and Methods

6.3.1 Data collection

Data used to develop equations for the prediction of Se movement from water to primary producers, primary consumers, and secondary consumers were obtained from two limnocorral experiments previously conducted at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA) in 2017 and 2018. Detailed descriptions of the experimental design and setup for each experiment are presented in Graves et al. (2019a) and Graves et al. (2020). Two separate lakes were used for the experiments. Lake 114 is a mesotrophic, shallow lake and Lake 239 is an oligotrophic lake. The physico-chemical characteristics of each lake are presented in Tables 2.1 and A.4.7. The experiments ran for 77 and 63 d in Lakes 114 and 239, respectively. Mean concentrations of aqueous TSe measured in enclosures ranged from 0.4 – 11.8 µg/L and background Se concentrations were 0.08 - 0.16 µg/L. Fathead minnow (*Pimephales promelas*) and finescale dace (*Phoxinus neogaeous*) were added to Lake 114 and Lake 239,

respectively, approximately half-way through the experimental period, and were exposed to Se for 42 to 44 d. In Lake 114, water, sediment, periphyton, benthic macroinvertebrates (BMI), zooplankton, and female fathead minnow muscle and ovary tissues were analyzed for TSe. In Lake 239, TSe in water, sediment, periphyton, phytoplankton, BMI, zooplankton and female finescale dace muscle and ovary tissue TSe were measured.

6.3.2 Se comparisons among lakes and model development

To determine if there were differences in Se bioaccumulation within a compartment between the two lakes, Se was first compared within each compartment using Analysis of Covariance (ANCOVA). Since compartment Se concentrations were analyzed at several time points, mean Se values for each compartment (sediment, periphyton, phytoplankton, zooplankton, BMI (by taxa), fish muscle, fish ovary) were calculated for each limnocorral. ANCOVA was used to determine if there were differences in the slopes or intercepts of the following relationships between lakes: 1) periphyton versus water TSe, 2) sediment versus water TSe, 3) benthic macroinvertebrates versus periphyton TSe (composite value for all BMI and Chironomid only), 4) fish muscle versus average invertebrate TSe and 5) fish ovary versus average invertebrate TSe. Phytoplankton was only collected from Lake 239 and therefore could not be compared between lakes. Zooplankton trophic transfer could not be compared between lakes without phytoplankton Se, so instead, zooplankton was compared between lakes as a function of water TSe.

When no differences in bioaccumulation between the two lakes were detected, Se data were pooled from both studies and used to assess log-log relationships between trophic levels. When differences were detected, lakes were kept separate and log-log relationships were analyzed for each lake. Log-log regression analysis used to assess relationships between primary producers (periphyton/phytoplankton/sediment) versus water, primary consumers (benthic macroinvertebrates/zooplankton) versus algae, and secondary consumers (small-bodied fish muscle) versus invertebrates. Benthic macroinvertebrates were regressed with periphyton as pooled values for all taxa, as well as with each order separately. Only Chironomidae and Heptageniidae were measured in Lake 239, whereas as several taxa within the orders Diptera, Ephemeroptera, Odonata, Trichoptera, Amphipoda, and Sphaeriida were measured in Lake 114. The equation of the line for each relationship was used as the predictive model for Se accumulation.

6.3.3 Out-of-model testing using literature data

To test the strength and accuracy of the Se distribution and trophic transfer models developed using limnocorral data, water, algae and invertebrate Se data were compiled from previous studies, in particular the reviews by Presser and Luoma (2010), DeForest et al. (2017), and Ponton et al. (2020). Additional data points from some other field-based studies conducted across the United States and Canada were included in the compiled literature and all data are listed in Appendix B. For the prediction of fish muscle Se, all fish and fish diet Se values used to test the models were obtained from Presser and Luoma (2010). For the prediction of Se transfer from water to primary producers, data from lotic systems were excluded from the analysis because Se bioaccumulation in these environments is known to differ greatly from accumulation in lentic conditions (Presser and Luoma 2010; Ponton et al. 2020). For the prediction of Se accumulation in invertebrates and fish, data from both lentic and lotic habitats were included in the analyses.

Models were used to calculate predicted values for the literature data, using the values reported for each compartment. For each trophic level, observed (dependent variable) and predicted (independent variable) values were compared using linear regression analysis to assess the accuracy and precision of the models. To assess accuracy, the 95% confidence intervals for the slope and intercept of the relationships between predicted and observed values were determined. Models with 95% confidence intervals that encompassed 0 for the intercept and 1 for the slope were considered to be accurate predictors of field collected data. To assess precision, the root mean square error (RMSE) was calculated as a measure of the absolute error associated with the models, where a lower RMSE indicates a better fit of the data.

6.4 Results

6.4.1 Comparison of Se bioaccumulation in Lake 114 and Lake 239

Periphyton Se bioaccumulation was similar between the two lakes, and did not differ significantly in slope (ANCOVA, $p=0.100$, Figure 6.1A) or intercept (ANCOVA, $p=0.357$, Figure 6.1A). Phytoplankton Se could not be compared between lakes, but Se accumulation in phytoplankton was significantly greater than in periphyton from the two lakes combined (Figure 6.1B), and differed significantly in slope (ANCOVA, $p=0.006$). Sediment TSe was greater in Lake 114 than Lake 239 (Figure 6.2), and the relationship between sediment and aqueous Se differed in slope (ANCOVA, $p<0.001$) between the two lakes.

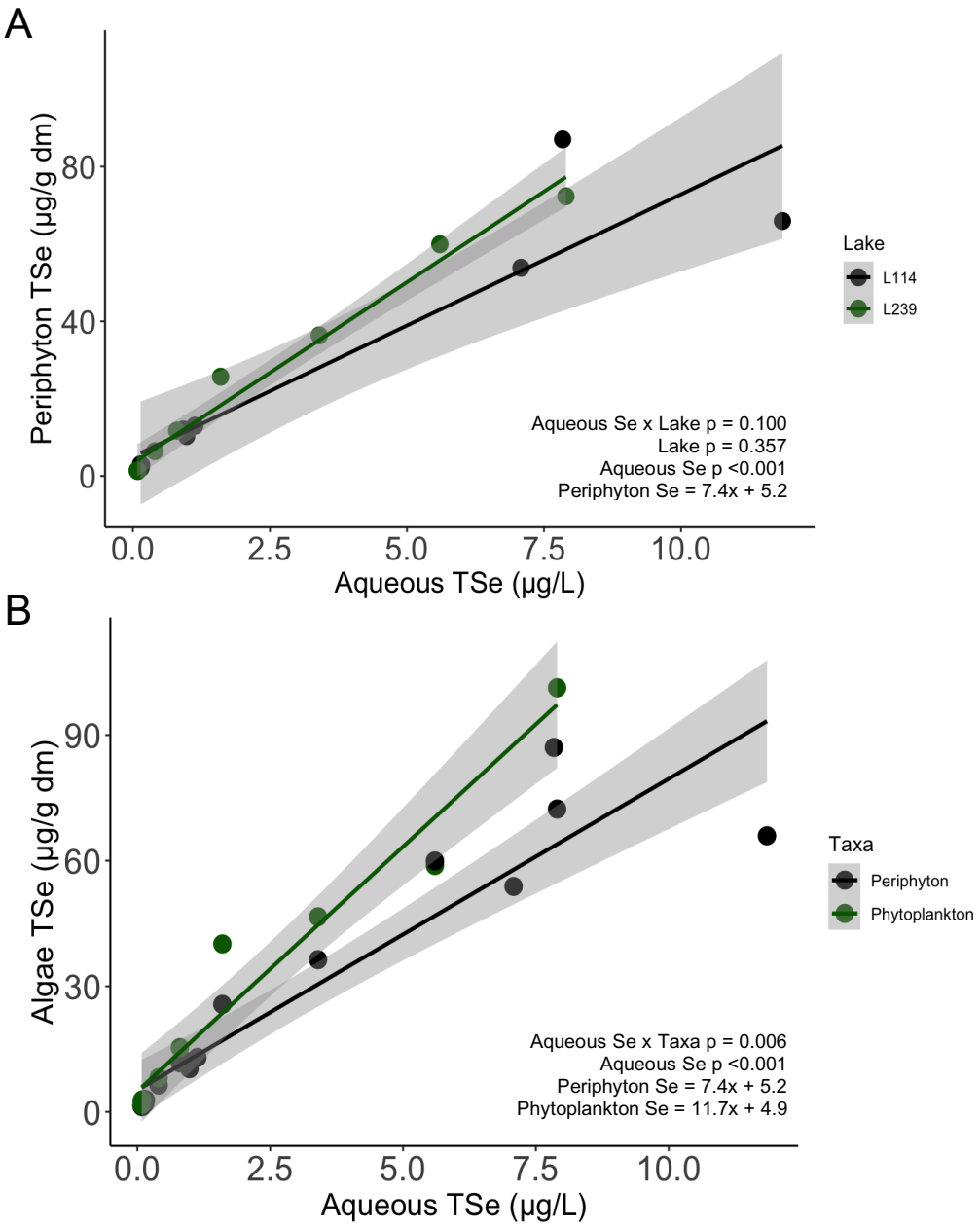

Figure 6.1: Analysis of covariance comparing Se bioaccumulation in periphyton versus aqueous total Se (TSe) from Lake 114 (black circles) and L239 (green circles) (A), and comparing Se bioaccumulation in periphyton (black circles) and phytoplankton (green circles) Se in Lakes 114 and 239 pooled (B). Grey shading indicates the 95% confidence interval for the equation of the line.

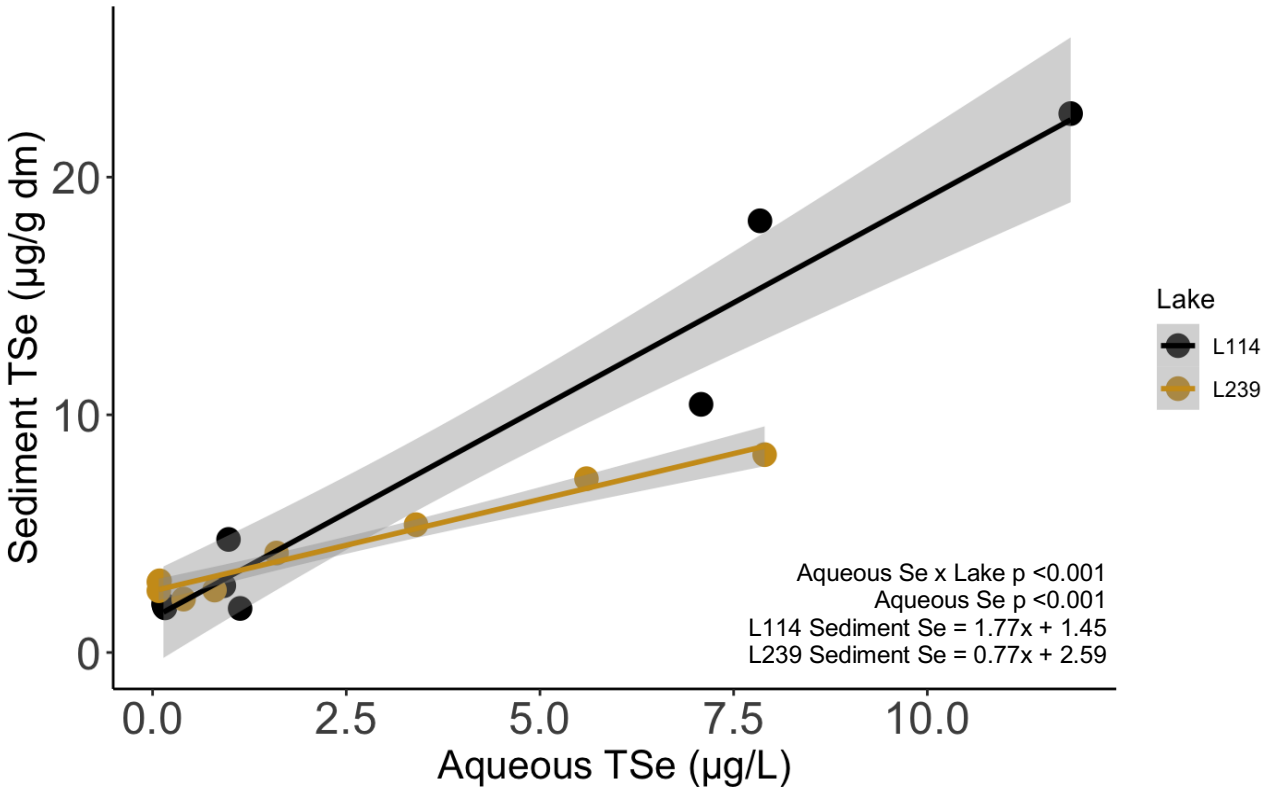

Figure 6.2: Analysis of covariance comparing sediment Se accumulation versus aqueous Se concentration in Lake 114 (black circles) and L239 (yellow circles). Grey shading indicates the 95% confidence interval for the equation of the line.

Invertebrate Se was difficult to compare between lakes because of the differences in taxa collected. With all BMI data pooled together, there were differences in slopes of the relationships between the two lakes (ANCOVA, p=0.027, Figure 6.3A), with invertebrate Se being greater in Lake 239 than Lake 114. The only BMI that could be compared between lakes was Chironomidae. There was no difference in the slope (ANCOVA, p=0.076, Figure 6.3B) or intercept (ANCOVA, p=0.450, Figure 6.3B) of the relationship between Chironomidae and periphyton Se between the two lakes, and there was a significant and positive relationship between Chironomidae and periphyton Se with both lakes pooled together (LR, $p<0.001$, $R^2=0.78$). Due to the differences in Se bioaccumulation among taxonomic groups, Se bioaccumulation was compared among certain invertebrate taxa (Figure 6.4), and equations were derived to predict a range of different invertebrate taxa (Table 6.1). Diptera and Ephemeroptera had significantly greater slopes than Amphipoda, Odonata, Sphaeridae, and Trichoptera (Figure 6.4).

Zooplankton trophic transfer could not be compared between lakes because phytoplankton Se was not measured in Lake 114. However, a comparison of zooplankton Se at different aqueous Se concentrations showed that bioaccumulation was similar in the controls and lower Se treatments (0.8 to 1 µg/L ; Figure 6.5), but zooplankton Se concentrations were much greater in Lake 114 after exposure to 8.9 µg/L than in Lake 239 at 7.9 µg/L (Figure 6.5). A significant difference in slope was observed between Lake 114 and Lake 239 (ANCOVA, $p<0.001$, Figure 6.5).

The greatest difference in bioaccumulation among lakes within a trophic level was observed for fish. Fish muscle and ovary Se bioaccumulation were both greater in Lake 114 than Lake 239 (Figure 6.6), with a significant difference in slopes between the two lakes for both tissues (ANCOVA, p=0.002 to 0.009, Figure 6.6).

6.4.2 Modelling selenium uptake

A summary of the models used for the prediction of Se in periphyton, phytoplankton, sediment, zooplankton, BMIs, and fish muscle are listed in Table 6.2. All of the linear regressions for observed versus predicted values were significant with p-values <0.05 for all tested models. The RMSE values for all models ranged from 0.12 to 0.52, indicating a range of variability in the datasets for different compartments.

The periphyton model predicted literature periphyton values with an accurate slope, but the intercept of the model test was greater than 0 (Figure 6.7A), indicating that model predictions were greater than actual values. There were fewer literature data to compare phytoplankton across

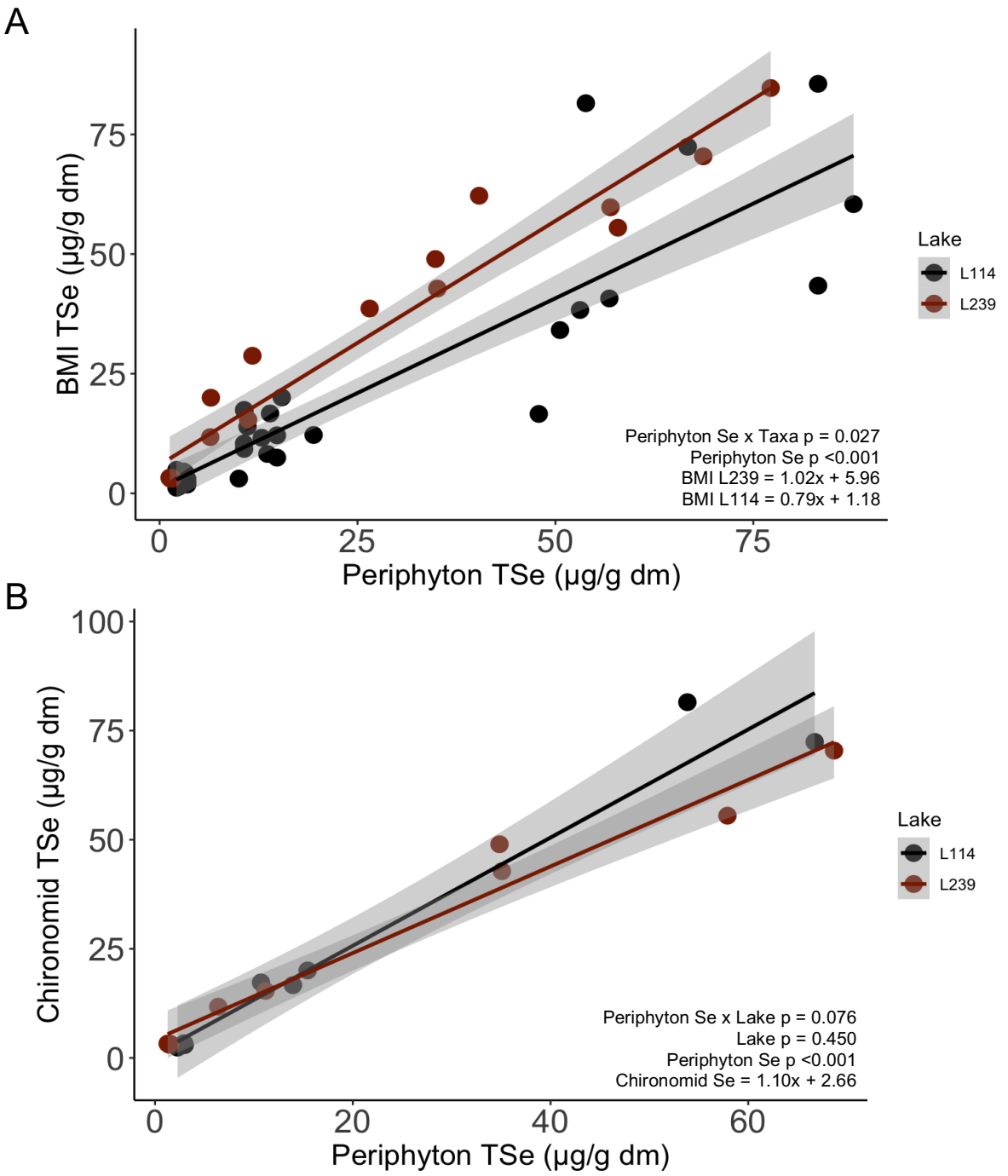

Figure 6.3: Analysis of covariance comparing Se bioaccumulation in all benthic macroinvertebrates (BMI) versus periphyton Se from Lake 114 (black circles) and L239 (red circles) (A), and comparing Se bioaccumulation in Chironomidae from Lake 114 (black circles) and Lake 239 (red circles) (B). Grey shading indicates the 95% confidence interval for the equation of the line.

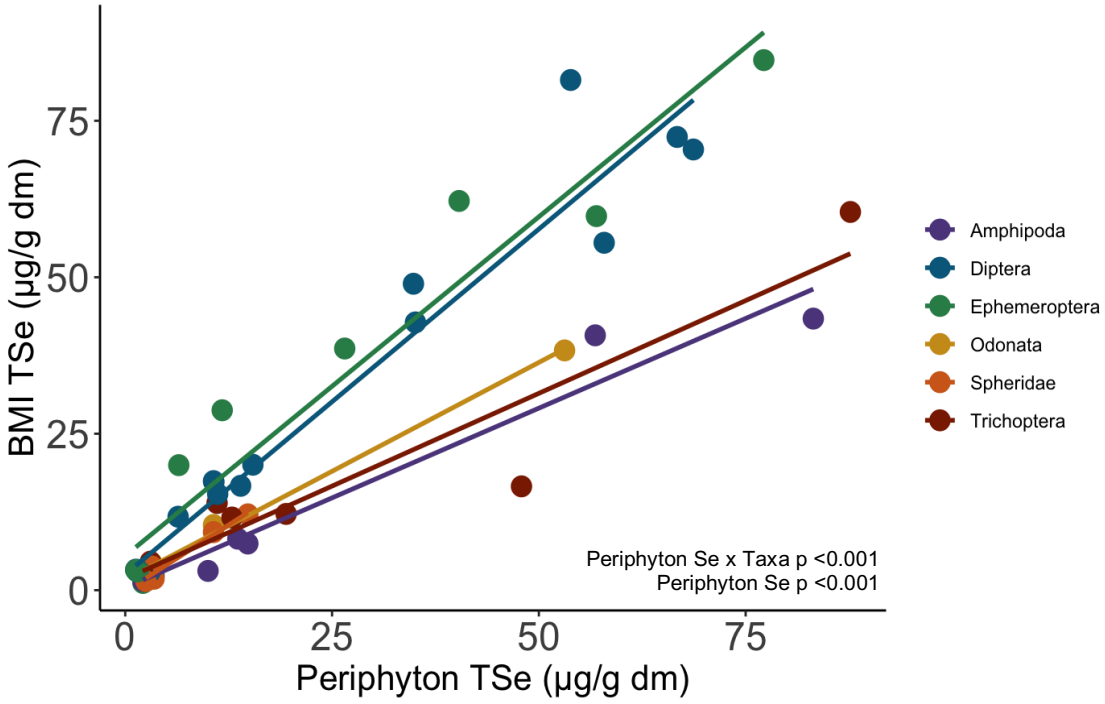

Figure 6.4: Relationship between benthic macroinvertebrate (BMI) Se and periphyton Se for several taxonomic groups pooled from Lake 114 and Lake 239. Taxa are colour coded according to the legend.

Table 6.1: Relationships between invertebrate Se and periphyton Se for several benthic macroinvertebrate taxa pooled from Lake 114 and Lake 239. In the equations of the line, y is the log invertebrate Se concentration in µg/g dm and x is log periphyton Se concentration in µg/g dm.

Taxonomic Group	Equation of the line	R^2	n
Amphipoda	y = 0.57x + 0.41	0.95	8
Diptera	y = 1.10x + 2.51	0.94	19
Ephemeroptera	y = 1.08x + 5.49	0.93	11
Odonata	y = 0.70x + 1.60	0.99	4
Sphaeriida	y = 0.85x − 0.22	0.96	5
Trichoptera	y = 0.59x + 1.81	0.86	7

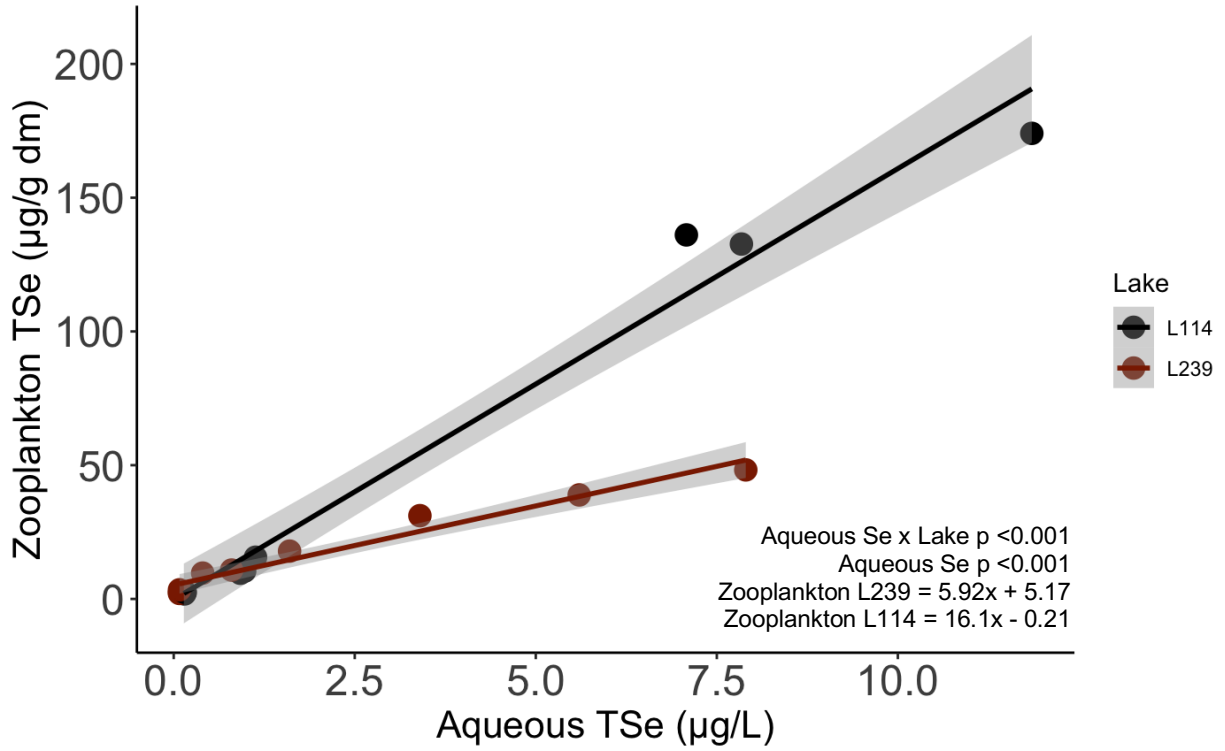

Figure 6.5: Analysis of covariance comparing Se bioaccumulation in composite zooplankton samples versus aqueous Se concentrations from Lake 114 (black circles) and Lake 239 (red circles). Grey shading indicates the 95% confidence interval for the equation of the line.

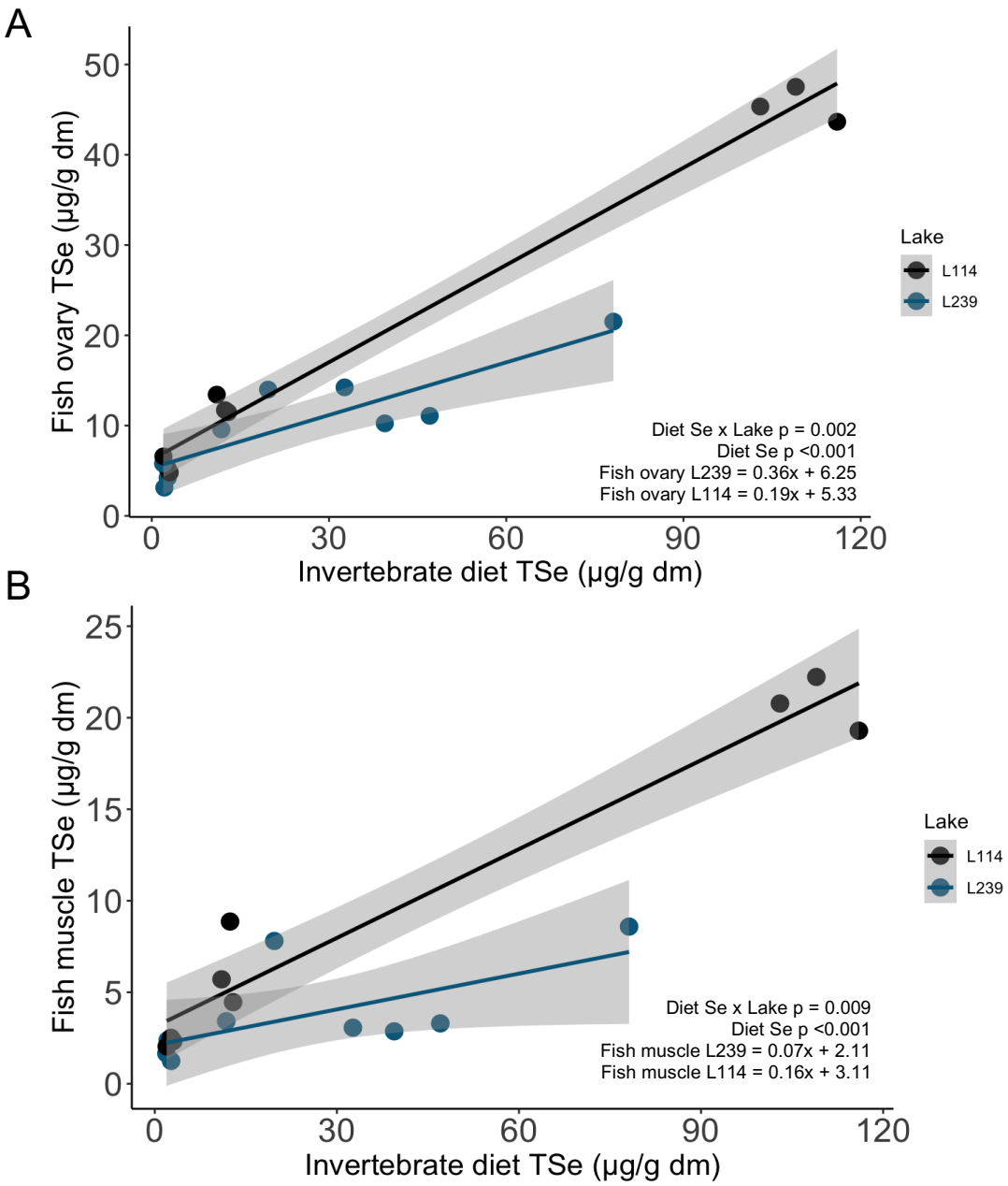

Figure 6.6: Analysis of covariance comparing fish ovary (A) and fish muscle (B) Se bioaccumulation versus average diet TSe in Lake 114 (black circles) and Lake 239 (blue circles). Grey shading indicates the 95% confidence interval for the equation of the line.

Table 6.2: Models to predict algae, sediment, invertebrate, and fish selenium derived from mesocosm data from Lakes 114 and 239, indicating the significance of the relationship between trophic levels (P-value), the fit of the relationship (R^2), the slope and intercept 95% confidence intervals (CI) for the observed versus predicted data tests and the root mean square error (RMSE) for the observed versus predicted tests.

Dependent variable	Independent variable	Model equation	Lake data	P-value	R^2	95% CI Slope	95% CI Intercept	RMSE
Periphyton	Water	y = 0.84x + 1.09	114, 239	<0.001	0.98	0.58, 1.02	-0.87, 0.21	0.36
Phytoplankton	Water	y = 0.82x + 1.26	239	<0.001	0.98	0.40, 1.18	-0.16, 0.62	0.18
Sediment	Water	y = 0.53x + 0.63	114	<0.001	0.81	0.35, 1.23	-0.40, 0.43	0.52
Sediment	Water	y = 0.22x + 0.62	239	0.004	0.67	0.86, 2.95	-1.44, 0.10	0.52
Zooplankton	Phytoplankton	y = 0.77x + 0.13	239	<0.001	0.93	0.73, 1.25	0.21, 0.59	0.20
Diptera	Periphyton	y = 0.92x + 0.21	114, 239	<0.001	0.94	0.94, 1.35	-0.23, 0.10	0.27
Ephemeroptera	Periphyton	y = 0.93x + 0.26	114, 239	<0.001	0.89	0.38, 0.76	0.49, 0.78	0.12
Amphipoda	Periphyton	y = 0.90x − 0.10	114	<0.001	0.90	0.35, 0.77	0.002, 0.22	0.16
Odonata	Periphyton	y = 0.93x + 0.02	114	0.020	0.94	0.78, 1.13	0.16, 0.53	0.24
Sphaeriidae	Periphyton	y = 1.13x − 0.21	114	0.015	0.86	N/A[a]	N/A	N/A
Trichoptera	Periphyton	y = 0.71x + 0.26	114	0.001	0.88	0.47, 1.81	-0.20, 0.78	0.27
Composite BMI	Periphyton	y = 0.89x + 0.13	114, 239	<0.001	0.86	0.57, 0.75	0.51, 0.63	0.26
Fish muscle	Invertebrate	y = 0.58x + 0.14	114	<0.001	0.96	1.47, 1.89	-0.36, -0.03	0.24

[a]N/A = Insufficient literature data to test model.

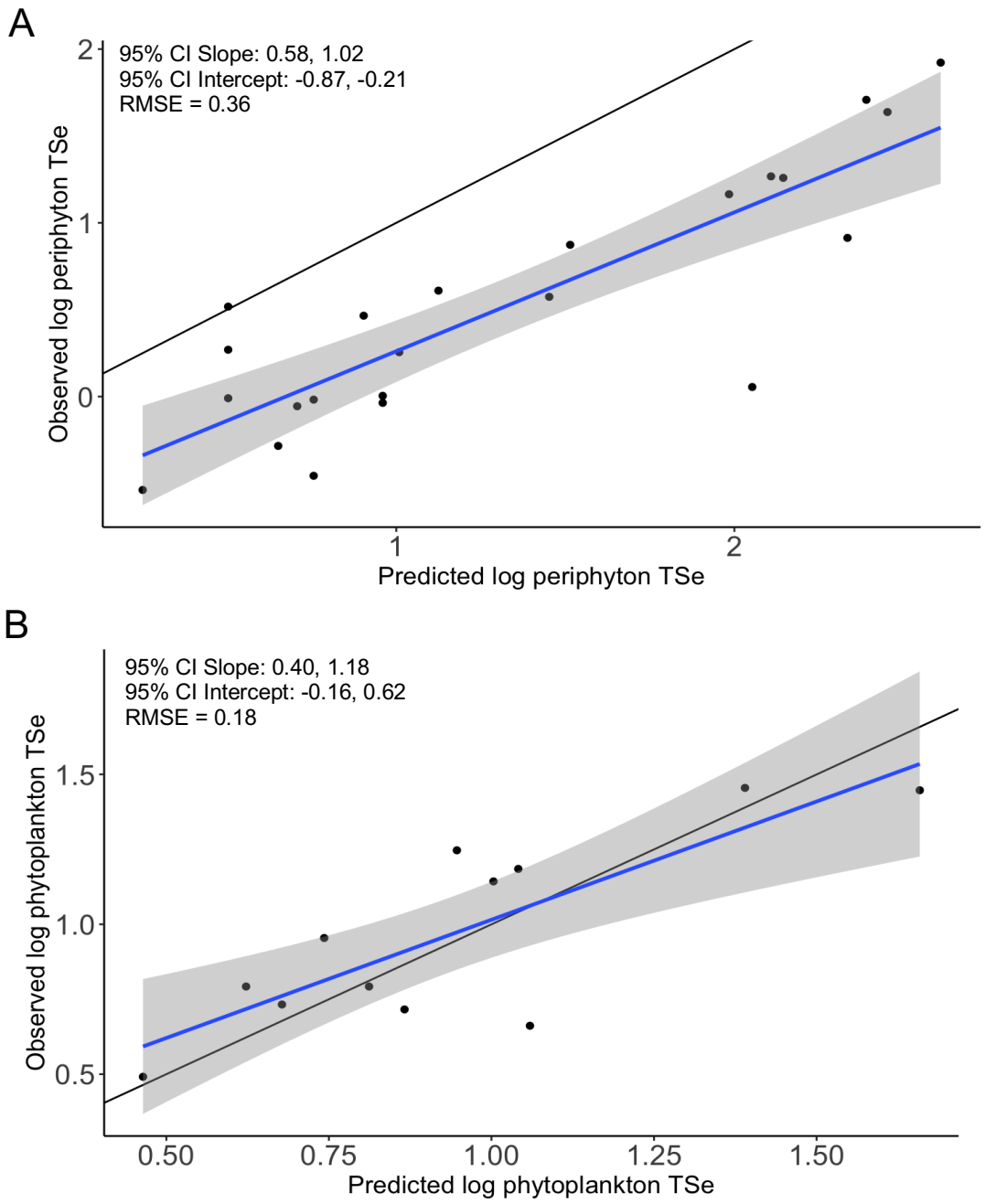

Figure 6.7: Observed versus predicted log periphyton TSe based on models derived from periphyton from Lake 114 and L239 data (A), observed versus predicted log phytoplankton TSe based on models derived from Lake 239 data (B). Blue line represents the line of fit for the two variables and the black line represents a 1:1 ratio for observed:predicted values. Observed values were gathered from the literature and are presented in Appendix B. All concentrations are in µg/g dm. Grey shading indicates the 95% confidence interval for the equation of the line.

studies, but the model derived from phytoplankton TSe in Lake 239 accurately predicted literature phytoplankton TSe values from a range of mine-affected lakes in the Canadian boreal forest (Figure 6.7B). The 95% CIs for the slope and intercept of the model were not significantly different from 1 and 0, respectively (Figure 6.7B).

Sediment models derived from Lake 114 and Lake 239 data were tested separately due to the significant difference in accumulation between the two lakes (Figure 6.8). The 95% CIs for slopes and intercepts of both models encompassed 1 and 0, respectively, but the Lake 114 model had smaller ranges for both, indicating a better fit to the dataset (Figure 6.8). The RMSE for sediment was 0.52, indicating that although the slopes and intercepts of the sediment model were accurate, there was a lot of unexplained variability in sediment data across studies.

The Diptera model was the only one of five BMI taxa models that accurately predicted literature values with the correct slope and intercept (Figure 6.9). Model tests for Odonata and Trichoptera had accurate slopes, but the intercepts for both were significantly greater than 0, indicating that the models under-predicted BMI Se from diet Se (Figure 6.9). For Ephemeroptera and Amphipoda, the models did not accurately predict the slope or the intercept of the model test. For composite values from the literature, in which the taxa collected were not known, the composite BMI model also did not accurately predict the slope or intercept of the model test (Figure 6.9). There were very few literature values for zooplankton to test the model. The slope of the model test for zooplankton was not significantly different from 1, but the intercept was greater than 0, indicating that literature zooplankton Se values were under-predicted by the model (Figure 6.10).

For fish muscle, the slope of the model test was significantly greater than 1, indicating that the model under-predicted fish Se values from the literature (Figure 6.11A). It is likely that fish in the limnocorral studies did not reach steady state Se concentrations, because based on the available literature data, fish muscle Se and diet Se are highly correlated with a 1:1 ratio (Figure 6.11B).

6.5 Discussion

The goals of this study were to compare Se bioaccumulation between two limnocorral studies, and to assess the predictability of Se bioaccumulation in aquatic systems using the empirically derived Se bioaccumulation data. Overall, I found that models for different taxa encompassing three trophic levels varied in their comparability and predictability. Some models were able to accurately predict Se bioaccumulation with correct slopes and intercepts (i.e.

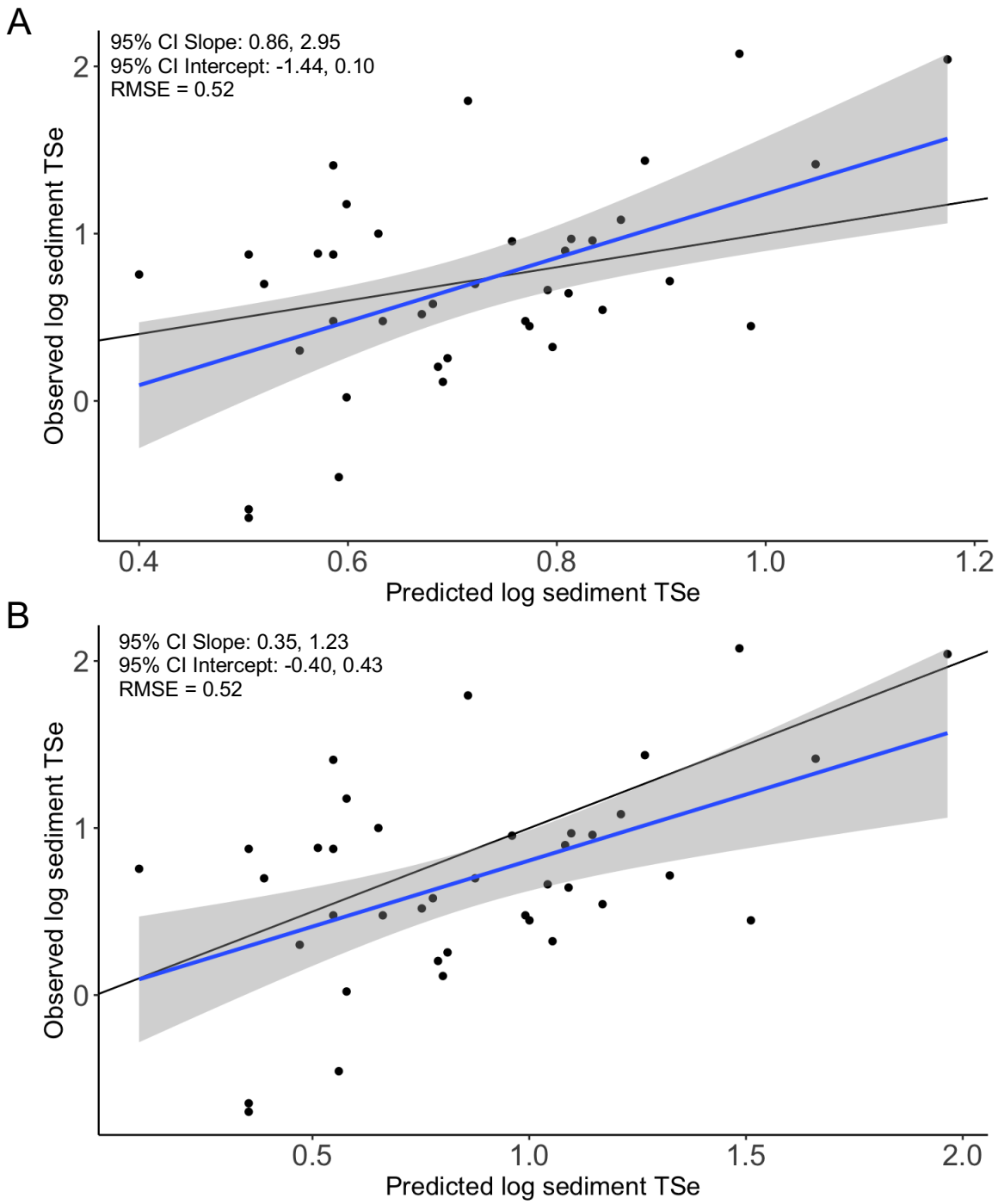

Figure 6.8: Observed versus predicted log sediment TSe based on models derived from sediment in Lake 239 (A) and Lake 114 (B). All concentrations are in µg/g dm. Blue line represents the line of fit for the two variables and the black line represents a 1:1 ratio for observed:predicted values. Grey shading indicates the 95% confidence interval for the equation of the line.

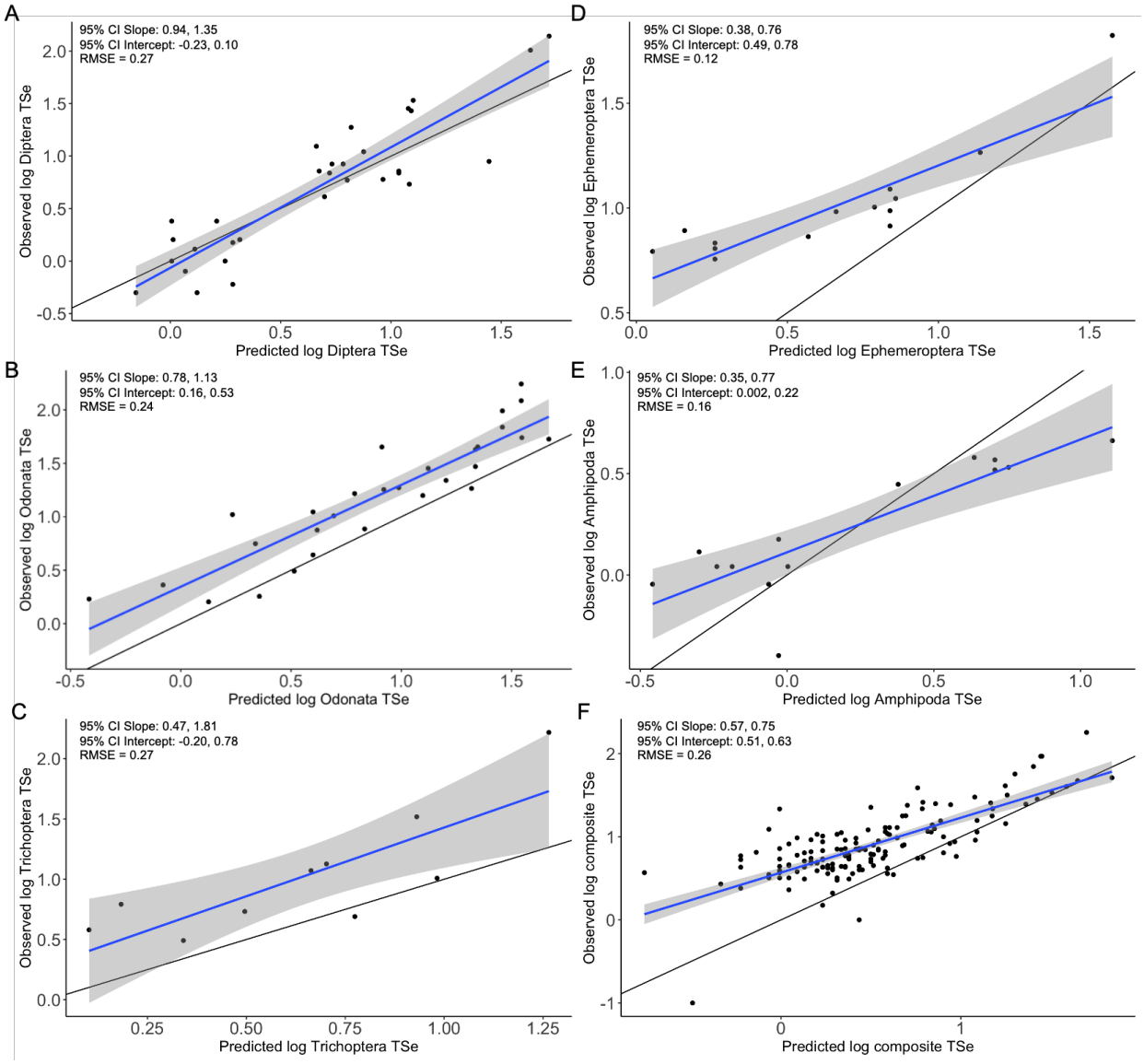

Figure 6.9: Observed versus predicted log invertebrate TSe based on models derived from benthic invertebrate Se from Lake 114 and 239. All concentrations are in µg/g dm. Blue line represents the line of fit for the two variables and the black line represents a 1:1 ratio for observed:predicted values. Grey shading indicates the 95% confidence interval for the equation of the line.

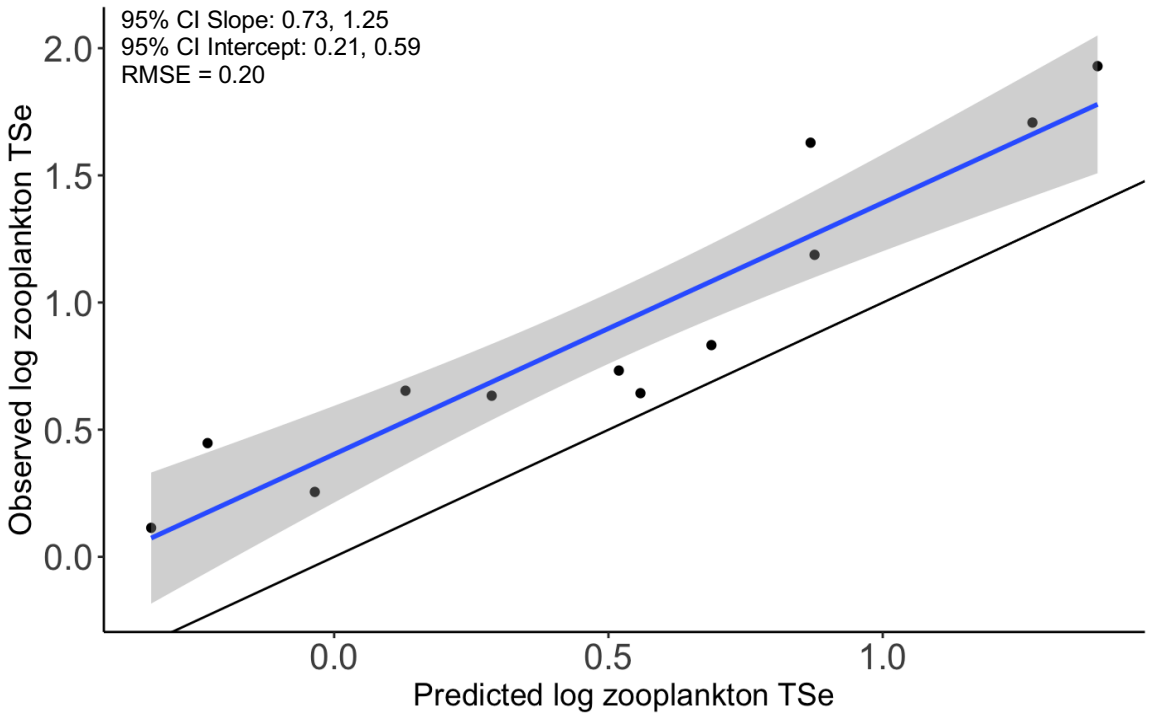

Figure 6.10: Observed versus predicted log zooplankton TSe based on models derived from zooplankton Se from Lake 239. All concentrations are in µg/g dm. Blue line represents the line of fit for the two variables and the black line represents a 1:1 ratio for observed:predicted values. Grey shading indicates the 95% confidence interval for the equation of the line.

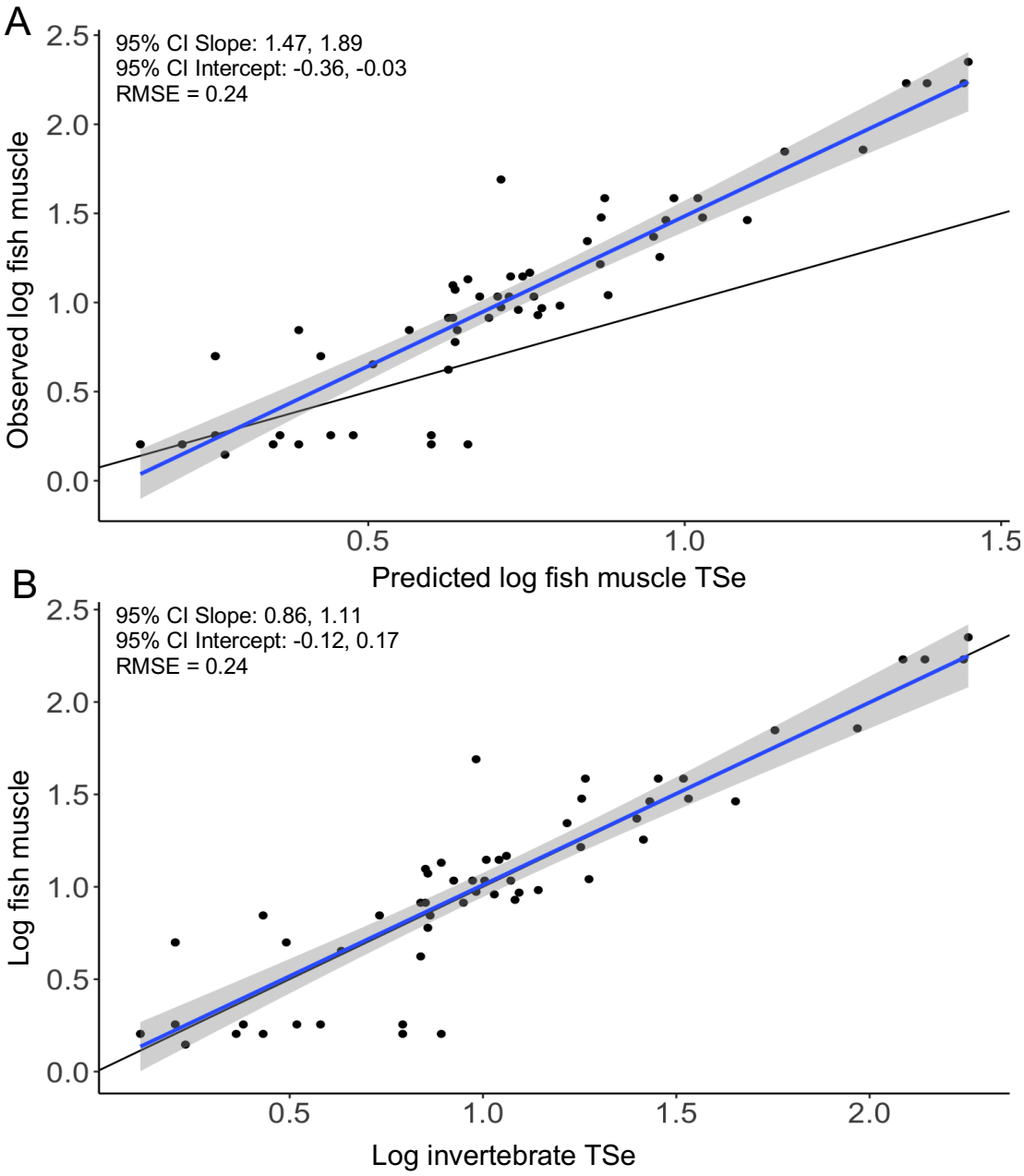

Figure 6.11: Observed versus predicted log fish TSe based on models derived from fish muscle Se from Lake 114 (A) and linear regression analysis of fish muscle TSe versus invertebrate TSe pooled from the literature (B). All concentrations are in µg/g dm. The blue line represents the line of fit for the two variables and the black line represents a 1:1 ratio of the dependent:independent variables. Grey shading indicates the 95% confidence interval for the equation of the line.

phytoplankton, sediment, Diptera), some were able to predict Se concentrations with similar slopes but not intercepts (i.e. periphyton, some BMIs, zooplankton), and other models were unable to predict Se bioaccumulation with correct slopes or intercepts (i.e. some BMIs, fish muscle). These results demonstrate that the extent of Se bioaccumulation is variable among systems, and that in future studies, additional information is needed to characterize systems of interest and improve our understanding Se bioaccumulation in different systems and among different taxa. At the base of the food web, periphyton Se bioaccumulation was similar between Lakes 114 and 239, but literature periphyton Se values were over-predicted using the mesocosm-derived model. This could be a result of several factors, including 1) speciation and bioavailability of Se in the limnocorrals versus natural systems, 2) physico-chemical characteristics of the receiving water, and 3) taxonomic differences in Se bioaccumulation. The speciation of Se in a system is likely one of the main factors contributing to overall variability in algal Se accumulation among systems. In a recent review of the algal Se bioaccumulation literature, the intercept of log particulate Se versus log aqueous Se was higher for selenite than for selenate (Ponton et al. 2020). Since bioaccumulation will increase as the proportion of reduced Se species present increases, the speciation of Se in water, and not just total Se, is one of the most important measurements required in the prediction of Se accumulation. In the studies used to build these predictive models, 100% selenite was added to limnocorrals. This is considered the more bioavailable of the two main oxyanions found in water. Selenium speciation was not measured in the limnocorrals over time, but this selenite could have been reduced further in these closed systems to organo-Se, which is taken up at an even higher rate than selenite (Simmons and Wallschlager 2011; Ponton et al. 2018). If future studies can report speciation of aqueous Se, then the intercept of this model could be corrected to account for the difference in accumulation between different Se species. Taxonomic characterization of samples may greatly improve our understanding of Se bioaccumulation at the base of the food web as well, although taxonomic identification of primary producers can be difficult and time-consuming. Even reporting some general characteristics of samples, such as chlorophyll *a* and organic matter content may help to refine this model.

Interestingly, the phytoplankton model, which could only be used to predict Se bioaccumulation in similar Canadian boreal lake systems across Ontario and Quebec due to a lack of data, did accurately predict Se. In these systems, reduced Se comprised a large portion of total Se, and the physico-chemical characteristics of the lakes were similar to Lake 239 (slightly basic

pH, oligotrophic, low sulfate; Ponton and Hare 2013). This may further suggest that the differences observed in periphyton uptake among systems are related to differences in some of these physico-chemical characteristics.

The sediment Se models both accurately predicted sediment Se bioaccumulation, but the RMSE was highest for sediment Se literature data, indicating that of all the compartments assessed, this dataset was the most variable. This may be due to differences in physico-chemical characteristics of the systems studied as discussed above, different depth sections at which sediment was collected among studies, the proportion of organic matter in the sediment, and the productivity or microbial activity of the sediment. This high variability demonstrates the need to better characterize collected samples. In order to compare Se bioaccumulation across systems, or to better understand bioaccumulation, there is a need for more standardization of samples and more description of sample types so that such comparisons across systems can be made.

Not surprisingly, the composite BMI model did not accurately predict composite BMI literature values. This is likely due to the taxonomic differences in Se accumulation among invertebrates (Presser and Luoma 2010; Graves et al. 2019a). Only one of the five models for different BMI taxa accurately predicted literature values; Diptera was the most abundant order of invertebrate taxa collected across the two limnocorral studies and it is likely that other models would improve if there was a greater amount of data available to construct them. For instance, the Odonata and Trichoptera models accurately predicted the intercept but not the slope of the literature data, but these models were only derived from sample sizes of 4 to 7. Trichoptera in the literature were largely the free-living predator Rhyacophilidae, which is different from the limnocorral taxa collected (Polycentropodidae and Phryganeidae). For the order Odonata, a large proportion literature values were for Zygoptera, while in the limnocorrals only Anisoptera were analyzed. The different patterns of accumulation for different invertebrate taxa highlight the importance of separating and analyzing individual taxa, and not pooled samples, to understand Se trophic dynamics. Without knowing the diet of fish or other predators, pooled invertebrate samples can dilute the actual Se amounts that are being transferred to higher trophic levels and decrease the predictability of Se bioaccumulation in invertebrates.

Relative to BMIs, zooplankton Se data were sparse in the literature, which is a concern considering the importance of zooplankton in aquatic food webs. Zooplankton in Lake 239 appeared to accumulate less Se than those in other systems, leading to the model under-predicting

zooplankton Se. Few studies report the taxa collected and zooplankton in all studies were collected as bulk samples. With bulk zooplankton it is difficult to 1) separate zooplankton from phytoplankton, 2) to know what zooplankton species are present and 3) to know if species differ in Se concentration. In the present limnocorral studies, biomass was too low to effectively sort zooplankton by taxa in all samples, but this information would be useful to determine taxonomic differences in bioaccumulation. In general, there were fewer data for plankton relative to benthos in the literature, and all data were from the United States, highlighting a need for the investigation of Se accumulation in more zooplankton taxa and in more regions.

Fish Se accumulation was the least predictable based on the mesocosm models developed herein. This is likely because fish did not reach steady state through these two limnocorral experiments, in which they were exposed to Se for 42 to 44 days. Particularly in cold-water, oligotrophic lakes, fish may consume very little food and would take a long time to reach steady state concentrations. The difference in productivity between Lake 114 and Lake 239 may explain why fish tissue Se was greater in Lake 114 (a mesotrophic lake) relative to Lake 239 (an oligotrophic lake). Interestingly, when fish muscle Se values were gathered from the literature and log-log regressed using invertebrate Se concentrations as diet items, there was a 1:1 ratio for fish diet:fish muscle Se. This further suggests that fish in the limnocorrals did not reach steady state, and that in most systems where fish are at steady state, fish muscle Se is expected to be similar to fish diet Se.

The distribution and trophic transfer models developed herein are a simplification of aquatic food webs. The interactions among organisms in an ecosystem are complex, some organisms feed on several different organisms that may occupy different trophic levels, either concurrently or temporally according to their life cycle or abundance of food items. Other organisms feed non-selectively, such that it is difficult to assign diet Se values to organisms. While these models do not capture the variability that exists in Se bioaccumulation due to these complicated interactions, they do provide a starting point for a method to predict Se bioaccumulation in organisms within a particular system. The models will benefit from several refinements, including adding water chemical characteristics that influence accumulation, taxonomic-specific bioaccumulation data, and information on diet preferences of individual organisms.

While some of these models may be useful in predicting the transfer of Se through each trophic level in aquatic systems, a more ideal bioaccumulation model would predict Se in organisms of concern (invertebrates and fish tissue) directly from water. This would completely eliminate the need to collect organisms from study systems. The models presented herein do provide some insight into the level of variability associated with each trophic level, and may be useful in constructing such a model in the future.

In the present study, I compared Se bioaccumulation between two limnocorral studies conducted in Canadian boreal lake ecosystems, and tested the ability of empirically-derived Se bioaccumulation models to predict Se distribution from water to algae/sediment, and trophic transfer from algae to invertebrates and invertebrates to fish across systems throughout the United States and Canada. For some compartments (i.e. invertebrates, algae) Se bioaccumulation was similar between the two study lakes. For others (i.e. sediment, fish), bioaccumulation differed between lakes and was higher in the mesotrophic study lake compared to the oligotrophic lake, suggesting that productivity may have an impact on Se bioaccumulation rates. Some models derived from limnocorral data accurately predicted the available literature data for Se bioaccumulation (i.e. phytoplankton, sediments, some BMI), while others did not (i.e. periphyton, most BMI, zooplankton, fish muscle). More information on bioaccumulation in different taxa, increased standardization of sample collection, and inclusion of other relevant endpoints (Se speciation, water chemistry characteristics, sample characteristics, dietary preferences) will help to increase the predictive ability of Se trophic transfer models in the future.

CHAPTER 7: GENERAL DISCUSSION

7.1 Project rationale and summary

Selenium is increasingly being recognized as a contaminant of global concern, and there is a growing need for environmentally relevant studies to improve the understanding of Se bioaccumulation and toxicity in aquatic organisms. The high site-specificity of Se accumulation renders general water quality guidelines less useful, and information about the food web dynamics of Se is essential to protecting areas exposed to excess Se. In particular, information about Se assimilation by organisms at the base of the food web is lacking although it is the largest and therefore most important accumulation step in the food web.

Selenium ecotoxicology has focused on the toxic effects on oviparous vertebrates, such as birds and fish. Though these organisms are indeed sensitive to Se, relatively few investigations have focused on assessing Se toxicity to other organisms, particularly those occupying lower trophic levels. Further, even fewer studies have focused on assessing Se toxicity at the ecosystem scale. Ecosystem-level studies are critical to understanding ecotoxicological impacts of contaminants. Particularly for those that bioaccumulate, such as Se, exposure and toxicity at higher trophic levels can depend on exposure and effects at lower trophic levels. Ecosystem-level studies also incorporate indirect effects and the complicated interactions between species and trophic levels that cannot be captured in the laboratory.

My research provided new information about Se dynamics in Canadian cold-water boreal lakes. High bioaccumulation was observed at the base of the food web that propagated to higher trophic levels. At a water Se level of 1 µg/L (the current CCME guideline), Se bioaccumulation in fish tissues was similar to, or exceeded, the BC MoE and US EPA fish tissue-based criteria for the protection of aquatic life. Saturable uptake curves and decreasing k_ds or TTFs with increasing Se exposure concentration were observed for all taxa, highlighting the dynamic nature of Se bioaccumulation. The similarity in bioaccumulation of Se across the two studied boreal lakes varied with compartment, and differences in bioaccumulation among taxa at each trophic level were observed. Toxicity of Se to invertebrates occurred at relatively low aqueous exposure levels. Exposure to 1 µg Se/L decreased diversity of BMIs, and zooplankton community composition decreased in both moderate (0.8 to 1.6 µg/L) and high (3.4 to 7.9 µg/L) exposures to Se. The density and biomass of both BMIs and zooplankton decreased with increasing Se concentration.

These results suggest that invertebrates may also be at risk for Se toxicity, in some cases at concentrations similar to, or lower than, those that would affect fish.

Finally, this research provided predictive models that may be useful in assessing the extent of Se exposure in similar lentic systems, by predicting the upper limits of Se bioaccumulation in algae, invertebrates, and fish. The models varied in similarity to previous literature collected across a range of study sites and highlight some of the needs of future Se bioaccumulation studies. For instance, measurement of water chemistry variables including Se speciation and standardization of sample collection will aid in the improvement of these models.

7.2 Regulatory implications of the present studies

The present research contributes information on the extent and patterns of Se bioaccumulation in representative boreal lakes. As discussed above, the data recorded could represent "maximum expected concentrations" at the current CCME guideline in lentic systems. The research shows that under certain conditions, i.e. high residence times, high redox potential, and high biogeochemical cycling leading to high proportions of reduced Se, bioaccumulation of Se near levels of concern could be reached in invertebrates and fish. As such, these systems may be at an increased risk for Se toxicity, and more strict guidelines may be necessary for these areas.

The kinetic models and log-log relationships between trophic levels developed using the data herein provide some means of predicting potential accumulation of Se in similar boreal lakes. Using these predictive equations, Se in periphyton, invertebrates, and fish could be predicted at a particular aqueous Se level of interest and used as a guide to determine what concentrations could be expected under similar conditions. Though the accuracy of the predictions should still be tested by collecting and measuring Se in organisms from an area of concern, the models could help to minimize the number of samples that need to be collected.

Due to the previous studies that have observed that food web pathways can have large implications for Se bioaccumulation in higher trophic level organisms, a major objective of the current study was to characterize taxonomic differences in Se uptake by lower trophic level organisms (i.e. algae and invertebrates). For algae, an important difference in the accumulation of Se by different compartments (periphyton and phytoplankton) was observed. Studies have noted differences in uptake by algal taxa previously, but few of these assessments have been conducted in the field. Though not new information, this is important to consider in future ecological risk assessments of Se, where the effect of food web pathways on Se bioaccumulation needs to be

considered (Stewart et al. 2004). It is also important to recognize that, because of the taxonomic differences in Se bioaccumulation, the choice of compartments sampled can influence the results and conclusions of an ecological risk assessment.

Differences in Se bioaccumulation among invertebrates were also characterized in the present study. These differences have been observed previously (Stewart et al. 2004; Presser and Luoma 2010), but this study was able to characterize Se bioaccumulation and TTFs over a gradient of concentrations and for several taxa within the same system. The differences in bioaccumulation observed among taxa highlight a few important points for future ecological risk assessment of Se. Based on the Se bioaccumulation observed, Se concentrations in fish could vary two-fold in a system due to differences in diet of the fish. As previous studies have found, food web pathways are extremely important in determining Se bioaccumulation, and assessing the ecological risk of Se in a particular system may require studying multiple invertebrates and fish species that represent multiple food web pathways and varying diets. Further, due to the observed taxonomic differences in invertebrate Se, these organisms should be collected and separated, at least by family, for Se measurements, so that differences among taxa are not masked or diluted by composite or bulk sampling.

This research also presents valuable information on the toxicity of Se to invertebrates, which has rarely been studied under field conditions. Due to the growing interest in protecting fish-less waters from Se toxicity, and the increasing recognition that certain invertebrates may be susceptible to Se toxicity at relatively low exposure levels, the results of the studies herein will be useful to determine field-derived effect concentrations for invertebrates. We were able to determine internal Se concentrations and aqueous Se levels at which toxicity occurred for benthic macroinvertebrates and zooplankton. Based on the data presented, toxicity to BMIs likely occurs at concentrations greater or similar to what is toxic for fish. For zooplankton, toxicity occurred at internal concentrations of just 11 μg/g dm, suggesting that zooplankton tissue guidelines may need to be considered in the protection of aquatic life.

These results helped to identify potential bioindicators of Se toxicity. For zooplankton, cladocerans appeared to be more sensitive to Se than copepods. As such, the presence or absence of typical cladoceran taxa, such as *Bosmina longirostris*, may be useful in identifying whether Se is having an impact on zooplankton communities. For BMIs, Heptageniidae was identified as being sensitive to Se exposure. The Order Ephemeroptera has been regarded as pollution intolerant and

is frequently used as a bioindicator as water quality, and these organisms could be used in a similar way to detect Se impacts on aquatic organisms.

7.3 Integration of ERASe project results

Given the site-specificity of Se bioaccumulation in aquatic systems due to Se speciation, water chemistry characteristics (ex. pH, competing ions, reducing conditions), and taxonomic differences in uptake of Se, there are considerable knowledge gaps requiring more research before ecological risk assessments of Se can be entirely effective. The ERASe project, of which my thesis research was one part, used several approaches to characterize bioaccumulation, trophic transfer, and toxicity of Se to organisms within Canadian boreal lake ecosystems. The overall goals of this large project were to address knowledge gaps and contribute to a better understanding of key processes related to Se exposure and effects in cold, freshwater systems.

The main goal of my thesis research in particular was to conduct field-based mesocosm experiments to better characterize Se uptake by algae and subsequent trophic transfer to invertebrates and fish. As expected due to previous observations that taxonomic differences in Se uptake by algal species are very high, Se bioaccumulation at the base of the food web was variable among the mesocosm studies and the laboratory studies using both algal monocultures and field-grown periphyton conducted as part of the ERASe project (Markwart 2019; Markwart et al. 2019; Raes 2020). For instance, in the present research, periphyton and phytoplankton Se concentrations ranged from approximately 40 to 50 µg/g dm at 5 µg Se/L (added as SeIV), whereas in the laboratory-grown monocultures, exposure to 5 µg SeIV/L resulted in mean Se concentrations of 8.9 µg/g in a chlorophyte *Stichococcus bacillarus* to 24.4 µg/g in a cyanophyte *Anabeana flos-aquea* (Markwart 2019). In field grown periphyton exposed to 5 µg SeIV/L, Se concentrations ranged from 14.5 µg/g OM dm in samples dominated by Bacillariophyta to 222.3 µg/g OM dm in samples composed of 55% Cyanophyta and 37% Chlorophyta (Markwart et al. 2019). The high accumulation in periphyton observed by Markwart (2019) was attributed to surface adsorption to iron hydroxides, and not due to internalized Se. In a separate study by Raes (2020), 5 µg SeIV/L resulted in mean periphyton Se concentrations up to 15 µg/g dm in samples composed of Bacillariophyta (40%), Cyanophyta (38%) and Chlorophyta (21%). Both the laboratory studies conducted by Markwart et al. (2019) and Raes (2020) and the field-based studies conducted herein illustrate the high variability in Se accumulation based on taxonomic differences in uptake, and it is important to note again that understanding the influence of periphyton community composition

on Se uptake is critical to better understanding and predicting bioaccumulation in systems with elevated Se.

The (generally) greater Se bioaccumulation in the mesocosm studies herein relative to the previously conducted laboratory studies could be due to differences in periphyton community composition and/or the conditions in the mesocosms. Though algae in the present research were not identified microscopically, historical data show that periphyton communities in Lake 239 are typically dominated by diatoms (Bacillariophyta) and phytoplankton communities are comprised largely of Chrysophyta and Dinophyta (IISD-ELA unpublished data). The mesocosms used herein, which were closed systems with relatively high residence times, may have provided greater reducing conditions and promoted the production of more bioavailable forms of Se. The mesocosms were open to the organic-rich sediment which could provide a means for the microbial reduction of inorganic Se to organo-Se. Organo-Se compounds, such as SeMet, typically accumulate to a higher extent than inorganic Se oxyanions and would result in greater overall Se uptake. In addition, the longer experimental period of the present studies (63 to 77 d) relative to the laboratory studies (7 to 14 d) may have resulted in greater Se uptake.

Trophic transfer of Se from algae to *Hyalella azteca* to fathead minnow was assessed in the laboratory by Raes (2020). They found that *H. azteca* fed *S. bacillarus* showed TTFs up to 7.7, unlike the mesocosm experiments where TTFs were generally around 1 for amphipods. Though in general much greater accumulation in algae and invertebrates were observed in the present mesocosm study at similar aqueous Se concentrations, the same trend of decreasing TTFs with increasing exposure Se was observed, suggesting again that Se uptake kinetics are concentration-dependent. Trophic transfer from invertebrates to fish appeared similar between the laboratory and mesocosm studies. In the laboratory, TTFs for fathead minnow females (based on muscle tissue Se) fed *H. azteca* supplemented with un-dosed chironomids was 0.78 in the controls, and decreased to 0.24 in fish fed a diet with 19 µg Se/g. A similar range in TTFs was observed for fathead minnow and finescale dace in the present research (0.5-1.4), and these TTFs also decreased with increasing Se exposure. As well, both the laboratory and field investigations found that ovary tissue Se concentrations were 2-3 times higher than muscle tissue. Similar egg/ovary concentrations were observed when dietary exposure concentrations were similar between the laboratory and mesocosm studies as well. At dietary concentrations of 19 µg/g, embryo Se concentrations were 15 µg/g dm (Raes 2020), while fathead minnow and finescale dace ovary concentrations ranged

from 12-20 µg/g among individuals when exposed to similar dietary Se concentrations in the studies herein.

Lane et al. (2019) and Raes (2020) assessed Se toxicity to developing fathead minnow embryos using three approaches: 1) maternal transfer following feeding with a naturally-selenized food, 2) maternal transfer via direct feeding with SeMet-laden food, and 3) direct SeMet embryo microinjection. Across all approaches, they found that incidences of embryo-larval deformities increased at embryo Se concentrations of 6.9 µg/g for naturally-selenized dietary exposure, 9.7 µg/g for microinjections, and 28 µg/g for maternal transfer of SeMet-laden food. As mentioned above, fish ovary tissue Se concentrations in the present research ranged from 12-20 µg/g when aqueous exposure levels were at or near the current CCME guideline (1 µg/L). Taken together, these results from laboratory and field studies suggest that, under certain conditions (i.e., large proportions of selenite in water, reducing conditions), high biological uptake and trophic transfer of Se may result in fish toxicity at aqueous concentrations near the CCME water quality guideline for the protection of aquatic life. Though not an intentional endpoint of the study, Raes (2020) also observed concentration-dependent decreases in *H. azteca* biomass at concentrations of 9 µg Se/L. This is corroborated by the results of the present thesis research, wherein decreased biomass and density of amphipods was observed at 8.9 µg/L. These results provide more evidence that invertebrates may be impacted by Se exposure at levels lower than previously thought, and that declines in invertebrate biomass may exacerbate Se toxicity to higher trophic level organisms.

Thus far, the ERASe project has contributed to the Se knowledge base and reduced uncertainty regarding Se bioaccumulation at different levels of exposure for both selenite and selenate, and differences in bioaccumulation among algae, invertebrate, and fish taxa. It has also identified the potential for toxicity to invertebrates and fish at environmentally relevant exposure levels. The data collected will aid in better prediction of Se distribution and effects in Canadian boreal lakes and thus, will be useful for future ecological risk assessments of selenium.

7.4 Advantages and limitations of mesocosms in Se ecotoxicology

Mesocosms are artificially constructed or "model" ecosystems designed to make observations or test ecological hypotheses at an intermediate level of environmental realism between laboratory microcosms and the real-world macrocosm (Odum et al. 1984; Caquet et al. 2000). The advantages of using mesocosms, particularly in the context of Se ecotoxicology, are numerous but not without trade-offs. Mesocosm studies are able to incorporate many trophic levels

and thus, interactions between trophic levels or different species (Parsons 1982). This is important in understanding the fate and effects of contaminants in ecosystems, for instance, Se is bioaccumulative, such that concentrations in higher trophic level organisms depend on uptake at the base of the food web. It is less realistic to conduct studies with multiple species in the laboratory, and it is difficult to incorporate complex species interactions into laboratory experiments. For the same reason, indirect effects of contaminants can be difficult to assess in the laboratory as well. For example, in the present study I observed decreased invertebrate density and biomass that were correlated with decreased fish condition factor. Since fish tissue Se concentrations observed herein have not been shown to directly impact the health of adult fish, I hypothesized that decreased condition factor was a result of decreased food availability. If fish were fed *ad libitum* in the laboratory, this effect would not have been observed.

Mesocosms also incorporate the effects of light, nutrients, and sediment and associated processes. The incorporation of these natural processes is essential to understanding the fate of Se, as these factors can all contribute to the uptake, movement between compartments, and re-cycling of the element. For instance, light and nutrient levels can impact the growth of primary producers and could therefore influence the uptake of Se (Riedel et al. 1996; Schlenk et al. 2007). Sediment, and associated processes that occur in surface sediment, are key to the biogeochemical cycling of Se. Sediment can represent a significant sink for Se (as shown in the mass balances in the present studies) through the processes of adsorption to sediment organic matter or iron hydroxides, assimilation and reduction by sediment-associated microorganisms, as well as detritus from dead and decaying organisms that have accumulated Se (Bruggeman et al. 2007; Luoma and Rainbow 2008; Peel et al. 2017). Sediment processes can also influence the speciation, and therefore the bioavailability and uptake, of Se. Sediment-associated microorganisms may reduce Se oxyanions to elemental or organo-Se. The incorporation of these variables into the present experiments increased realism of the studies and provided a means to study the distribution of Se, which would not be possible in the laboratory.

Another advantage of the use of mesocosms is that they can be manipulated in a desired way, such that they maintain some control, for instance, in the exposure of a contaminant. The effects of a particular contaminant or different mixtures of contaminants are difficult to assess in natural systems because there are often multiple stressors present and finding appropriate replication or reference sites is difficult. The ability to replicate mesocosms is beneficial, since this

allows an increase in the statistical power and ability to detect or discern effects and patterns. In mesocosms, one can choose one or several aspects of a system to manipulate in a known and controlled manner. This is relevant to Se in particular because some of the anthropogenic activities that contribute to excess Se release in aquatic systems, such as mining, are associated with the release or presence of several metals, metalloids, and other stressors simultaneously. This can make discerning the effects of Se from other correlated stressors difficult. In addition, mesocosms allow the assessment of time-dependent effects, since the contaminant of interest can be studied both before and after the additions (Riebesell et al. 2010).

It is also important not to over-state the applicability of these mesocosm-based studies to the real-world, since mesocosms are unable to represent all of the processes and interactions of a whole ecosystem. A comparison of results of various mesocosm versus whole-lake studies highlighted that, regardless of the size of a mesocosm, there are some components of natural ecosystems that just cannot be incorporated and as such, results of mesocosm experiments do not always match that of whole-lake studies (Schindler 1998). One factor of mesocosms that can impact the fate of chemicals, and could have affected the kinetics of Se uptake, is that there is less wave energy transferred within limnocorrals compared to the rest of the lake, leading to less mixing and perhaps less air-water gas exchange within enclosures (Schindler 1998).

Another particularly important limitation of mesocosm studies is that, especially in the boreal forest, these studies are often limited to one season due to the difficulty of over-wintering enclosures in a lake that freezes. I attempted to conduct an overwinter experiment as part of my thesis research, but the logistical difficulties associated with accessing, adding Se, and sampling the limnocorrals throughout the winter made this attempt unsuccessful. As such, in the present studies, we were limited to ecological phenomena that occur seasonally. In the present work, both sediment and fish tissue concentrations did not appear to reach a steady state, and these compartments likely take longer than the season allowed to reach an equilibrium. Even if I did continue the study for the full length of the ice-off period (approximately end of May to October/November), this would present additional issues with long-term studies in mesocosms. Longer-term studies are difficult because the mesocosms can be separated from nutrient sources (Schindler 1998). Typically, as the length of mesocosms studies increase, the more different the dynamics inside the enclosures are from the rest of the lake, decreasing the realism of the experiment. The relatively short duration of mesocosm studies is also not sufficient to encompass

the life-span of many invertebrates and vertebrates, such that there may not even be time to see all potential effects of the contaminant exposure.

Further, while it is possible to incorporate lower trophic levels into mesocosms, it is difficult to incorporate higher trophic levels in the relatively small area of a mesocosm due to insufficient food biomass. For instance, in the present study, the sample size and the species of fish studied was limited by what could be supported within the enclosures. The low biomass may have affected the time to steady state for fish, and as discussed in Chapter 3, the even lower biomass of food in Se-treated limnocorrals likely had an impact on overall fish health. In 2018, I attempted to conduct a reproductive life cycle bioassay with fathead minnows in Lake 114. This experiment was unsuccessful as only a total of 5 egg clutches were collected from six limnocorrals from June to August. I was not able to determine a cause for the lack of reproduction occurring in the limnocorrals, but it is possible that the low reproductive rates were related to low food biomass within the ~3000-L enclosures.

7.5 Comparison of ANOVA and regression experimental designs

In this thesis, two different experimental approaches were used. In the first field season, an ANOVA design with three experimental groups in triplicate was used. In the second field season, a regression design in which the control group was triplicated and there were six different, unreplicated treatment levels. Though ANOVA is distinct type or subset of regression and these two techniques ultimately share the same underlying mathematical model, they did have some different strengths and weaknesses.

In the present study, the ANOVA design was particularly useful when no prior information was available to indicate the amount of variability that could be expected between mesocosms at the same exposure concentration. Without knowing the relationship between the dependent variable (Se bioaccumulation) and independent variable (Se source concentration), a linear regression design would be a gamble in terms of being able to statistically analyse the results. The ANOVA design was useful in assessing the variability both in bioaccumulation and in toxicity. With this approach we were also able to set the mesocosms in three areas of the lake that, based on visual observation, varied in organic matter content. Specifically, in the context of Se, the ANOVA design was useful because the site-specificity of Se is very high, and at the outset of these experiments, I was unsure how much variability in bioaccumulation I could expect in different

limnocorrals in different areas of a lake. The 10-fold difference between each of the treatments provided information about the range of values that could be expected.

In contrast, while the un-replicated regression design could not inform about the variability between limnocorrals at a given concentration, it gave much more information about the concentration-response relationships of both bioaccumulation and toxicity. In general, this design was beneficial because it provided more information on the direction of the relationship between variables, and not just that there were differences. This design was more useful in developing predictive equations between dependent and independent variables, and will ultimately be more useful in extrapolating to other systems or as a predictive tool.

The ideal experimental design for a limnocorral experiment such as this would be a fully replicated regression (Cottingham et al. 2005). With this approach, there are several levels of exposure that are each replicated at least once. This would better control for the inherent variability in each limnocorral, and, were linear regression judged to not be an appropriate analysis of the data, then ANOVA could be used as a fallback to compare among treatments (Cottingham et al. 2005).

7.6 Use of selenite versus selenate in experiments

In the present studies, selenite was used as the species of Se added to enclosures due to its particular relevance and greater concern in lentic systems. While selenite and selenate are the two most common forms of Se found in water, the relative proportions of each differ in systems as a result of the source speciation of Se and the conditions of the receiving system (Presser and Luoma 2010). Selenate is more common in agricultural drainage, mountaintop coal mining, and metals mining discharges, while selenite is more often found in oil refinery and fly-ash disposal effluents (Presser and Luoma 2010). In terms of receiving system influence on Se speciation, typically selenate, the more oxidized form of Se, is more common in lotic systems and selenite, the more reduced Se oxyanion, is more common in lentic systems. Few studies have measured Se speciation in Canadian boreal lakes, but selenite in mine-affected lakes in Quebec and Ontario ranged from <7 to 68% of total Se (Ponton and Hare 2013), while in the present study, selenite in pristine Canadian boreal lakes ranged from 52 to 79% of total Se. The high proportions of selenite provide justification for the use of this species of Se. However, since selenite is taken up at a higher rate than selenate, the addition of 100% selenite likely means that these experiments represent a “worst

case scenario" for Se exposure, and that bioaccumulation of Se in organisms observed herein likely represents the upper limits of what would be expected in similar systems.

No Se speciation analysis was conducted in these limnocorral studies, so it is not possible to know whether dissolved Se in the limnocorrals stayed in the form of selenite, or if a significant portion was reduced further to organo-Se. The potential for biogeochemical of cycling of Se in these mesocosms is reasonable, considering, particularly in the mesotrophic study lake, that productivity was fairly high and that the high organic matter surface sediment provided substrate for the microbial reduction of Se. Considering that organo-Se is taken up at greater rate than inorganic Se, this would contribute to the high Se bioaccumulation observed in the present study.

In hindsight, based on the aqueous Se speciation data available, the most realistic scenario for Se exposure in most lentic systems would be a mixture of selenate and selenite. Exposing organisms to Se in different proportions of selenite and selenate would better mimic several different sources and site-specific conditions. However, conducting these experiments with mixtures of these two Se species would also add a significant amount of logistical difficulty to the experiments. One approach to improve the realism of these experiments would have been to tag selenite and selenate with different stable Se isotopes to determine the relative uptake of each Se species under the same conditions.

Due to the fact that relative proportions of selenite and selenate are likely to vary at each specific site, a better approach might be to model Se uptake under conditions of 100% selenate or 100% selenite exposure separately, and then correct for the proportion of each Se species present. Differences in Se speciation are likely a major reason for the observation that periphyton Se accumulation herein was greater than for other study systems, and perhaps using the correction above is necessary. In addition, due to the high proportion of selenite, the aqueous Se concentrations at which Se toxicity occurred herein are likely lower than would be observed elsewhere. In terms of relating the toxicity data to real-world scenarios, it would be best to use internal Se concentrations, rather than aqueous Se concentrations, to express Se levels of concern for invertebrates.

7.7 Future research needs and recommendations

Several gaps remain in the literature that would aid our understanding of Se ecotoxicology and improve the site-specific prediction of Se bioaccumulation. First, more information on the differences in bioaccumulation between selenate, selenite, and organo-Se in natural systems is

necessary. Given the importance of Se speciation to bioaccumulation, it seems prudent to collect this information with every field study moving forward. This information will be critical to improving predictive models of Se bioaccumulation in the future. The inclusion of other pertinent water chemistry characteristics in field studies is also recommended, given the potential effects of factors such as nutrient levels, pH and competitive ions like sulphate or phosphate.

As discussed in more detail in Chapter 6, differences in Se bioaccumulation by different taxa were evident at several trophic levels. Thus, a greater focus on characterizing species composition of samples is necessary to better predict Se bioaccumulation. Related to this, there is currently little understanding of the mechanisms by which some organisms accumulate greater amounts of Se than others. A combination of both field and laboratory studies could be used to determine mechanisms of uptake for several organisms including phytoplankton and periphyton communities, and invertebrate taxa. Based on these taxonomic differences, future studies should prioritize separation of taxonomic groups when measuring Se, and more data regarding Se bioaccumulation in different taxa is necessary. For invertebrates, the literature currently seems to be biased towards benthic macroinvertebrates. Much less data on zooplankton Se bioaccumulation is available, and this is perhaps due to more of a focus on lotic systems. The Se literature would benefit from more information on zooplankton, especially considering that some zooplankton taxa appear to be quite sensitive to Se.

Future studies should aim to improve the modelling and prediction of Se bioaccumulation in lentic and lotic systems. The models presented herein can be refined in many ways. Including water chemistry parameters of interest, such as pH, sulfate, and nutrient levels would help to identify the variance in Se bioaccumulation due to competition and other water chemistry factors. The types of taxa and characteristics of the taxa being sampled, at all trophic levels, will help to decrease variability in bioaccumulation as well. This can be done more easily for invertebrates and fish, but more work is required to determine taxonomic composition of periphyton and phytoplankton. Having more information for individual taxa will also help to identify dietary preferences, which would also help improve the current models. In particular, fish Se bioaccumulation is likely largely dependent on dietary preferences and habitat use. By expanding our knowledge of all of these parameters, Se prediction can be expanded to several different systems.

Due to the finding that Se can impact invertebrates at relatively low Se concentrations (11 µg/g dm in zooplankton), there is a need for more field studies to better characterize the effects of Se on different invertebrate taxa and to investigate the longer-term impacts on ecosystems. Studies should focus on dietary exposures and assess toxicity based on internal concentrations. More longer-term studies are needed to encompass the life span of longer-lived organisms. In addition, more field-based studies with wild organisms are necessary to improve the environmental relevance of results. Currently, very few field studies assess the effects of Se alone on invertebrates and extremely few studies are conducted in lake systems. The invertebrate studies to date are largely mining-related, where it is difficult to tease apart the effects of Se from other stressors, and most studies are focused on lotic systems. To increase the diversity of taxa and habitats being studied, more studies should be done in other types of systems. Further studies related to the mechanism of action for Se toxicity to invertebrates are needed as well. While it is plausible that the mechanism may be similar to vertebrates, where metabolism of SeMet leads to the production of reactive oxygen species and an increase in oxidative stress, this has not been tested for invertebrates.

Ultimately, the best way to test the fate and effects of Se in a representative boreal lake would be to conduct a whole-lake Se experiment. Though the cost and effort would be greater, several unanswered questions could be addressed in a manner more realistic than mesocosm or laboratory studies. This design would better incorporate the hydrodynamic aspects of the speciation and fate of Se such as mixing and redox conditions of a representative lentic system. It would also better incorporate several different species and trophic interactions that are essential to understanding the bioaccumulation and trophic transfer of Se through food webs. To further increase the realism of the experiment, selenite and selenate could be added in proportions that best represent anthropogenic sources of Se in these lakes, for instance, perhaps 50% selenate and 50% selenite could be added to the lake. As discussed above, using different Se stable isotopes to label the two species of Se would help to better understand the fate and relative uptake rates of the two oxyanions.

Further, an important factor that has not been previously been incorporated into the studies of Se effects on aquatic ecosystems in Canada is the impact of winter conditions on both bioaccumulation and toxicity. In the boreal lakes studied herein, the ice-on season can span from November to May, and this is expected to have significant effects on Se trophic dynamics, where

organisms that are known to be more active throughout the winter may accumulate different levels of Se than those that are relatively dormant. In addition, Winter Stress Syndrome has been proposed previously as a way in which the combination of elevated Se and lower temperatures may increase toxicity to fish. In this case, energy stores (as lipids) decrease due to a combination of decreased feeding and increased energetic demands due to lower temperatures (Lemly 1993).

A whole-lake study would enable a study of Se fate and effects over multiple years of study, allowing for a longer-term investigation of Se impacts over the complete lifespan of several invertebrates and fish. Finally, one of the most important benefits of conducting a whole-lake study to determine the fate and effects of Se in Canadian boreal lakes is that such a study could encompass three time periods: before, during, and recovery from Se additions. This powerful before-after control-impact design would allow more certainty in attributing impacts on aquatic organisms directly to Se, and the recovery portion of the study would be of great interest, for instance, to decommissioned mines where it is important to know how long it takes for an ecosystem to recover from Se-related impacts.

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APPENDIX A

SUPPORTING INFORMATION FOR CHAPTER 2

Supporting Information A.2.1: Spiking limnocorrals with selenium

For spiking, sodium selenite (Sigma Aldrich, Oakville, ON, Canada) was dissolved in ultrapure water (Barnstead® DiamondTM NANOpure, 18 MΩ/cm; Barnstead International, Massachusetts, USA) to create a stock solution of 20 g Se/L. Secondary stock solutions were made on the day of spiking by adding a calculated amount of stock (using limnocorral water volume and target concentration) to a pre-cleaned 1-L high density polyethylene (HDPE) Nalgene bottle containing 1 L of limnocorral water. The secondary stock solutions for each limnocorral were transported to the field in a cooler on ice, added to the limnocorral water, and mixed gently with a wooden paddle. Surface water was sampled from each limnocorral 3 h post-spike to determine initial Se concentrations.

Supporting Information A.2.2: Mass balance calculations

Filtered aqueous TSe mass was calculated as the Se concentration measured in water multiplied by the water volume of the limnocorral measured at d 77 (volume calculated by taking the mean of 5 depth measurements per enclosure). Periphyton Se mass was calculated as the Se concentration measured in the material from the curtain strip multiplied by periphyton dry mass on a known area of suspended curtain strip and the surface area of the curtain for the entire limnocorral curtain. Benthic macroinvertebrate Se mass was calculated as the Se concentration in benthic macroinvertebrates multiplied by the mass of benthic macroinvertebrates collected at d 77 per Hester-Dendy sampler and the surface area of the limnocorral sediment. Zooplankton Se mass was calculated as the zooplankton dry mass in a known volume of sampled water multiplied by the zooplankton Se concentration on d 77 multiplied by the volume of water in the limnocorral. For fathead minnow, Se mass was calculated as the whole body dry mass of a fish (assumed to be 75% moisture; McPhee and Janz 2014) multiplied by the whole body TSe concentration (converted from muscle TSe; US EPA 2004). Selenium mass in sediment was calculated as the measured sediment Se concentration in the top 3 cm of a core multiplied by the density of the sediment (dry mass of sediment/volume of core) multiplied by the volume of the limnocorral surface sediment (2 m diameter, 3 cm core). To account for background Se, background water and sediment Se masses were subtracted from quantified treatment water and sediment Se masses. The total Se

accounted for was calculated as the sum of the Se in these six compartments divided by the total Se mass added to each enclosure.

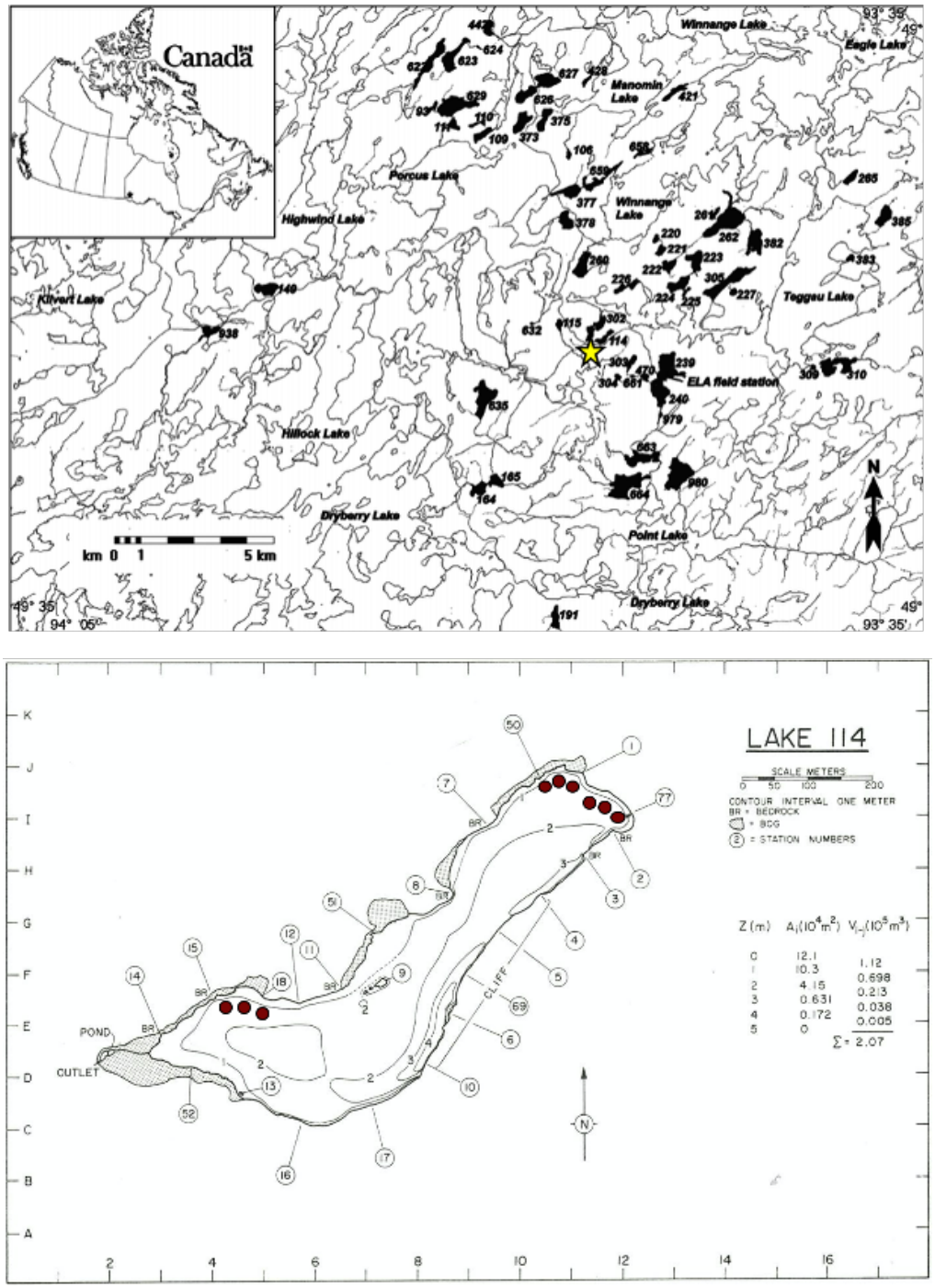

Figure A.2.1: Map of the Experimental Lakes Area (reproduced from Blanchfield et al. 2009) with a yellow star indicating the study lake (Lake 114), and a bathymetric map of Lake 114 with red circles indicating the areas of limnocorral placement.

Table A.2.1: Summary of sampling days and matrices collected from each limnocorral during the experimental period.

Day of Experimental Period	Water	Periphyton	Zooplankton	Benthic Macroinvertebrates	Fathead Minnow	Surface Sediment
-2	X	X	X	X		X
0	X					
3	X					
7	X	X	X	X		
14	X	X	X	X		
21	X					
28	X					
33		X	X	X	X	X
35	X					
42	X					
49	X	X	X	X	X	
56	X					
63	X	X	X	X	X	
70	X					
77	X	X	X	X	X	X

Table A.2.2: Masses of each matrix (g) per limnocorral used in mass balance calculations.

Treatment	0.12 µg/L			1.0 µg/L			8.9 µg/L		
Replicate	1	2	3	1	2	3	1	2	3
Sediment	31.2	39.6	17.7	24.3	42.7	24.3	15.1	26.0	25.7
Periphyton	16.4	53.8	20.9	8.7	24.6	25.9	20.8	95.2	52.5
Zooplankton	0.0020	0.0018	0.0008	0.0016	0.0004	0.0010	0.0005	0.0009	0.0018
Benthic Macroinvertebrates	0.0047	0.0166	0.0318	0.0872	0.0078	0.0107	0.0000	0.0848	0.0029
Fish	1.63	1.31	0.25	0.79	0.96	0.81	0.55	0.19	0.57

SUPPORTING INFORMATION FOR CHAPTER 4

Supporting Information A.4.1: Limnocorral set-up and artificial substrates

In May 2018, artificial substrates for periphyton (strips of six 4.8 x 4.8 cm unglazed clay tiles) and benthic macroinvertebrates (Hester-Dendy samplers with 0.001 m^2 surface area and cobble baskets filled with 20 fist-sized rocks) were deployed in the same area as the limnocorrals to colonize for two weeks before transfer to limnocorrals. On d -10 of the experimental period, artificial substrates were transferred to the limnocorrals. Clay tiles were suspended 40 cm from the water surface and Hester-Dendy samplers were suspended so that they sat at the sediment-water interface without sinking into sediment. Rock baskets were also suspended at the sediment water interface. Strips of limnocorral curtain material (40 x 10 cm) were suspended in the water column 40 cm from the water surface as well in order to estimate periphyton growth on the limnocorral walls. Baited minnow traps were deployed within each limnocorral prior to the beginning of the experiment to remove any endemic fish. In an effort to start the experiment with similar plankton communities across all enclosures, each limnocorral was seeded with zooplankton collected using a 150 µm horizontal tow net in areas near the enclosures (details below in section 4).

Supporting Information A.4.2: Leakage estimation using sodium chloride

Although the side curtains of enclosures were anchored to the lake bottom with sandbags, small amounts of the inert compound sodium chloride (NaCl) were added to each enclosure to monitor potential leakage throughout the experimental period. Sodium concentrations were measured in the same water samples collected for TSe analysis using ICP-MS (see Materials and Methods section ICP-MS analysis). Sodium concentrations increased over the experimental period as a result of evaporation. Therefore, in order to assess leakage, Na^+ concentrations were first adjusted to account for the observed changes in depth. After depth correction, the percent change in Na^+ from the beginning to end of the experiment ranged from 0 to 11%, indicating minimal leakage in the enclosures (Figure A.4.2).

Supporting Information A.4.3: Seeding enclosures with plankton

Prior to the experiment start date, a 150-µm mesh tow net was pulled ~ 50 cm from the water surface in areas of 1-2 m depth near the enclosure set-up area to collect representative littoral zooplankton. Several tows were conducted to collect zooplankton biomass, which was then rinsed

into a clean bucket. The zooplankton was concentrated using the 150 µm mesh tow net and diluted into 10 L of lake water. For each enclosure, one liter of concentrated zooplankton was added by swirling the bucket and collecting the organisms in a clean nalgene bottle.

Supporting Information A.4.4: Speciation of selenium in a range of Experimental Lakes Area (ELA) lakes

In September 2019, five ELA lakes that ranged in water chemistry characteristics were sampled for water selenium speciation. Sample collection and analysis followed the methods detailed in Donner and Siddique (2019). Briefly, a 250 mL acid-washed polypropylene sampling bottle was used to collect a sample of surface water 20 cm below the water surface in an area with a total water depth of approximately 1 m. Water was immediately filtered on site through a 0.45 um polyethersulfone (PES) filter into an acid-cleaned 8 mL Nalgene bottle with no head space (12 mL of water total added). Two replicate samples per lake were taken. Sample blanks, which were two 250 mL acid-washed polypropylene sampling bottle filled with reverse osmosis water taken to the field and opened during sampling, were analyzed with the lake sample. Water samples were kept on ice for transport to the lab, then were stored on ice for transport and analyzed within one week of collection at the University of Alberta using previously validated methods from Donner and Siddique (2019). Total Se and Se speciation (SeIV, SeVI, selenomethionine and methylselenocysteine) were measured. Percent selenite was calculated as: 100 x (SeIV (µg/L)/total Se (µg/L)).

Supporting Information A.4.5: Mass balance of Selenium

The mass of Se measured in each compartment within each limnocorral was calculated as follows: filtered aqueous TSe mass was calculated as the Se concentration measured in water multiplied by the water volume of the limnocorral measured at d 63. Phytoplankton Se mass was calculated as the Se concentration measured in 1 L of filtered water multiplied by the mass of phytoplankton/particulate matter on the filter and the total volume of water in the limnocorral. Periphyton Se mass was calculated as the Se concentration measured in the material collected from a strip of limnocorral curtain material multiplied by periphyton dry mass on a known area of suspended curtain strip and the surface area of the curtain for the entire limnocorral curtain. Benthic macroinvertebrate Se mass was calculated as the Se concentration in benthic macroinvertebrates multiplied by the mass of benthic macroinvertebrates collected at d 63 per Hester-Dendy sampler and the surface area of the limnocorral sediment. Zooplankton Se mass was

calculated as the zooplankton dry mass in a known volume of sampled water multiplied by the zooplankton Se concentration on d 63 multiplied by the volume of water in the limnocorral. For finescale dace, Se mass was calculated as the whole body dry mass of a fish (assumed to be 75% moisture based on fathead minnow; McPhee and Janz 2014) multiplied by the whole body TSe concentration (converted from muscle TSe; US EPA 2004). Selenium mass in sediment was calculated as the measured sediment Se concentration in the top 1 cm of a core multiplied by the density of the sediment (dry mass of sediment/volume of core) multiplied by the volume of the limnocorral surface sediment (2 m diameter, 3 cm core). To account for background Se, background water and sediment Se masses were subtracted from quantified treatment water and sediment Se masses. The total Se accounted for was calculated as the sum of the Se in these seven compartments divided by the total Se mass added to each enclosure.

Supporting Information Figures:

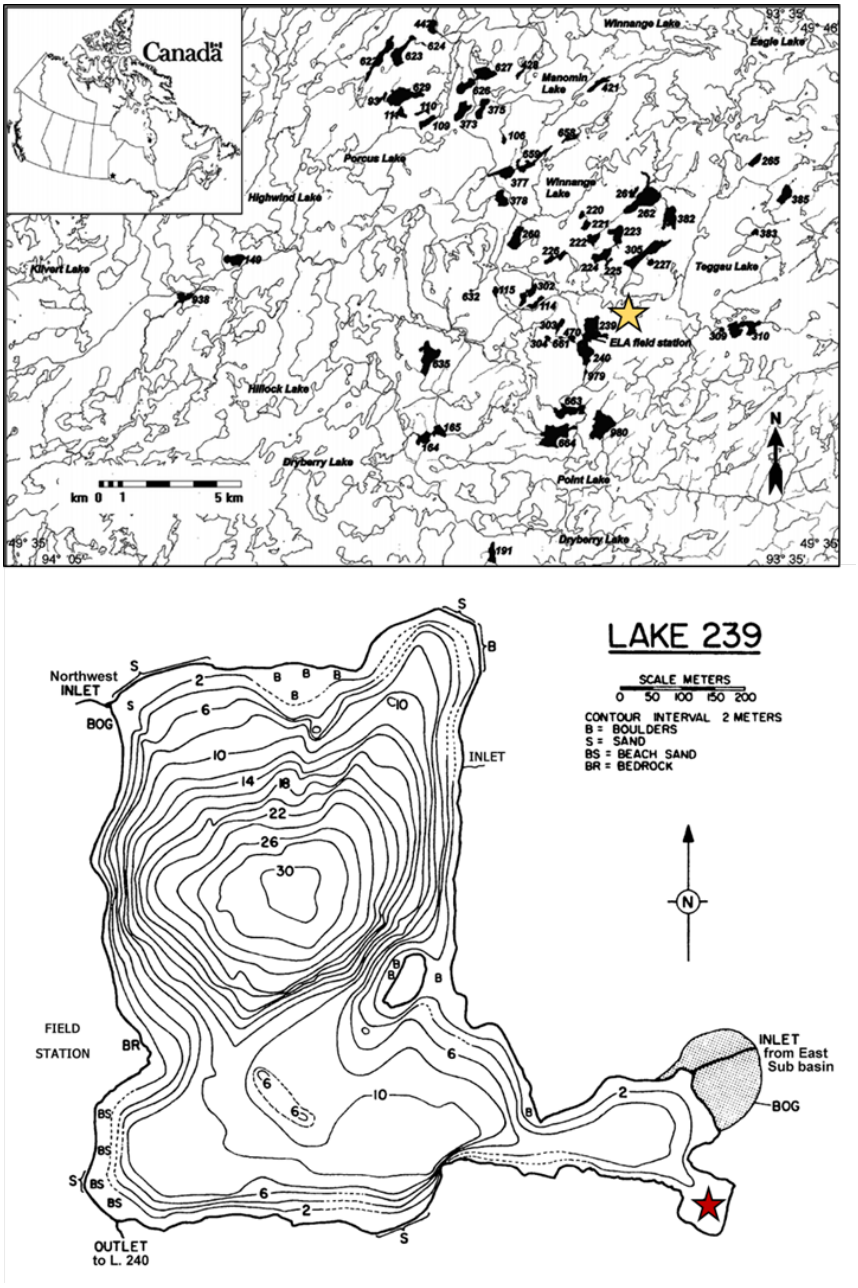

Figure A.4.1: Map of the Experimental Lakes Area (reproduced from Blanchfield et al. 2009) with a yellow star indicating the study lake (Lake 239), and a bathymetric map of Lake 239 with a red star indicating the area of limnocorral placement.

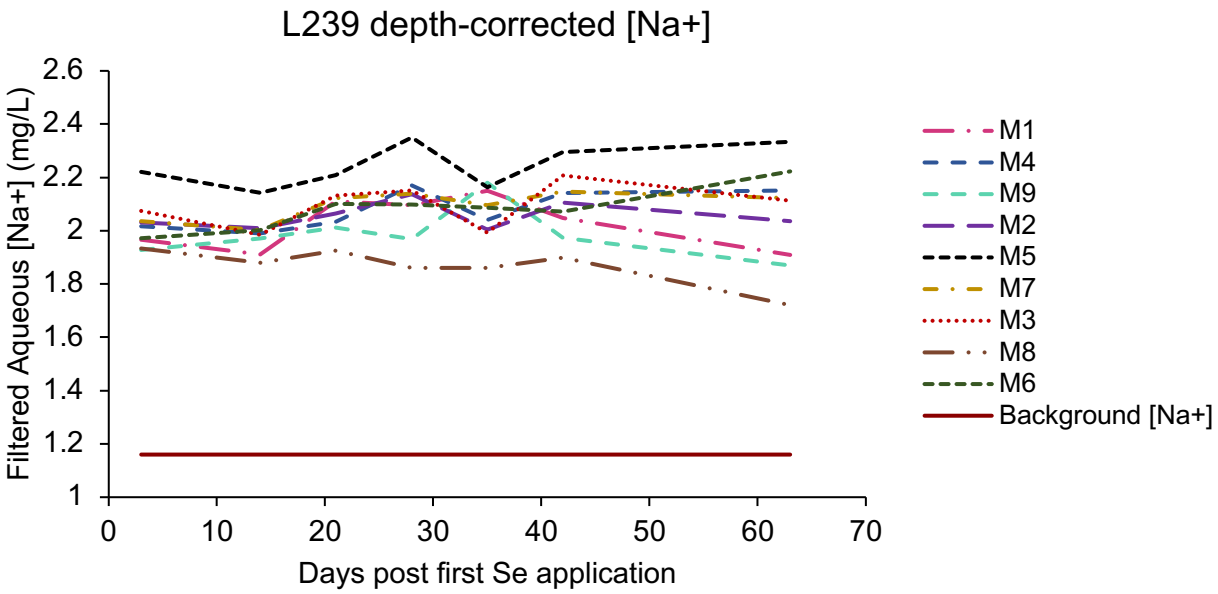

Figure A.4.2: Aqueous sodium ion concentrations corrected for the known change in water depth in each limnocorral throughout the experimental period. Background [Na^+] represents the mean background Na^+ concentration measured in enclosures before the addition of NaCl.

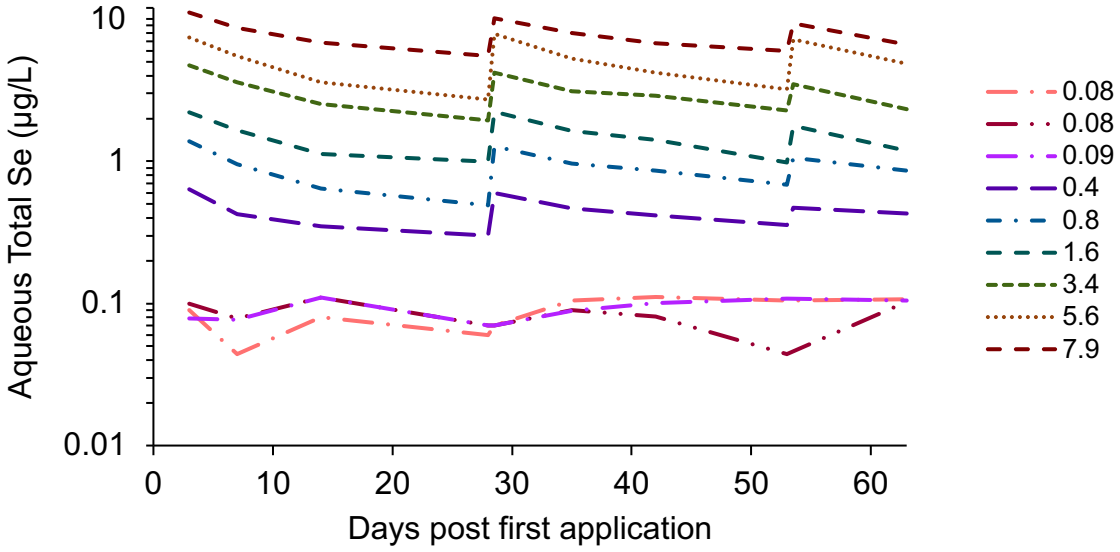

Figure A.4.3: Measured aqueous dissolved total selenium within each limnocorral throughout the experimental period. Legend shows mean measured TSe for each limnocorral.

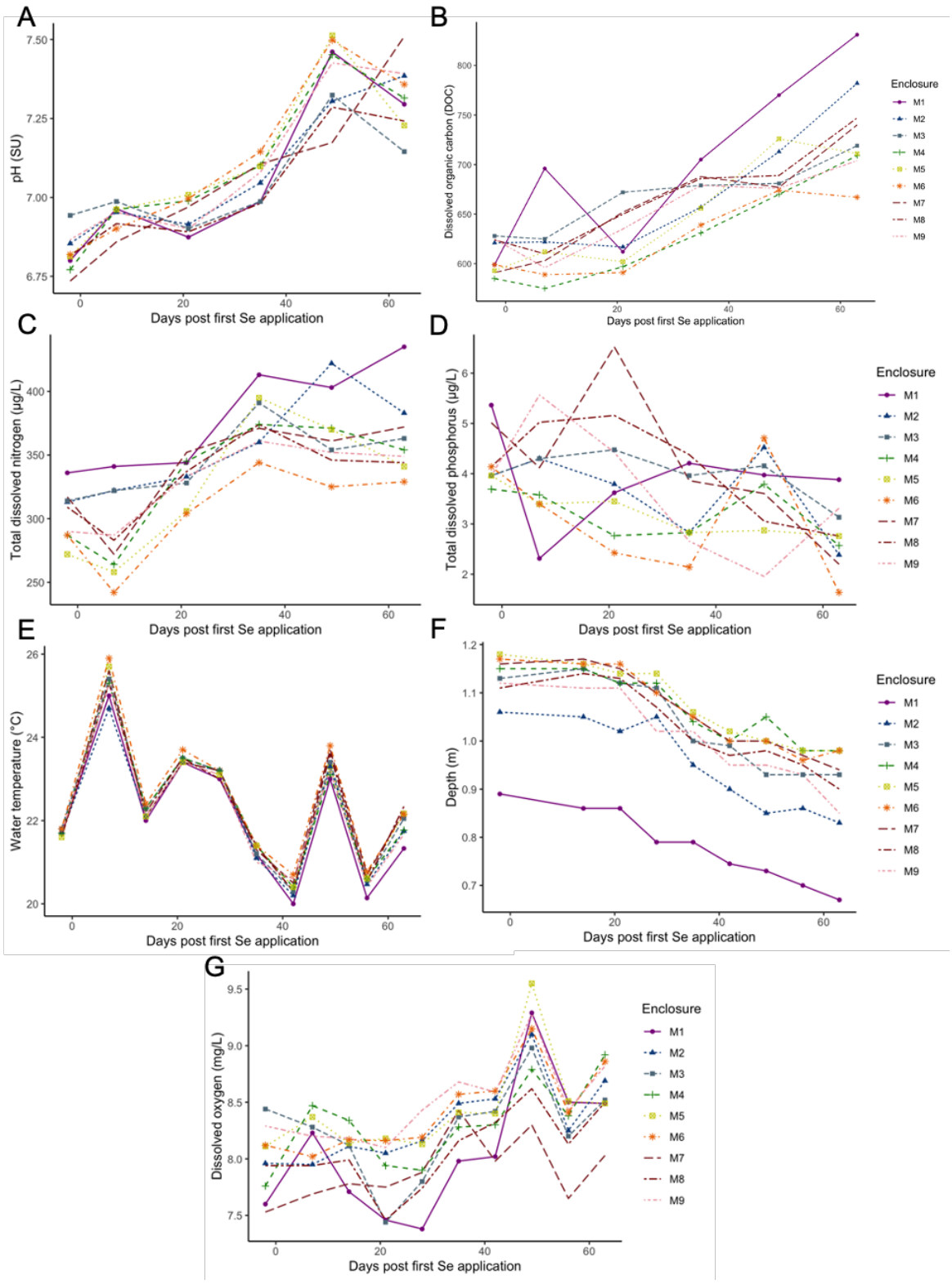

Figure A.4.4: Water chemical and physical parameters measured throughout the experimental period from days -2 to 63 for each enclosure labelled M1 to M9.

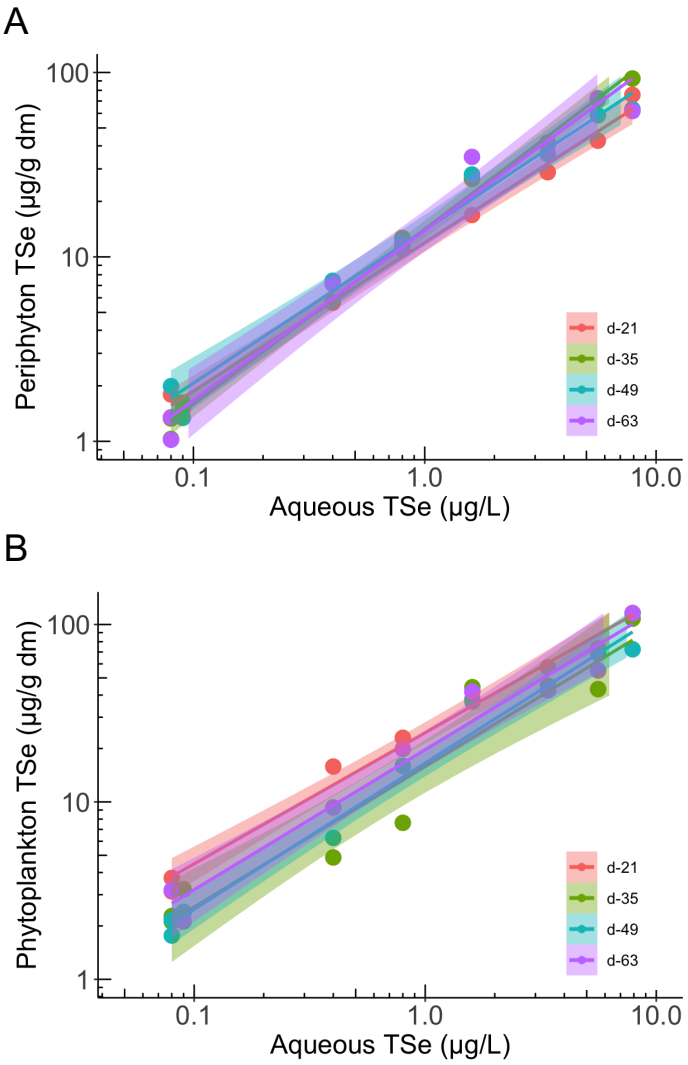

Figure A.4.5: Analysis of covariance of log periphyton (A) and phytoplankton (B) total Se versus log aqueous total Se on days 21, 35, 49 and 63 of the experimental period. Solid lines represent lines of best fit for each time point and shading represents the 95% confidence interval for the equation of the line. No significant differences in slopes or intercepts were detected between time points.

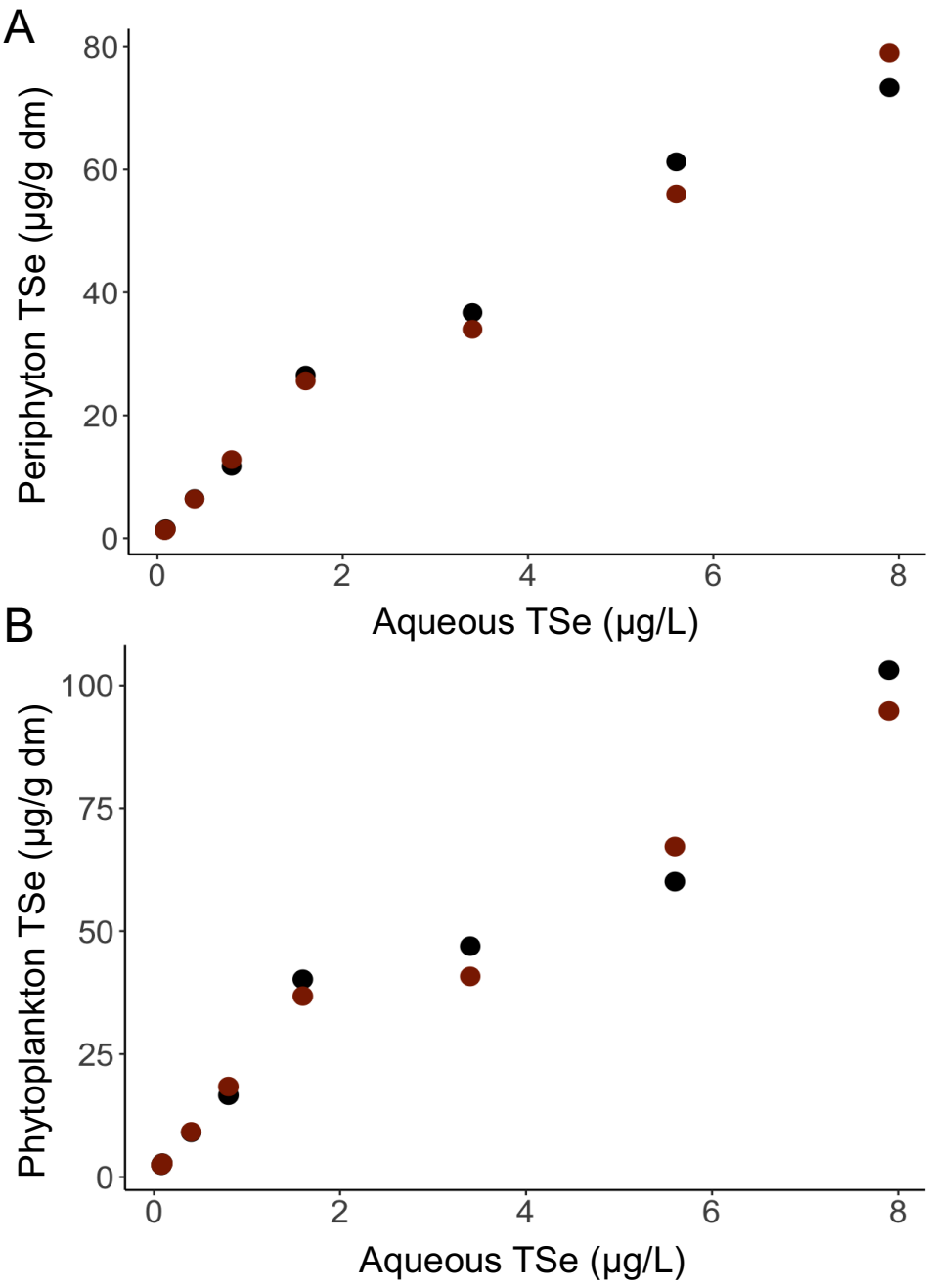

Figure A.4.6: Comparison of actual (black circles) versus predicted (red circles) periphyton (A) and phytoplankton (B) total Se bioaccumulation. Predicted values were calculated using two-compartment models, with two different k_ds used for periphyton, and three different k_ds used for phytoplankton.

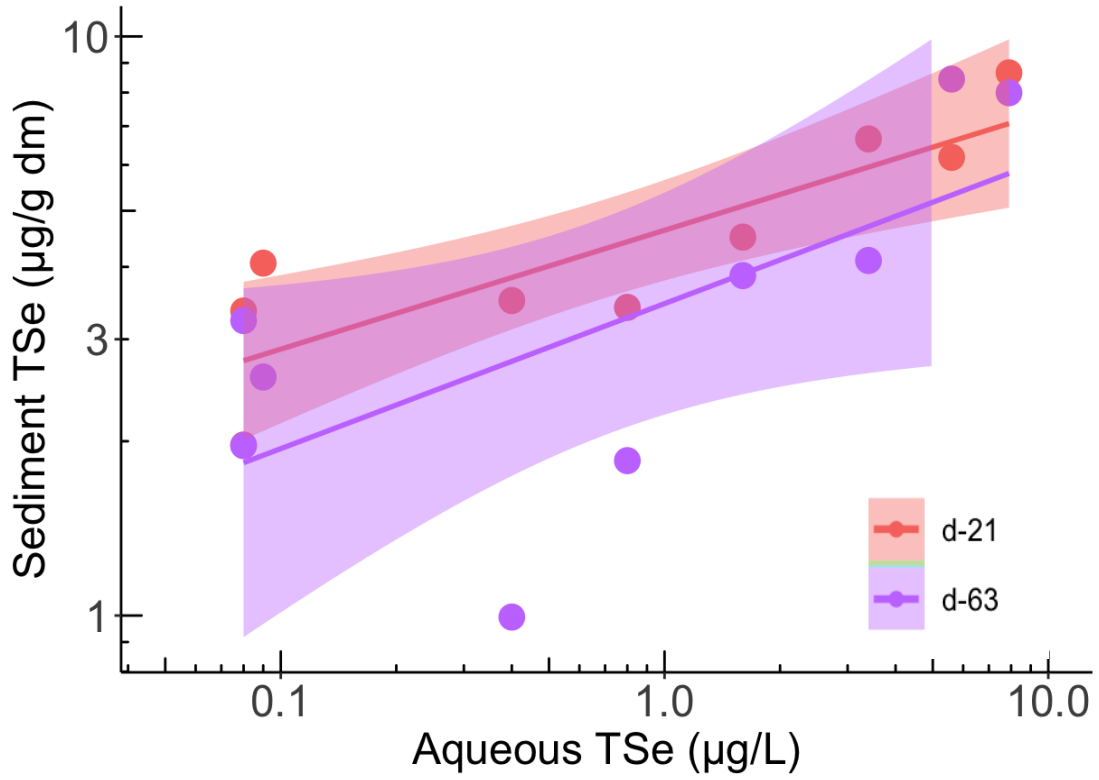

Figure A.4.7: Analysis of covariance of log sediment total Se and log aqueous total Se from day 21 and day 63 of the experimental period. Solid lines represent lines of best fit for each time point and shading represents the 95% confidence interval for the equation of the line. No significant differences in slopes or intercepts were detected between time points.

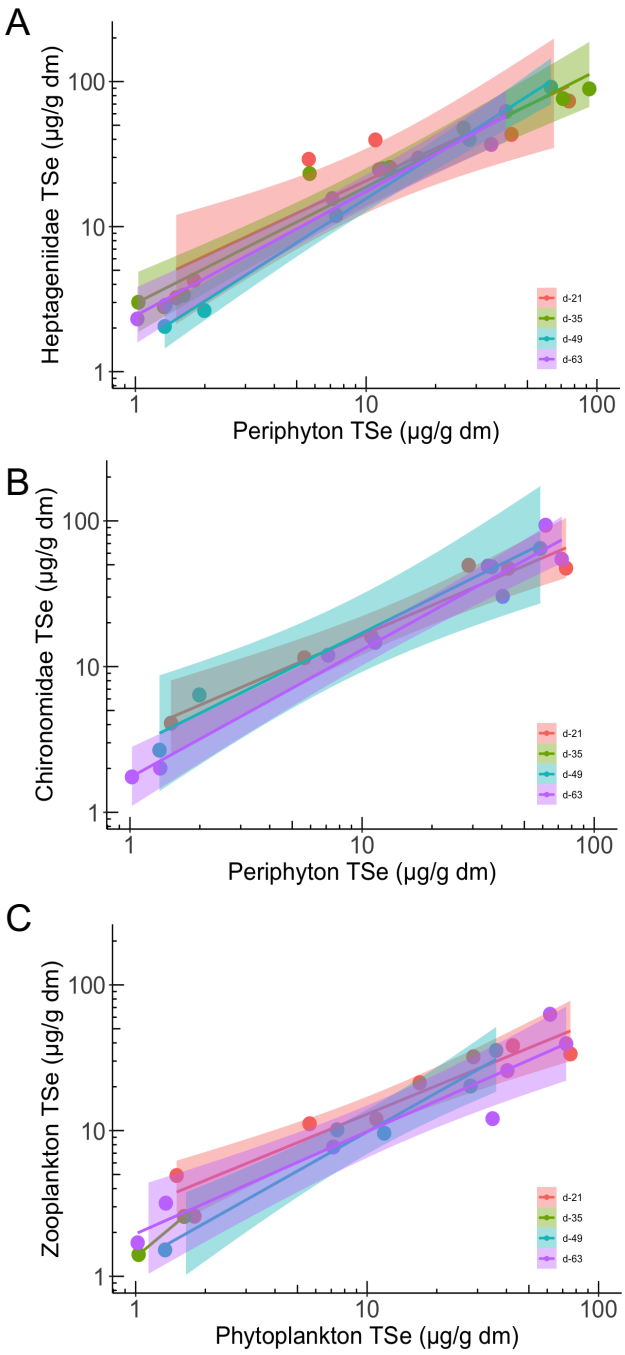

Figure A.4.8: Analysis of covariance of Heptageniidae (A), Chironomidae (B), and zooplankton (C) total Se and periphyton or phytoplankton total Se on days 21, 35, 49, and 63 of the experimental period. Solid lines represent lines of best fit for each time point and shading represents the 95% confidence interval for the equation of the line. No significant differences in slopes or intercepts were detected between time points.

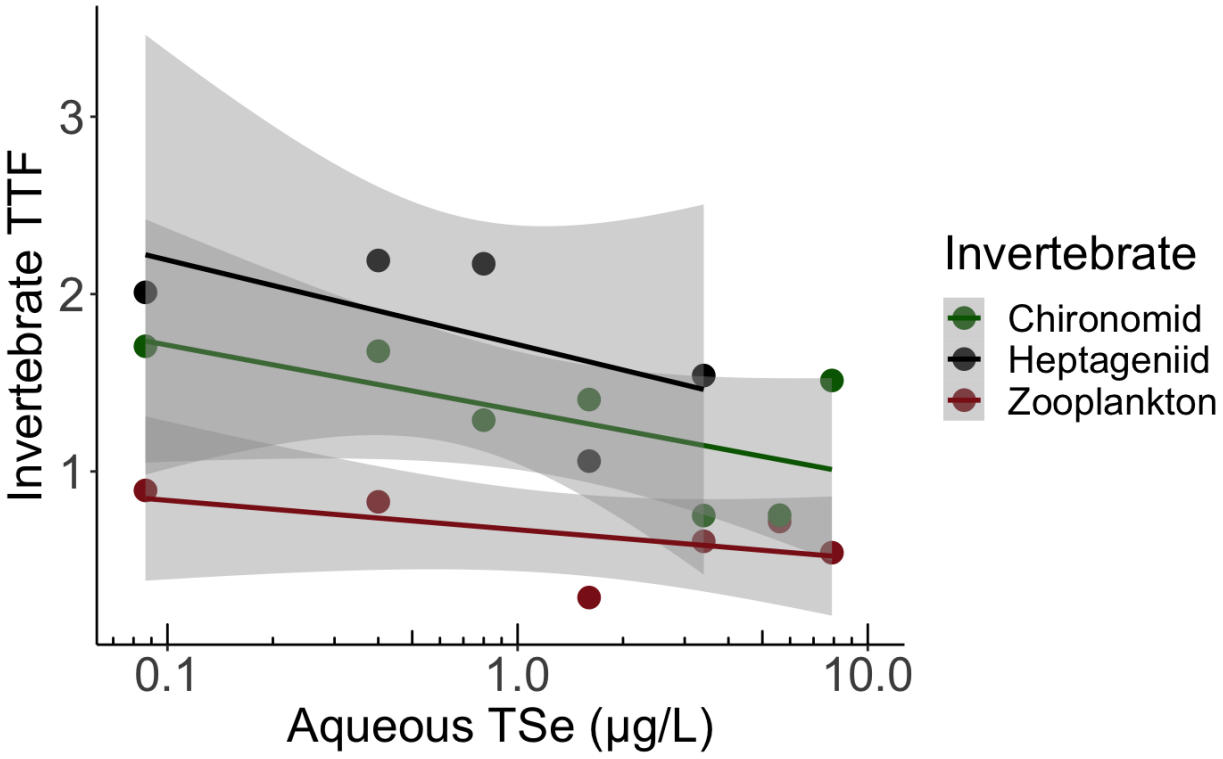

Figure A.4.9: Linear regression analysis of the relationship between invertebrate trophic transfer factor (chironomid, green circles; heptageniid, black circles; zooplankton, red circles) and aqueous total Se concentration on day 63 of the experimental period.

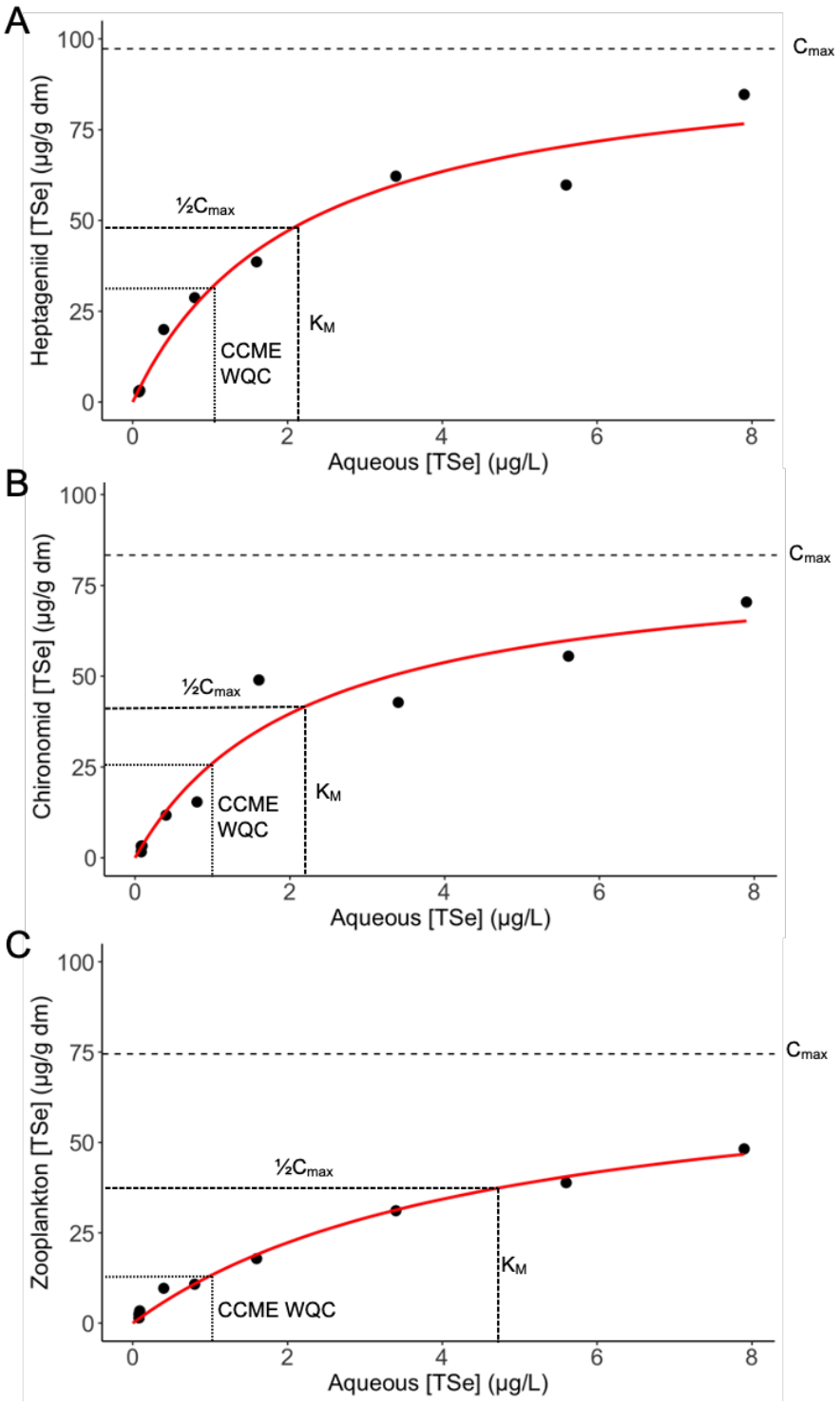

Figure A.4.10: Michaelis-Menten uptake curves showing the relationship between aqueous Se concentration and invertebrate Se bioaccumulation for Heptageniidae (A), Chironomidae (B), and zooplankton (C). C_{max} = modelled maximum concentration, K_M = concentration of aqueous Se at ½ C_{max}, CCME WQC = Canadian water quality criteria (set at 1 µg/L).

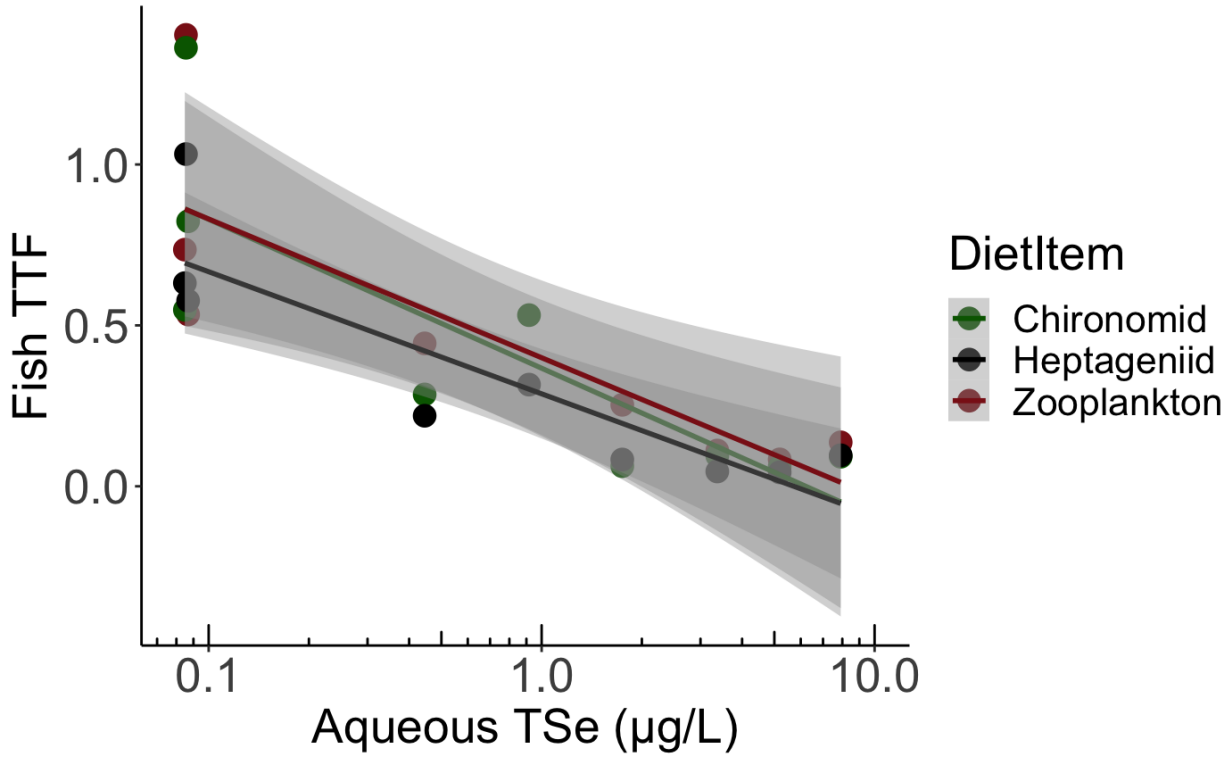

Figure A.4.11: Linear regression analysis of the relationship between finescale dace trophic transfer factor (TTF) and aqueous total Se concentration on day 63 of the experimental period (42 d of exposure for fish) based on three potential diet items (chironomid, green circles; heptageniid, black circles; zooplankton, red circles).

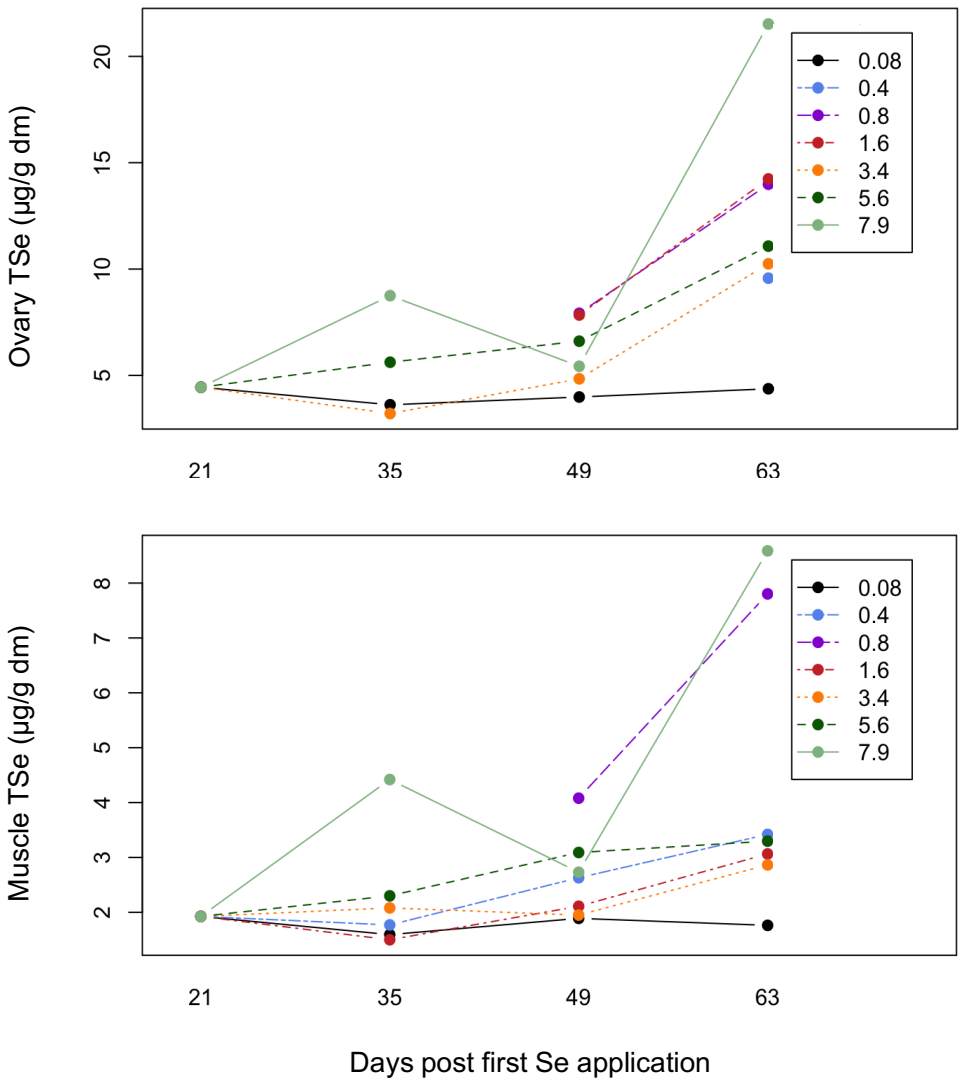

Figure A.4.12: Finescale dace ovary (A) and muscle (B) tissue total Se accumulation on days 21, 35, 42, and 63 of the experimental period after exposure to aqueous Se concentrations ranging from 0.08 to 7.9 µg/L within limnocorrals. On day 21, symbols represent n=3 baseline fish samples, symbols on day 35 and 49 symbols represent n=1/limnocorral and symbols on day 63 represent n=2-3/limnocorral.

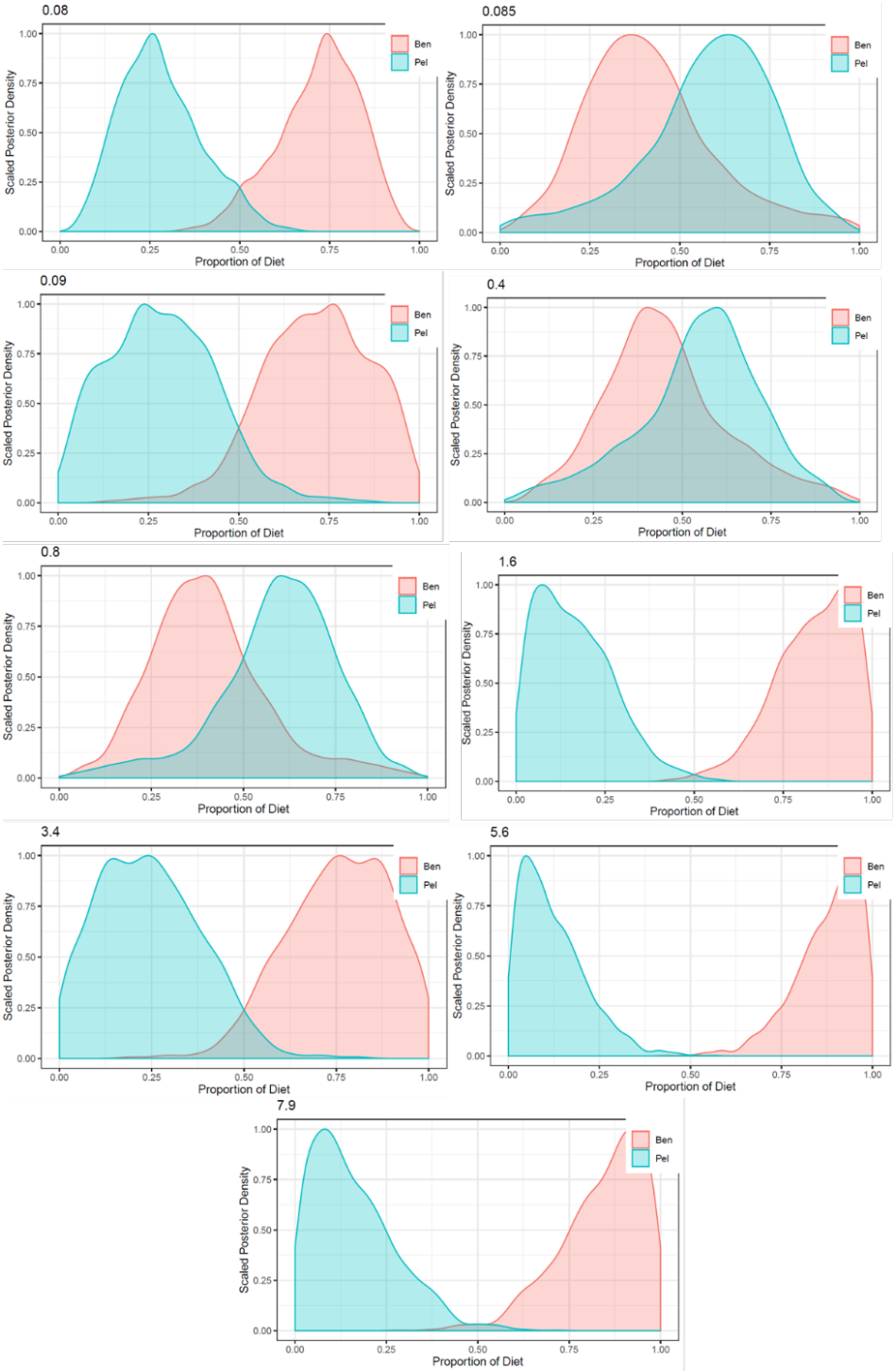

Figure A.4.13: Density distributions representing the proportion of benthic (red) versus pelagic (blue) food sources of finescale dace fish within each limnocorral after 42 d of exposure to mean aqueous Se concentrations ranging from 0.08 to 7.9 µg/L.

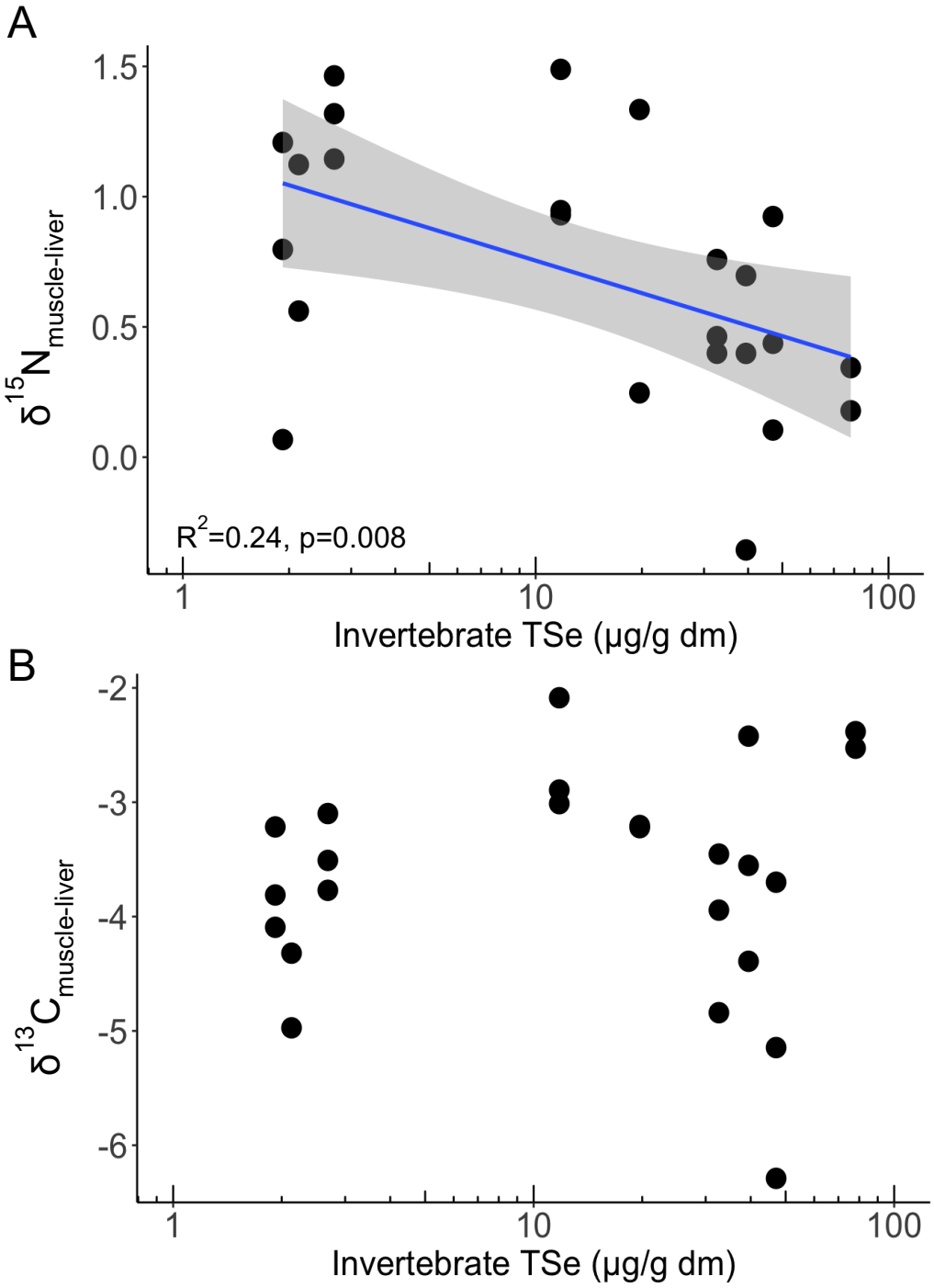

Figure A.4.14: Relationships between the change in fish liver relative to muscle tissue $\delta^{15}N$ ($\delta^{15}N_{muscle\text{-}liver}$; A) or $\delta^{13}C$ ($\delta^{13}C_{muscle\text{-}liver}$; B) and log invertebrate total Se after 42 d of exposure to aqueous Se concentrations ranging from 0.08 to 7.9 µg/L within limnocorrals.

Supporting Information Tables:

Table A.4.1: Morphometrics of finescale dace (*Phoxinus neogaeus*) collected from Lake 239 east inflow on day 21 (baseline; n=3), and retrieved from limnocorrals on days 35, 49 and 63 (n=1 on day 35 and 49; n=2-3 on day 63). Body mass of fish was not measured prior to addition to limnocorrals.

Experiment day	Treatment	Fork length (mm)	Body mass (g)	Fulton's condition factor (%)[a]
21	Baseline	60 ± 2	2.17 ± 0.10	0.97 ± 0.07
21	0.08 µg/L	70 ± 5		
21	0.08 µg/L	64 ± 5		
21	0.09 µg/L	62 ± 7		
21	0.4 µg/L	64 ± 6		
21	0.8 µg/L	61 ± 7		
21	1.6 µg/L	63 ± 3		
21	3.4 µg/L	66 ± 7		
21	5.6 µg/L	63 ± 6		
21	7.9 µg/L	65 ± 6		
35	0.08 µg/L	70	2.83	0.83
35	0.08 µg/L	69	3.27	0.99
35	0.09 µg/L	65	2.55	0.93
35	0.4 µg/L	63	2.39	0.96
35	0.8 µg/L			
35	1.6 µg/L	66	2.50	0.87
35	3.4 µg/L	72	3.27	0.88
35	5.6 µg/L	72	3.63	0.97
35	7.9 µg/L	66	2.72	0.95

49	0.08 µg/L	73	4.08	1.05
49	0.08 µg/L	67	3.07	1.02
49	0.09 µg/L			
49	0.4 µg/L	65	2.03	0.74
49	0.8 µg/L	70	3.06	0.89
49	1.6 µg/L	64	2.17	0.83
49	3.4 µg/L	68	2.93	0.93
49	5.6 µg/L	63	2.51	1.00
49	7.9 µg/L	67	2.39	0.79
63	0.08 µg/L	70 ± 3	3.36 ± 0.50	0.99 ± 0.22
63	0.08 µg/L	66 ± 5	2.91 ± 0.72	1.01 ± 0.04
63	0.09 µg/L	65 ± 9	2.20 ± 0.66	0.78 ± 0.09
63	0.4 µg/L	63 ± 5	2.26 ± 0.44	0.89 ± 0.05
63	0.8 µg/L	63 ± 6	2.51 ± 0.91	0.96 ± 0.07
63	1.6 µg/L	64 ± 4	2.40 ± 0.24	0.94 ± 0.22
63	3.4 µg/L	64 ± 8	2.32 ± 0.93	0.83 ± 0.09
63	5.6 µg/L	62 ± 6	2.23 ± 0.68	0.92 ± 0.02
63	7.9 µg/L	68 ± 10	3.01 ± 1.22	0.93 ± 0.02

[a]Fulton's condition factor = 100 × (body mass (g)/fork length (mm)^3)

Table A.4.2: Mass balance for TSe calculated at the end of the 63 d experimental period for experimental groups ranging from 0.08 to 7.9 µg/L Se. All masses shown in µg. Sediment and water Se masses were corrected for background (control) Se levels.

Treatment (µg/L)	0.08	0.08	0.09	0.4	0.8	1.6	3.4	5.6	7.9
Mass of Se Added	0	0	0	2947	7283	12858	23932	51083	61062
Filtered water	225	317	280	892	2412	3271	6556	13456	20208
Sediment[a]	2691	4387	5102	0	5955	3024	3352	14254	30821
Periphyton	6	5	6	33	52	350	307	1433	708
Particulate matter[b]	7	8	12	17	83	134	45	470	624
Zooplankton	59	64	76	79	15	19	6	27	1712
Benthic macroinvertebrates	1.4	0.8	0.7	18	8.7	6.5	1.9	1.2	2.3
Finescale dace	1.2	2.8	2.7	3.3	8.5	3.2	2.2	3.3	7.5
Total mass of Se measured	2991	4784	5480	1042	8535	6808	10270	29644	54083
Percent of added Se measured	n/a	n/a	n/a	35	117	53	43	58	89

[a]Sediment refers to only the top 1 cm of the sediment core samples

[b]Particulate matter includes phytoplankton, organic matter, and inorganic matter >0.6 µm and <53 µm

Table A.4.3: Percentage of added Se that was measured in each compartment (filtered water, sediment, periphyton, particulate matter, zooplankton, benthic macroinvertebrates, and finescale dace) at the end of the 63 d experimental period for experimental groups ranging from 0.4 to 7.9 µg/L Se. All masses shown in µg. Sediment and water Se masses were corrected for background (control) Se levels.

Treatment (µg/L)	0.4	0.8	1.6	3.4	5.6	7.9
Mass of Se Added	2947	7283	12858	23932	51083	61062
Filtered water	30.3	33.1	25.4	27.4	26.3	33.1
Sediment[a]	0.0	81.8	23.5	14.0	27.9	50.5
Periphyton	1.12	0.71	2.72	1.28	2.81	1.16
Particulate matter[b]	0.58	1.14	1.04	0.19	0.92	1.02
Zooplankton	2.68	0.21	0.15	0.03	0.05	2.80
Benthic macroinvertebrates	0.611	0.119	0.051	0.008	0.002	0.004
Finescale dace	0.112	0.117	0.025	0.009	0.006	0.012
Percent of added Se measured	35	117	53	43	58	89

[a]Sediment refers to only the top 1 cm of the sediment core samples

[b]Particulate matter includes phytoplankton, organic matter, and inorganic matter >0.6 µm and <53 µm

Table A.4.4: Parameters used in stable isotope mixing models: sources are mean $\delta^{13}C$ and $\delta^{15}N$ for benthic invertebrates (Ephemeroptera from present study, Chironomidae from Mailman, 2009) and littoral zooplankton (from Mailman, 2009). Trophic enrichment functions are mean values for aquatic organisms.

	Sources		Trophic Enrichment Function	
	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Benthic Invertebrates	-24.92 ± 1.97	2.11 ± 1.26	1.3 ± 0.4	3.4 ± 2
Zooplankton	-26.53 ± 1.61	1.98 ± 0.3	1.3 ± 0.4	3.4 ± 2

Table A.4.5: Diagnostic results from mixing models.

Gelman Diagnostic	DIC	Epsilon.1	Epsilon.2
18 > 1.01, 0 > 1.05	103.4	1.51 ± 0.80	0.068 ± 0.028

Table A.4.6: Total selenium concentrations and percent selenite (SeIV) from five lakes at the Experimental Lakes Area with varying water chemistry characteristics.

Lake	TSe (µg/L)	% SeIV	% SeVI	DOC (µM)	TDN (µg/L)	TDP (µg/L)	DIC (µM)	Water Renewal (y)
L114	0.099 ± 0.008	52 ± 9.9	48 ± 10	609	364	3.80	52.4	2.1[a]
L239	0.159 ± 0.010	54 ± 26	46 ± 26	601	267	2.49	161	9.2[a]
L470	0.132 ± 0.013	79 ± 5.7	21 ± 5.7	1000	492	5.10	65.4	0.07[b]
L227	0.155 ± 0.006	73 ± 2.6	27 ± 2.6	643	350	8.69	51.9	3.9[a]
L224	0.071 ± 0.001	58 ± 11	42 ± 11	291	196	0.52	114	17.4[a]

Abbreviations: TSe=Total Selenium, DOC = Dissolved organic carbon, TDN=Total dissolved nitrogen, TDP=Total dissolved phosphorus, DIC=Dissolved inorganic carbon.

[a]Reported in Curtis and Schindler (1997)

[b]Estimated using IISD-ELA unpublished data

Table A.4.7: Aqueous total Se (TSe, µg/L; calculated using area under the curve for each enclosure), enclosure water volume (L), and mean (minimum-maximum) values for temperature (°C), pH (SU), dissolved oxygen (%), total dissolved nitrogen (µg/L), total dissolved phosphorus (µg/L), and dissolved organic carbon (mg/L) for each enclosure during the experimental period.

Enclosure	Aqueous TSe	Volume	Temperature	pH	DO	TDN	TDP	DOC
M1	0.08	2764	22.1 (20.0 - 23.4)	7.1 (6.8 - 7.5)	93.7 (86.0 - 114)	379 (336 - 435)	3.89 (2.31 - 5.36)	8.43 (7.19 - 9.98)
M4	0.08	3611	22.3 (20.4 - 25.3)	7.1 (6.8 - 7.5)	96.5 (88.3 - 108)	332 (264 - 374)	3.20 (2.57 - 3.96)	7.54 (6.90 - 8.52)
M9	0.09	3517	22.2 (20.6 - 25.3)	7.1 (6.7 - 7.4)	98.4 (93.5 - 114)	329 (287 - 361)	3.65 (1.96 - 5.57)	7.84 (7.15 - 8.46)
M2	0.4	3328	22.2 (20.2 - 24.7)	7.1 (6.8 - 7.3)	97.0 (90.3 - 112)	356 (305 - 422)	3.63 (2.83 - 4.52)	8.03 (7.40 - 9.39)
M5	0.8	3705	22.4 (20.4 - 25.7)	7.1 (6.8 - 7.6)	98.2 (92.0 - 116)	324 (258 - 395)	3.21 (2.76 - 3.96)	7.81 (7.12 - 8.72)
M7	1.6	3642	22.4 (20.5 - 25.6)	7.1 (6.7 - 7.2)	92.2 (85.5 - 103)	340 (272 - 372)	4.22 (2.19 - 6.52)	7.92 (7.09 - 8.64)
M3	3.4	3548	22.3 (20.3 - 25.4)	7.0 (6.9 - 7.4)	96.4 (87.6 - 109)	345 (314 - 391)	3.99 (3.13 - 4.48)	8.01 (7.50 - 8.64)
M8	5.6	3485	22.4 (20.3 - 25.3)	7.0 (6.8 - 7.8)	94.3 (87.8 - 106)	332 (283 - 374)	4.09 (2.76 - 5.16)	8.02 (7.32 - 8.97)
M6	7.9	3674	22.6 (20.7 - 25.9)	7.1 (6.8 - 7.5)	98.6 (92.6 - 112)	305 (242 - 344)	3.07 (1.64 - 4.71)	7.52 (7.07 - 8.09)

Abbreviations: TSe = total Selenium, DO = dissolved oxygen, TDN = total dissolved nitrogen, TDP = total dissolved phosphorus, DOC = dissolved organic carbon

Table A.4.8: Mean (± standard deviation) total Se concentrations (µg/L for water and µg/g dm for other compartments) in all measured compartments in each limnocorral with aqueous Se concentrations ranging from 0.08 to 7.9 µg/L.

Water	Sediment	Periphyton	Phytoplankton	Zooplankton	Chironomid	Heptageniid	Fish muscle	Fish ovary
0.08	3.0 ± 0.5	1.3 ± 0.4	2.6 ± 0.8	1.8 ± 0.7	2.5 ± 0.3	2.8 ± 1.1	1.3 ± 0.2	4.2 ± 0.8
0.08	2.6 ± 0.9	1.4 ± 0.5	2.8 ± 0.6	2.5 ± 0.8	3.2 ± 2.7	3.0 ± 0.7	2.4 ± 1.7	3.1 ± 1.3
0.09	3.0 ± 1.5	1.3 ± 0.2	2.4 ± 0.7	3.4 ± 1.4	3.3 ± 1.1	3.2 ± 0.5	1.7 ± 0.4	5.8 ± 2.6
0.4	2.2 ± 1.8	6.5 ± 0.9	9.1 ± 4.8	9.7 ± 1.8	12 ± 0.3	20 ± 7.7	3.4 ± 2.1	9.6 ± 3.2
0.8	2.6 ± 1.1	12 ± 0.7	17 ± 6.6	11 ± 1.7	15 ± 0.9	29 ± 7.2	7.8 ± 1.9	14 ± 1.4
1.6	4.2 ± 0.5	27 ± 7.4	40 ± 3.5	18 ± 5.0	49	39 ± 7.5	3.1 ± 1.3	14 ± 8.5
3.4	5.4 ± 1.8	37 ± 5.8	47 ± 7.0	31 ± 4.9	43 ± 11	62	2.9 ± 0.4	10 ± 1.4
5.6	7.3 ± 1.6	61 ± 14	60 ± 14	39 ± 0.9	56 ± 8.7	60 ± 23	3.3 ± 0.8	11 ± 4.2
7.9	8.3 ± 0.5	73 ± 15	103 ± 21	48 ± 21	70 ± 35	85 ± 9.8	8.6 ± 1.5	22 ± 5.5

Table A.4.9: Mean (± standard deviation) distribution coefficients (k_ds; L/kg dry mass) and trophic transfer factors (TTFs; unitless) of Se calculated for each compartment in each limnocorral with aqueous Se concentrations ranging from 0.08 to 7.9 µg/L.

Water	Sediment	Periphyton	Phytoplankton	Zooplankton	Chironomid	Heptageniid	Fish muscle	Fish ovary
0.08	28,517 ± 7,476	15,422 ± 4,270	28,784 ± 8,563	0.7 ± 0.1	1.8 ± 0.2	2.3 ± 0.7	0.5 ± 0.1	1.6 ± 0.3
0.08	23,359 ± 11,882	17,020 ± 4,734	29,173 ± 4,447	0.9 ± 0.2	2.5 ± 1.1	1.9 ± 0.5	1.1 ± 0.8	1.5 ± 0.6
0.09	24,363 ± 12,348	13,499 ± 2,054	25,927 ± 8,839	1.1 ± 0.2	2.7 ± 1.2	2.6 ± 1.0	0.9 ± 0.2	3.0 ± 1.4
0.4	3,418 ± 2,115	13,028 ± 3,467	17,576 ± 7,949	1.1 ± 0.5	1.8 ± 0.3	3.2 ± 1.7	0.3 ± 0.2	0.8 ± 0.3
0.8	1,985 ± 470	11,011 ± 1,621	15,630 ± 6,399	0.6 ± 0.1	1.4 ± 0.1	2.5 ± 0.8	0.4 ± 0.1	0.7 ± 0.1
1.6	1,721 ± 272	14,998 ± 6,164	22,082 ± 3,951	0.5 ± 0.2	1.4	1.5 ± 0.3	0.1 ± 0.04	0.4 ± 0.3
3.4	1,155 ± 98	10,171 ± 2,683	12,682 ± 967	0.7 ± 0.1	1.2 ± 0.5	1.5	0.1 ± 0.01	0.3 ± 0.1
5.6	997 ± 436	10,518 ± 3,317	10,129 ± 2,444	0.6 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	0.1 ± 0.01	0.2 ± 0.1
7.9	734 ± 128	7,932 ± 1,123	11,250 ± 2,646	0.4 ± 0.2	1.1 ± 0.6	1.1 ± 0.3	0.1 ± 0.02	0.3 ± 0.1

Table A.4.10: Michaelis-Menten and non-linear regression (power) equations for total Se (TSe) bioaccumulation in algae and invertebrates. For all equations, aqueous Se is in µg/L, and periphyton, phytoplankton, and invertebrate TSe are in µg/g dm.

X variable	Equation of the line
Aqueous TSe	Periphyton TSe = 174x/(10.9+x)
Aqueous TSe	Chironomid TSe = 83.4x/(2.2+x)
Aqueous TSe	Heptageniid TSe = 97.3x/(2.1+x)
Aqueous TSe	Zooplankton TSe = 74.5x/(4.7+x)
Periphyton TSe	Chironomid TSe = 114x/(52+x)
Periphyton TSe	Heptageniid TSe = 121x/(43+x)
Aqueous TSe	Phytoplankton TSe = $20x^{0.8}$
Phytoplankton TSe	Zooplankton TSe = $1.46x^{0.8}$

Table A.4.11: Sediment loss on ignition (%) for three baseline sediment cores, and from each limnocorral on d 21 and 63 of the experimental period.

Experimental day	Enclosure ID	Replicate	Experimental group	Loss on ignition (%)
-2	Baseline 1	1	Lake	80.5
-2	Baseline 5	1	Lake	85.5
-2	Baseline 9	1	Lake	81.0
21	M1	2	0.08	75.9
21	M4	3	0.08	90.6
21	M9	1	0.09	81.5
21	M2	1	0.4	82.9
21	M5	1	0.8	85.8
21	M7	1	1.6	74.4
21	M3	1	3.4	81.0
21	M8	3	5.6	78.2
21	M6	1	7.9	81.8
63	M1	2	0.08	71.6
63	M4	2	0.08	81.0
63	M9	2	0.09	78.6
63	M2	3	0.4	85.1
63	M5	2	0.8	82.1
63	M7	2	1.6	76.8
63	M3	2	3.4	82.1
63	M8	2	5.6	79.5
63	M6	2	7.9	80.6

Supporting Information References for Chapter 4:

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US Environmental Protection Agency. 2004. Draft aquatic life water quality criteria for selenium—2004. Washington, DC.

SUPPORTING INFORMATION FOR CHAPTER 5

Table A.5.1: Zooplankton species codes and corresponding species names used in Principal Component Analysis (PCA) and presented in red text in Figure 5.3.

Species Name	Species code
Acroperus harpae	Aharp
Bosmina longirostris	Blong
Chydorus sp.	Chyd
Copepod nauplii	cycnaup
Copepodid	cycope
Diacyclops bicuspidatus	Dbic
Diaphanosoma birgei	Dbirg
Daphnia galeata mendotae	Dgal
Diaptomus minutus	Dmin
Epischura lacustris	Elac
Holopedium gibberum	Hgibb
Mesocyclops edax	Medax
Ophryoxus gracilis	Ograc
Polyphemus pediculus	Pped
Sida crystallina	Scrys
Scapheloberis kingii	Sking
Tropocyclops extensus	Text

Table A.5.2: List of benthic macroinvertebrate taxa used in Principal Component Analysis (PCA) and presented in Figure 5.9.

Family Name
Aeshnidae
Caenidae
Ceratopogoniidae
Chironomidae
Corduliidae
Dytiscidae
Gammaridae
Heptageniidae
Hirudinea
Hydrachnidae
Notonectidae
Perlidae
Phryganeidae
Planorbidae
Polycentropodidae
Caenogrionidae

APPENDIX B

SELENIUM LITERATURE DATA USED TO TEST BIOACCUMULATION MODELS

Table B.6.1: Water and algae/particulate Se values gathered from the literature and used to test models predicting algae Se from water Se.

Matrice	Aqueous Se (µg/L)	Particulate Se (µg /g dm)	Reference
Particulates	3.8	6	Birkner, 1978
Sediment	0.3	0.2	Birkner, 1978
Sediment	0.3	0.225	Birkner, 1978
Sediment	0.8	1.05	Birkner, 1978
Sediment	1.7	3.3	Birkner, 1978
Sediment	2.1	1.3	Birkner, 1978
Sediment	2.2	1.8	Birkner, 1978
Sediment	4.2	9	Birkner, 1978
Sediment	4.8	3	Birkner, 1978
Sediment	6	4.6	Birkner, 1978
Sediment	7.6	9.3	Birkner, 1978
Sediment	9.4	9.1	Birkner, 1978
Sediment	12.5	12.1	Birkner, 1978
Sediment	15.9	27.3	Birkner, 1978
Particulates	11.5	27	Bowie et al., 1996
Particulates	0.92	1.1	California Resources Agency, 2006
Particulates	10.9	30.5	Cumbie, 1984; Lemly, 1985
Particulates	8.8	11.8	Fan et al., 2002
Sediment	7.4	4.4	Hansen and others, 1998
Sediment	1.9	3.8	Hillwalker et al. 2006
Sediment	2.9	5	Hillwalker et al. 2006
Sediment	5	2.8	Hillwalker et al. 2006
Sediment	6.3	2.1	Hillwalker et al. 2006
Periphyton	0.3	0.52	Mailman Thesis
Periphyton	0.35	0.88	Mailman Thesis
Periphyton	0.6	2.92	Mailman Thesis
Periphyton	0.7	0.92	Mailman Thesis
Periphyton	0.8	1.8	Mailman Thesis
Periphyton	1.1	4.07	Mailman Thesis
Sediment	0.3	7.5	Mailman Thesis
Sediment	0.35	5	Mailman Thesis
Sediment	0.6	7.6	Mailman Thesis
Sediment	0.7	7.5	Mailman Thesis
Sediment	0.8	15	Mailman Thesis
Sediment	1.1	10	Mailman Thesis
Particulates	0.54	0.95	Marden, 2008
Particulates	25.6	9.925	Moore et al., 1990
Periphyton	0.1	0.29	Muscatello et al. 2008
Periphyton	0.7	1.01	Muscatello et al. 2008
Periphyton	2.7	3.75	Muscatello et al. 2008
Sediment	0.1	5.7	Muscatello et al. 2008

Sediment	0.7	25.6	Muscatello et al. 2008
Sediment	2.7	62.2	Muscatello et al. 2008
Particulates	0.5	4.4	Orr et al. 2006
Particulates	7.15	5.55	Orr et al. 2006
Particulates	46	5.49	Orr et al. 2006
Particulates	88	3.21	Orr et al. 2006
Sediment	0.5	2	Orr et al. 2006
Sediment	0.7	3	Orr et al. 2006
Sediment	1.15	3	Orr et al. 2006
Sediment	7.15	7.9	Orr et al. 2006
Sediment	46	2.8	Orr et al. 2006
Sediment	88	26	Orr et al. 2006
Periphyton	0.2	0.978	Orr et al. 2012
Periphyton	0.2	1.86	Orr et al. 2012
Periphyton	0.2	3.294	Orr et al. 2012
Periphyton	0.4	0.96	Orr et al. 2012
Periphyton	0.4	0.35	Orr et al. 2012
Periphyton	3.2	7.471	Orr et al. 2012
Periphyton	11.6	14.608	Orr et al. 2012
Periphyton	14	1.135	Orr et al. 2012
Periphyton	16.3	18.508	Orr et al. 2012
Periphyton	18	18.147	Orr et al. 2012
Periphyton	30.3	8.19	Orr et al. 2012
Periphyton	35.3	51.042	Orr et al. 2012
Periphyton	41.9	43.41	Orr et al. 2012
Periphyton	64.49	83.58	Orr et al. 2012
Phytoplankton	0.107	3.1	Ponton and Hare, 2018
Phytoplankton	0.167	6.2	Ponton and Hare, 2018
Phytoplankton	0.195	5.4	Ponton and Hare, 2018
Phytoplankton	0.234	9	Ponton and Hare, 2018
Phytoplankton	0.284	6.2	Ponton and Hare, 2018
Phytoplankton	0.331	5.2	Ponton and Hare, 2018
Phytoplankton	0.415	17.65	Ponton and Hare, 2018
Phytoplankton	0.486	13.9	Ponton and Hare, 2018
Phytoplankton	0.541	15.3	Ponton and Hare, 2018
Phytoplankton	0.569	4.587	Ponton and Hare, 2018
Phytoplankton	1.44	28.5	Ponton and Hare, 2018
Phytoplankton	3.063	27.9825	Ponton and Hare, 2018
Sediment	2	1.6	Presser and Luoma, 2009
Sediment	20.4	5.2	Presser and Luoma, 2009
Sediment	330	110	Presser and Piper, 1998
Particulates	9	32	Saiki and Lowe, 1987
Sediment	41	119	Saiki and Lowe, 1987
Particulates	3.7	6.7	USGS, 2008
Particulates	0.035	0.5	Velinsky and Cutter, 1991
Particulates	0.18	0.11	Ware, 2008
Particulates	0.67	0.93	Ware, 2008
Sediment	0.74	0.35	Zhang and Moore, 1996
Sediment	10.4	3.5	Zhang and Moore, 1996

Table B.6.2: Invertebrate and algae Se values gathered from the literature and used to test models predicting invertebrate Se from algae Se.

Taxa	System	Dietary Se	Invertebrate Se	Reference
Ephemeroptera	Lotic	4.2	8.2	Casey 2005
Ephemeroptera	Lotic	1	6.4	Casey 2005
Ephemeroptera	Lotic	4.2	12.3	Casey 2005
Ephemeroptera	Lotic	1	6.8	Casey 2005
Ephemeroptera	Lotic	4.2	9.7	Casey 2005
Ephemeroptera	Lotic	1	5.7	Casey 2005
Trichoptera	Lotic	4.2	13.4	Casey 2005
Diptera	Lotic	4.2	8.4	Casey 2005
Diptera	Lotic	1	2.4	Casey 2005
Plecoptera	Lotic	10.4	8.4	Casey 2005
Plecoptera	Lotic	5.3	4	Casey 2005
Plecoptera	Lotic	10.4	10.4	Casey 2005
Plecoptera	Lotic	2.6	2.6	Casey 2005
Trichoptera	Lotic	10.4	10.2	Casey 2005
Trichoptera	Lotic	5.3	4.9	Casey 2005
Odonata	Lentic	3.4	3.1	Birkner 1978
Odonata	Lentic	2.3	1.8	Birkner 1978
Odonata	Lentic	4.2	11.1	Birkner 1978
Odonata	Lentic	4.2	4.4	Birkner 1978
Odonata	Lentic	7.5	7.7	Birkner 1978
Odonata	Lentic	15.3	28.4	Birkner 1978
Odonata	Lentic	11	18.7	Birkner 1978
Odonata	Lentic	14.4	15.8	Birkner 1978
Odonata	Lentic	24.9	18.4	Birkner 1978
Odonata	Lentic	18.7	21.9	Birkner 1978
Odonata	Lentic	26.7	45.1	Birkner 1978
Odonata	Lentic	43.8	55	Birkner 1978
Odonata	Lentic	59.3	53.3	Birkner 1978
Diptera	Lotic	1.2	0.6	Saiki et al. 1993
Diptera	Lotic	1.1	1	Saiki et al. 1993
Diptera	Lentic	22	8.9	Saiki et al. 1993
Diptera	Lentic	7.9	7.2	Saiki et al. 1993
Diptera	Lentic	8.9	5.4	Saiki et al. 1993
Diptera	Lentic	7.9	6.9	Saiki et al. 1993
Diptera	Lotic	0.4	0.5	Saiki et al. 1993
Diptera	Lotic	0.6	1	Saiki et al. 1993
Diptera	Lotic	6.6	6	Saiki et al. 1993

Diptera	Lotic	3.4	4.1	Saiki et al. 1993
Diptera	Lotic	1.2	1.5	Saiki et al. 1993
Diptera	Lotic	1.3	1.6	Saiki et al. 1993
Diptera	Lotic	0.8	0.5	Saiki et al. 1993
Diptera	Lotic	0.7	0.8	Saiki et al. 1993
Amphipoda	Lotic	1.2	0.4	Saiki et al. 1993
Amphipoda	Lotic	1.1	0.9	Saiki et al. 1993
Amphipoda	Lentic	22	4.6	Saiki et al. 1993
Amphipoda	Lentic	7.9	3.3	Saiki et al. 1993
Amphipoda	Lentic	8.9	3.4	Saiki et al. 1993
Amphipoda	Lentic	7.9	3.7	Saiki et al. 1993
Amphipoda	Lotic	0.4	0.9	Saiki et al. 1993
Amphipoda	Lotic	0.6	1.3	Saiki et al. 1993
Amphipoda	Lotic	6.6	3.8	Saiki et al. 1993
Amphipoda	Lotic	3.4	2.8	Saiki et al. 1993
Amphipoda	Lotic	1.2	1.5	Saiki et al. 1993
Amphipoda	Lotic	1.3	1.1	Saiki et al. 1993
Amphipoda	Lotic	0.8	1.1	Saiki et al. 1993
Amphipoda	Lotic	0.7	1.1	Saiki et al. 1993
Crayfish	Lotic	1.2	0.7	Saiki et al. 1993
Crayfish	Lotic	1.1	0.8	Saiki et al. 1993
Crayfish	Lentic	22	5.2	Saiki et al. 1993
Crayfish	Lentic	7.9	4.4	Saiki et al. 1993
Crayfish	Lentic	8.9	3.1	Saiki et al. 1993
Crayfish	Lentic	7.9	3.2	Saiki et al. 1993
Crayfish	Lotic	0.4	0.5	Saiki et al. 1993
Crayfish	Lotic	0.6	0.7	Saiki et al. 1993
Crayfish	Lotic	6.6	1.7	Saiki et al. 1993
Crayfish	Lotic	3.4	1.9	Saiki et al. 1993
Crayfish	Lotic	1.2	0.8	Saiki et al. 1993
Crayfish	Lotic	1.3	1.3	Saiki et al. 1993
Crayfish	Lotic	0.8	0.9	Saiki et al. 1993
Crayfish	Lotic	0.7	0.9	Saiki et al. 1993
Composite	Lotic	0.8	2.3	Orr et al. 2012
Composite	Lotic	2.3	7.2	Orr et al. 2012
Composite	Lotic	2.6	6.2	Orr et al. 2012
Composite	Lotic	1.5	4.2	Orr et al. 2012
Composite	Lotic	1.3	4.3	Orr et al. 2012
Composite	Lotic	2.7	6.1	Orr et al. 2012
Composite	Lotic	1.3	1.5	Orr et al. 2012
Composite	Lotic	0.3	2.7	Orr et al. 2012

Composite	Lotic	0.7	3.2	Orr et al. 2012
Composite	Lotic	0.8	4.6	Orr et al. 2012
Composite	Lotic	2	3.6	Orr et al. 2012
Composite	Lotic	1.9	3.1	Orr et al. 2012
Composite	Lotic	1.6	10.7	Orr et al. 2012
Composite	Lotic	8.9	5.8	Orr et al. 2012
Composite	Lotic	3.8	9.7	Orr et al. 2012
Composite	Lotic	1.3	10.2	Orr et al. 2012
Composite	Lotic	2.6	5.4	Orr et al. 2012
Composite	Lotic	4.2	8	Orr et al. 2012
Composite	Lotic	1.3	8.7	Orr et al. 2012
Composite	Lotic	3.3	10.9	Orr et al. 2012
Composite	Lotic	2.1	8.4	Orr et al. 2012
Composite	Lotic	1.5	4.7	Orr et al. 2012
Composite	Lotic	1.3	6.8	Orr et al. 2012
Composite	Lotic	1.3	5.4	Orr et al. 2012
Composite	Lotic	5.1	5.5	Orr et al. 2012
Composite	Lentic	8.2	24.3	Orr et al. 2012
Composite	Lentic	43.4	40.2	Orr et al. 2012
Composite	Lentic	1	6.4	Orr et al. 2012
Composite	Lentic	1	4.3	Orr et al. 2012
Composite	Lentic	83.6	51.2	Orr et al. 2012
Composite	Lentic	51	47.3	Orr et al. 2012
Composite	Lentic	14.6	25.5	Orr et al. 2012
Composite	Lentic	18.1	14.4	Orr et al. 2012
Composite	Lentic	1.1	9.2	Orr et al. 2012
Composite	Lentic	3.3	3.6	Orr et al. 2012
Composite	Lotic	0.5	6.5	Golder 2011
Composite	Lotic	0.1	3.7	Golder 2011
Composite	Lotic	0.9	6	Golder 2013a
Composite	Lotic	1.8	4.3	Golder 2011
Composite	Lotic	1.3	5.3	Golder 2013a
Composite	Lotic	1.3	4.3	Golder 2011
Composite	Lotic	1.1	6.6	Golder 2011
Composite	Lotic	0.8	8.2	Golder 2013a
Composite	Lotic	0.4	5.9	Golder 2013a
Composite	Lotic	0.9	9.7	Golder 2013a
Composite	Lotic	2.2	6.9	Golder 2009
Composite	Lotic	1.6	3	Golder 2011
Composite	Lotic	2.3	4	Golder 2013a
Composite	Lotic	3.2	6	Golder 2009

Composite	Lotic	1.1	5.5	Golder 2011
Composite	Lotic	1.7	4.4	Golder 2011
Composite	Lotic	2.7	7.9	Golder 2009
Composite	Lotic	3.2	6.6	Golder 2011
Composite	Lotic	1.6	3.2	Golder 2013a
Composite	Lotic	3.8	12.9	Golder 2011
Composite	Lotic	1.8	9.1	Golder 2013a
Composite	Lotic	1.8	5.9	Golder 2013a
Composite	Lotic	1	5.1	Golder 2011
Composite	Lotic	0.4	5.3	Golder 2013a
Composite	Lotic	2.1	7.1	Golder 2011
Composite	Lotic	0.7	7.2	Golder 2013a
Composite	Lotic	1.4	3.6	Golder 2009
Composite	Lotic	0.7	3.9	Golder 2011
Composite	Lotic	2.2	7.2	Golder 2013a
Composite	Lotic	1.4	4.2	Golder 2013a
Composite	Lotic	1.9	5.5	Golder 2009
Composite	Lotic	1.5	4	Golder 2011
Composite	Lotic	1.1	5	Golder 2013a
Composite	Lotic	1.9	6	Golder 2009
Composite	Lotic	2.2	4	Golder 2013a
Composite	Lotic	0.6	3.3	Golder 2011
Composite	Lotic	1.4	4.5	Golder 2013a
Composite	Lotic	0.7	4.4	Golder 2013a
Composite	Lotic	1.6	7.1	Golder 2009
Composite	Lotic	1.1	4.8	Golder 2011
Composite	Lotic	0.8	4.6	Golder 2013a
Composite	Lotic	0.4	4.3	Golder 2011
Composite	Lotic	0.7	5.4	Golder 2013a
Composite	Lotic	0.9	3.8	Golder 2011
Composite	Lotic	1.2	3.9	Golder 2013a
Composite	Lotic	0.4	2.4	Golder 2011
Composite	Lotic	0.7	3.7	Golder 2013a
Composite	Lotic	2.2	4.4	Golder 2013a
Composite	Lotic	5.5	5.6	Golder 2013a
Composite	Lotic	5.1	38.8	Golder 2009
Composite	Lotic	4.1	17.8	Golder 2011
Composite	Lotic	2.6	5	Golder 2013a
Composite	Lotic	1.8	4.4	Golder 2013a
Composite	Lotic	0.8	3.2	Formation and HabiTech 2012
Composite	Lotic	0.6	3.2	Formation and HabiTech 2012

Composite	Lotic	1.2	4.8	Formation and HabiTech 2012
Composite	Lotic	0.2	0.1	Formation and HabiTech 2012
Composite	Lotic	1	3.1	Formation and HabiTech 2012
Composite	Lotic	2.7	4.5	Formation and HabiTech 2012
Composite	Lotic	0.6	3.5	Formation and HabiTech 2012
Composite	Lotic	1.2	4.9	Formation and HabiTech 2012
Composite	Lotic	1.4	4.5	Formation and HabiTech 2012
Composite	Lotic	0.8	4.5	Formation and HabiTech 2012
Composite	Lotic	2.4	7	Formation and HabiTech 2012
Composite	Lotic	0.7	21.6	Formation and HabiTech 2012
Composite	Lotic	1.5	2.1	Formation and HabiTech 2012
Composite	Lotic	3.3	4.2	Formation and HabiTech 2012
Composite	Lotic	3.4	10.6	Formation and HabiTech 2012
Composite	Lotic	0.6	12.3	Formation and HabiTech 2012
Composite	Lotic	1.2	10.7	Formation and HabiTech 2012
Composite	Lotic	7.4	6.4	Formation and HabiTech 2012
Composite	Lotic	8.7	8.7	Formation and HabiTech 2012
Composite	Lotic	1.7	7	Formation and HabiTech 2012
Composite	Lotic	2.2	1	Formation and HabiTech 2012
Composite	Lotic	12	15.7	Formation and HabiTech 2012
Composite	Lotic	15	21.7	Formation and HabiTech 2012
Composite	Lotic	35.2	33.9	Formation and HabiTech 2012
Composite	Lotic	6.5	12.5	Formation and HabiTech 2012
Composite	Lotic	12	11.4	Formation and HabiTech 2012
Composite	Lotic	6.2	11.4	Formation and HabiTech 2012
Composite	Lotic	28.5	28.4	Formation and HabiTech 2012
Composite	Lotic	24.2	24.7	Formation and HabiTech 2012
Composite	Lotic	2.6	22.6	Formation and HabiTech 2012
Composite	Lotic	8.1	8.3	Formation and HabiTech 2012
Composite	Lotic	18.5	31.7	Formation and HabiTech 2012
Composite	Lotic	11.6	30	Formation and HabiTech 2012
Composite	Lotic	4.4	23.9	Formation and HabiTech 2012
Composite	Lotic	7.4	10	Formation and HabiTech 2012
Composite	Lotic	11.7	9.1	Formation and HabiTech 2012
Composite	Lotic	3.6	3.5	Formation and HabiTech 2012
Composite	Lotic	3.4	12.9	Formation and HabiTech 2012
Composite	Lotic	3.2	12.9	Formation and HabiTech 2012
Composite	Lotic	7.1	15.5	Formation and HabiTech 2012
Composite	Lotic	5.9	11.6	Formation and HabiTech 2012
Composite	Lotic	3.1	5.5	Formation and HabiTech 2012
Composite	Lotic	1.9	5.4	Formation and HabiTech 2012

Composite	Lotic	14.9	17.8	Formation and HabiTech 2012
Composite	Lotic	1.7	11.2	Formation and HabiTech 2012
Composite		1.3	4.3	Presser and Luoma 2010
Composite		1.6	10.7	Presser and Luoma 2010
Composite		2.2	7.1	Presser and Luoma 2010
Composite		2.9	7.1	Presser and Luoma 2010
Composite		2.9	6.9	Presser and Luoma 2010
Composite		2.9	8.9	Presser and Luoma 2010
Composite		27	70	Presser and Luoma 2010
Composite		58	180	Presser and Luoma 2010
Composite		18	41	Presser and Luoma 2010
Composite		20.7	56.9	Presser and Luoma 2010
Composite		2.5	12.1	Presser and Luoma 2010
Trichoptera		8.8	33	Presser and Luoma 2010
Trichoptera		26	165.4	Presser and Luoma 2010
Trichoptera		2.15	5.4	Presser and Luoma 2010
Trichoptera		1.3	3.1	Presser and Luoma 2010
Trichoptera		3.7	11.8	Presser and Luoma 2010
Trichoptera		0.6	3.8	Presser and Luoma 2010
Trichoptera		0.78	6.2	Presser and Luoma 2010
Diptera		43.5	139	Presser and Luoma 2010
Diptera		1.3	1.6	Presser and Luoma 2010
Diptera		4.6	18.8	Presser and Luoma 2010
Diptera		3.1	12.4	Presser and Luoma 2010
Diptera		5.3	11	Presser and Luoma 2010
Diptera		0.61	1.6	Presser and Luoma 2010
Diptera		0.25	1	Presser and Luoma 2010
Diptera		3.6	6.9	Presser and Luoma 2010
Diptera		3.2	7.2	Presser and Luoma 2010
Diptera		9.1	27	Presser and Luoma 2010
Diptera		9.3	34	Presser and Luoma 2010
Diptera		8.8	28.4	Presser and Luoma 2010
Diptera		4.4	5.9	Presser and Luoma 2010
Diptera		3.7	8.4	Presser and Luoma 2010
Diptera		0.6	2.4	Presser and Luoma 2010
Diptera		0.78	1.3	Presser and Luoma 2010
Odonata		9.1	45	Presser and Luoma 2010
Odonata		35.2	98	Presser and Luoma 2010
Odonata		43.5	175	Presser and Luoma 2010
Odonata		4.4	7.5	Presser and Luoma 2010
Odonata		9.3	18	Presser and Luoma 2010

Odonata	0.34	1.7	Presser and Luoma 2010
Diptera	35.2	102	Presser and Luoma 2010
Odonata	26	42.6	Presser and Luoma 2010
Odonata	26	29.5	Presser and Luoma 2010
Odonata	35.2	69	Presser and Luoma 2010
Odonata	43.5	122	Presser and Luoma 2010
Odonata	1.3	1.6	Presser and Luoma 2010
Odonata	2.2	5.6	Presser and Luoma 2010
Odonata	5.3	10.2	Presser and Luoma 2010
Odonata	0.78	2.3	Presser and Luoma 2010
Odonata	1.7	10.5	Presser and Luoma 2010
Odonata	6.7	16.5	Presser and Luoma 2010
Composite	30.5	93	Presser and Luoma 2010
Composite	6.6	25	Presser and Luoma 2010
Composite	30	93	Presser and Luoma 2010
Composite	3.2	11.5	Presser and Luoma 2010
Composite	6.3	13.9	Presser and Luoma 2010
Composite	4.3	17.9	Presser and Luoma 2010
Composite	1.8	7.2	Presser and Luoma 2010
Composite	2.1	9.6	Presser and Luoma 2010
Composite	5.45	21.7	Presser and Luoma 2010
Composite	5.5	26	Presser and Luoma 2010
Composite	4.1	7.8	Presser and Luoma 2010
Ephemeroptera	2.7	9.6	Presser and Luoma 2010
Ephemeroptera	4.4	11.1	Presser and Luoma 2010
Ephemeroptera	8.8	18.4	Presser and Luoma 2010
Ephemeroptera	26	66.6	Presser and Luoma 2010
Ephemeroptera	2.15	7.3	Presser and Luoma 2010
Ephemeroptera	3.7	10.1	Presser and Luoma 2010
Ephemeroptera	0.6	6.2	Presser and Luoma 2010
Ephemeroptera	0.78	7.8	Presser and Luoma 2010
Plecoptera	2.15	2.7	Presser and Luoma 2010
Plecoptera	3.7	9.4	Presser and Luoma 2010
Plecoptera	0.6	3.3	Presser and Luoma 2010
Plecoptera	0.78	2.7	Presser and Luoma 2010