DIFFERENTIAL SELENIUM UPTAKE BY PERIPHYTON IN BOREAL LAKE ECOSYSTEMS

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ABSTRACT

Selenium (Se) is a naturally occurring trace element with a narrow margin between essentiality and toxicity in many organisms. Selenium is a contaminant of concern in the boreal forest region of North America because certain anthropogenic activities increase the loading of Se into cold-water aquatic ecosystems, which can have adverse effects on higher trophic levels such as fish, amphibians, and birds. Selenium is rapidly and efficiently assimilated from the water column into organisms at the base of the food web and transferred to higher trophic levels through dietary pathways. This initial step of aqueous Se uptake by organisms at the base of the food web is the greatest step in Se assimilation into aquatic food webs and has much uncertainty surrounding it. Complex assemblages of algae, bacteria, fungi, and detritus that exist at the sediment-water interface, also known as periphyton, play a key role in Se incorporation and biotransformation to more harmful organic forms and in energy cycling in aquatic systems. There are significant site-specific differences that exist in Se enrichment into aquatic food webs by organisms at the base of the food web, which makes predicting the ecotoxicological effects of elevated Se loading uncertain, varying 10^2 to 10^6-fold among different systems. Most field studies focused on the ecological risk assessment of Se have been conducted in warm-water systems and more research is needed regarding the effects of increased Se loading in cold freshwater ecosystems, including how certain water quality variables influence the incorporation of Se into food webs by organisms like periphyton. Additionally, boreal lakes specifically can be at a greater risk to Se toxicity at elevated levels due to the generally low presence of buffering ions like sulfate and phosphate which are known to interfere with Se uptake by various organisms. The goals of my research were to further address these research gaps to better understand the biodynamics of Se assimilation by organisms at the base of cold freshwater food webs. Specifically, an experiment was performed examining the bioaccumulation of low environmentally relevant concentrations of Se as selenite reflecting the current Se guidelines in naturally grown periphyton from multiple boreal lakes. The Se exposure concentrations used were 0.5, 1, 2, 4 µg/L, corresponding to the current freshwater lentic Se guidelines of 1 µg/L in Canada, 1.5 µg/L in the United States, and 2 µg/L in British Columbia. The results of the research revealed that periphyton rapidly and variably accumulated Se at low aqueous Se concentrations in a concentration-dependent manner. A range of periphyton tissue Se concentrations of 8.0 – 24.9 µg/g dm was seen in the 1 – 2 µg/L treatments surrounding the

current freshwater Se guidelines, reaching 30.9 – 50.2 µg/g dm in the highest treatments in certain boreal lake systems. Previous studies have reported adverse effects in invertebrates fed periphyton at similar Se concentrations, suggesting that systems exposed to low levels of Se could experience adverse effects in certain higher trophic level populations. Differential uptake of Se into periphyton among the five studied lakes was also observed, where periphyton from mesotrophic lakes generally accumulated more Se than periphyton from oligotrophic lakes. The differences in Se uptake were likely explained by periphyton community composition and water chemistry differences, however significant correlations between these variables were observed. Higher proportions of the specific algal phylum known as the charophytes in periphyton grown in more oligotrophic lakes corresponded to decreased periphyton Se uptake, as well as in the presence of water with higher dissolved inorganic carbon content. Increased proportions of another algal phyla known as the bacillariophytes or diatoms in periphyton from more mesotrophic lakes corresponded to increased periphyton Se uptake, as well as in the presence of higher total dissolved phosphorus content. The trends demonstrated by different water chemistry and periphyton community variables in this experiment among multiple boreal lakes could serve as representative factors to consider when assessing potential risks of Se toxicity in different lentic systems. The results of this research provide further insight on the biodynamics of Se assimilation at the base of boreal lake food webs at environmentally relevant concentrations, which can potentially inform Se ecological risk assessments in cold, freshwater ecosystems in North America.

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DEDICATION

This thesis is dedicated with love to Virginia, Arno, and Kyle Oldach.

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LIST OF ABBREVIATIONS

ºC	degrees Celsius
[TSe]	total selenium concentration
µg	microgram
µg/g	micrograms per gram
µg/L	micrograms per litre
µL	microlitre
µmol	micromole
α	alpha
AIC	Akaike Information Criterion
ANOVA	analysis of variance
ATP	adenosine triphosphate
ATRF	Aquatic Toxicology Research Facility
BC MoE	British Columbia Ministry of Environment
CCME	Canadian Council of Ministers of the Environment
Chl*a*	chlorophyll *a*
cm^2	centimetres squared
COVID-19	Coronavirus disease 2019
CPCC	Canadian Phycological Culture Centre
DIC	dissolved inorganic carbon
df	degrees of freedom
dm	dry mass
DOC	dissolved organic carbon
DO	dissolved oxygen
EF	enrichment function
g	gram
GH/KH	general and carbonate hardness

GLM	generalized linear model
GLMM	generalized linear mixed model
Ha	hectare
H_2O_2	hydrogen peroxide
HNO_3	nitric acid
HDPE	high-density polyethylene
ICP-MS	inductively coupled plasma mass spectrometry
IC-ICP-MS	ion chromatography inductively coupled plasma mass spectrometry
IISD-ELA	International Institute for Sustainable Development – Experimental Lakes Area
kg	kilogram
km	kilometre
KW	Kruskal-Wallis test statistic
L	litre
LR	linear regression
LTER	long-term ecological research
m	metre
mg	milligram
mL	millilitre
mm	millimetre
NLR	non-linear regression
NO_3^-	nitrate
NO_2^-	nitrite
NH_3	ammonia
PCA	principal component analysis
PO_4^{3-}	phosphate
ppm	parts per million

pR^2	pseudo R squared
RH	rectangular hyperbola
RO	reverse osmosis
ROS	reactive oxygen species
rpm	revolutions per minute
S	sulfur
SD	standard deviation
Se	selenium
Se^0	elemental selenium
Se^{2-}	selenide
SeIV	selenite (selenium 4^+ oxidation state)
SeCys	selenocysteine
SeMet	selenomethionine
SeVI	selenate (selenium 6^+ oxidation state)
TDN	total dissolved nitrogen
TDP	total dissolved phosphorus
TORT-3	lobster hepatopancreas reference material for trace metals
TP	total phosphorus
TTF	trophic transfer factor
US EPA	United States Environmental Protection Agency

NOTE TO READERS

This thesis was prepared in a manuscript style. Therefore, some redundancies may exist among the chapters. Chapter 1 serves as a comprehensive introduction to the topics later discussed in further detail. Chapter 2 is a data chapter has been made into a manuscript for submission to the journal *Environmental Pollution.* The accompanying supplemental information for the publication is included in Appendix A. The anticipated citation for this paper is noted below. Chapter 2 is a summary of the combined results from five distinct experiments conducted in the summer of 2018 and is therefore the only data chapter. Chapter 3 serves as a general integration of how the present research relates to the current research and expands on future research directions. Included in chapter 3 is a proposed experiment that was unable to be carried out due to various unforeseen circumstances. A complete list of references can be found at the end of this thesis to avoid repetition among chapters. Appendix B includes additional results from the study performed and outlined in Chapter 2 that were omitted in the manuscript prepared for publication.

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CHAPTER 1

INTRODUCTION

1.1 Properties of selenium

Selenium (Se) is a trace element with an atomic number of 34 and molecular weight of 78.96 located in group 16 (chalcogens) on the periodic table and exists in chemical forms that have properties similar to sulfur (Lide, 1994; Young et al., 2010a). Selenium was discovered in 1818 by a chemist named Jöns Jacob Berzelius as an unknown impurity causing worker toxicity in a sulfuric acid factory (Young et al., 2010a). Selenium is classified as a non-metal but exists in various physical forms that can behave as metalloids or non-metals. There are four main categories of species that Se can be classified as: 1) inorganic, 2) volatile and methylated, 3) proteins and amino acids, and 4) non-protein amino acids and biochemical intermediates (Maher et al., 2010). Selenium has four oxidation states and exists both inorganically (elemental selenium (Se^0), selenides (Se^{-2}), selenite (Se^{+4} or SeIV) and selenate (Se^{+6} or SeVI) and organically as selenoproteins, selenium containing amino acids and methylated compounds (Cutter, 1989; Young et al., 2010a; Bodnar et al., 2012). In the water column, Se is hydrolyzed to form the oxyanions selenate (SeVI) and selenite (SeIV) which display increased solubility with increasing pH (Young et al., 2010a; Janz, 2011).

1.2 Sources of selenium

Selenium is a naturally occurring unevenly distributed global contaminant found at low levels (0.05ppm) in several forms including black shales, organic-rich marine deposits, metal ores, and crustal rock (Lemly, 2002; Maher et al., 2010; Young et al., 2010a; Bodnar et al., 2012). While some geographic areas are rich in Se and Se toxicity can be a threat, some areas including Finland, New Zealand and certain areas in China and the United States, are deficient in Se and can be at risk of Se deficiencies (Winkel et al., 2015). Selenium is released into the environment through natural processes such as rock weathering, volatilization, and wildfires, but certain anthropogenic activities release greater levels of Se into the environment in comparison

(Maher et al., 2010). Industrial activities practiced worldwide such as crude oil refinement, coal, metal, uranium and phosphate mining, agricultural irrigation of seleniferous soils, and fossil fuel combustion release Se in various chemical forms into many environments (Lemly, 2002; Maher et al., 2010; Young et al., 2010a; Janz et al., 2014). Selenium can enter waterbodies near or distant to the release site through deposition of fly ash from coal-fired power plants (aqueous and vapour phases of Se), run-off from mining waste rock and agricultural land, municipal wastewater discharge and release of certain oil refinery effluents or directly into tailings ponds (Maher et al., 2010, Janz, 2011).

Selenium is generally released by industry as the inorganic forms of selenate or selenite depending on the release source but can exist in various phases and transform into various species depending on specific site characteristics (Maher et al., 2010, Janz, 2011). This variability in natural and anthropogenic sources, variability of Se species and phase, as well as environmental factors that can influence Se, makes it difficult to predict the risk of Se contamination in different environments.

A notable example of Se contamination and subsequent toxicity came from Belews Lake in North Carolina in the 1970s, which received fly ash from a nearby coal-fired power plant. Almost all resident fish species in this reservoir had been impacted due to increased and persistent levels of Se which caused reproductive failure in these populations, and thus extirpation (Lemly, 2002; Young et al., 2010a). Fish are not the only vertebrates affected by elevated levels of Se in aquatic systems, as was seen in the case of the Kesterson Reservoir in California. This reservoir received inputs of agricultural drainage containing Se and the resident adult birds experienced direct toxicity from elevated Se levels, as well as significant reproductive failure through severe deformities and mortality of their embryos and hatchlings (Ohlendorf et al., 1986; Young et al., 2010a). Generally, oviparous (yolk-bearing) vertebrates are more sensitive to Se toxicity than other vertebrates, such as mammals.

1.3 Biological relevance of selenium

1.3.1 Essentiality of selenium

Since Se is an essential element, a certain level of intake is required to maintain certain physiological processes in almost all living organisms, from primary producers such as algae and bacteria, to higher vertebrates including fish and mammals. Selenium is required to make various selenoproteins including glutathione peroxidases and thioredoxin reductases, which provide protection against cellular damage from oxidative stress (Rotruck et al., 1973; Janz et al., 2010), iodothyronine deiodinases which regulate thyroid hormone homeostasis (Bianco and Larsen, 2006), and formate dehydrogenase in bacteria (Böck et al., 1991). Another well studied selenoprotein is selenoprotein P, which is involved in regulating selenium distribution and homeostasis in mammals (Burk and Hill, 2009). Many selenoproteins have been identified and studied, but the functions of many of these Se-containing proteins currently remains unclear (Araie and Shiraiwa, 2016).

Selenium can be substituted into sulfur-containing amino acids to form selenomethionine (SeMet) and actively incorporated into the active site of cysteine to form selenocysteine (SeCys), which has been recognized as the 21st essential amino acid (Böck et al., 1991; Janz et al., 2010). The requirements of dietary Se for maintenance of regular physiological processes including maintaining cell viability varies among different species and classes of organisms (Araie and Shiraiwa, 2009). Fish have the largest selenoproteome, consisting of 32-37 selenoproteins and require between 5-25 μg Se/kg body weight per day depending on the species (Janz, 2011). Humans have 25 selenoprotein families (Janz, 2011) and adults are recommended to consume 55 μg (0.7 μmol)/day, with a tolerable upper intake level set at 400 μg (5.1 μmol/day) (Institute of Medicine (US), 2000). Varying numbers of selenoproteins have been identified in a large range of organisms including bacteria, archaea, and several eukaryotes, however, there have been no selenoproteins yet found in higher plants or fungi (Araie and Shiraiwa, 2009). Interestingly, aquatic organisms generally have more selenoproteins than terrestrial organisms, potentially due to more efficient utilization of Se in aquatic habitats (Araie and Shiraiwa, 2009).

Many knowledge gaps remain regarding the requirements and essentiality of Se in organisms at the base of the food web like algae, but recently selenoproteins and growth stimulating effects of Se has been identified in various algal species (Araie and Shiraiwa, 2009; Araie and Shiraiwa, 2016). In a review identifying Se requirements in phytoplankton growth, 33 species from six distinct phyla including diatoms, chlorophytes, dinoflagellates and chrysophytes

demonstrated stimulated growth in the presence of added Se (Araie and Shiraiwa, 2009). However, even if a species has selenoproteins and demonstrates increased growth in the presence of Se, some do not actually require Se for growth, such as the green algae *Chlamydomonas reinhardtii* (Araie and Shiraiwa, 2016). Another study by Baines and Fisher (2001) found that differing phytoplankton species concentrated Se regardless of variable Se requirements, with species in the same family (thus assumed similar Se requirements) exhibiting significantly different Se uptake, concluding therefore that some microalgae take up far more Se than physiologically required. The variability among algae in requirements of Se and the many knowledge gaps remaining in this field, along with the significance these organisms have in respect to incorporation of Se into food webs warrants further investigation and research.

In addition to algae, many other organisms like aerobic and anaerobic bacteria require Se for maintaining a regular functioning metabolism. Bacteria assimilate Se oxyanions which are reduced to SeCys and SeMet and incorporated into selenoproteins which have structural and enzymatic functions against reactive oxygen species (ROS) (Staicu et al., 2017). Some anaerobic bacteria are also able to use Se as a terminal electron acceptor for cellular respiration, creating energy for bacterial growth in these conditions (Staicu et al., 2017). A study by Kousha et al., (2017) found that increasing selenite concentrations increased growth and total amino acids in lactic acid bacteria, where incorporated selenite was biotransformed mainly into SeCys.

1.3.2 Toxicity of selenium

While required for various processes, Se has a very narrow margin between essentiality and toxicity and can cause detrimental effects to many organisms when present at high concentrations. Dietary exposure is the primary route of exposure for chronic toxicity for primary consumers and vertebrates, specifically through exposure to organic selenium compounds (Stewart et al., 2010; Young et al., 2010a; Janz, 2011). Oviparous (egg-laying) vertebrates are very sensitive to toxic effects from elevated dietary Se exposure (Lemly, 2002; Janz et al., 2010). When too much Se is present in an organism, Se can behave as a sulfur (S) analog and replace S in some proteins and enzymes, which can cause functional problems and/or toxicity (Stewart et al., 2010). Specifically, teratogenicity from chronic exposure can be a highly detrimental effect to these populations, where Se is maternally transferred to developing embryos (Janz et al., 2010; Maher et al., 2010).

In birds, Se is concentrated in the albumin and embryos are exposed to Se during yolk sac resorption, whereas in fish, they are exposed during yolk absorption after vitellogenesis (Spallholz and Hoffman, 2002; Janz et al., 2010). Embryo-larval deformities are a common effect of Se exposure in fish and birds, and these deformities can lead to population declines through impaired survivability and reproduction (Spallholz and Hoffman, 2002; Janz et al., 2010; Young et al., 2010a; Janz, 2011). Oxidative stress is another mechanism of toxicity from elevated Se exposure that may have negative impacts on these vertebrate populations (Spallholz and Hoffman, 2002; Palace et al., 2004).

In many aquatic invertebrates, dietary exposure to Se can be responsible for 90% of Se in body burdens (Stewart et al., 2010). Invertebrates have previously been regarded as a fairly tolerant group of taxa to Se toxicity, whose main concern lies in being a contaminated food source to higher vertebrates (Lemly, 2002; deBruyn and Chapman, 2007; Conley et al., 2009). However, deBruyn and Chapman (2007) reported invertebrate toxicity from Se exposure at guidelines considered 'safe' for birds and fish, which resulted in mortality and reductions in growth in various invertebrate species. Conley et al. (2013) found that the mayfly *Centroptilum triangulifer* experienced significant detrimental effects on survival, development time and secondary production from elevated Se exposure. Another study by Conley et al. (2009) found that when *C. triangulifer* was exposed to environmentally relevant levels of Se-loaded natural periphyton from a lotic system as a food source, significant decreases in fecundity and adult body mass was observed. While knowledge gaps still remain in this area, it is apparent that toxic effects occur in some invertebrates at Se levels considered safe, and that they should be regarded as more than simply an exposure route for higher vertebrates.

Organisms at the base of the food web accumulate Se rapidly and to a greater extent than any other aquatic community (Graham et al., 1992; Janz, 2011), through incorporation of inorganic Se directly from the water column into aquatic food chains (Bottino et al., 1984; Young et al., 2010a). These organisms are generally regarded as tolerant to high levels of Se and do not often exhibit symptoms of toxicity (Riedel et al., 1991), however, many exceptions have been reported. A more recent study with the green alga *Chlamydomonas reinhardtii* reported reduced cell growth, cell bloating and formation of starch granules as a result of Se toxicity at high concentrations (Vriens et al., 2016). A review by Schiavon et al., (2017) reports toxic

effects of excess Se exposure in various algal species, including reduced growth, impaired primary production, potential damages to chloroplast structure from Se-generated ROS, malformation of proteins and inhibition of cell division. Many algal species possess detoxification mechanisms to cope with excess Se, including promoting enzymatic and non-enzyamatic antioxidant activity, and biomethylation/transformation of Se to less toxic species such as Se^0 and dimethyl selenides (Vriens et al., 2016; Schiavon et al., 2017).

Bacterial communities are also generally regarded as quite tolerant organisms to Se (Young et al., 2010a; Janz, 2011), but some exceptions have been reported in this case also. Various bacterial species exposed to Se as selenite demonstrated impaired growth, phenotypic changes and altered cell morphology (Staicu et al., 2017). Although Se can be toxic to bacteria, bacteria have developed effective detoxification strategies to overcome Se toxicity in some cases. Bacteria are able to reduce Se oxyanions to elemental Se via glutathione and thioredoxin systems, and dissimilatory, sulfide-mediated or siderophore-mediated reduction (Staicu et al., 2017). Kousha et al., (2017) reported that increasing selenite concentrations did not inhibit growth in the bacteria *Pediococcus acidilactici* but did result in lower and more plateaued growth patterns when compared to bacteria in lower Se treatments. This pattern was likely observed due to the activation of bacterial detoxification mechanisms at higher Se treatments in comparison to lower Se treatments, which involve transformation of accumulated selenite to Se^0 and subsequent deposition in the outer edges of bacterial cells (Kousha et al., 2017).

1.4 Selenium in aquatic environments

1.4.1 Biogeochemical cycling of selenium in freshwater

The cycling and speciation of Se is complex, and environmental compartments like sediments, water, and aquatic biota all play key roles in how Se is distributed within a freshwater system. Selenium enters water bodies in various concentrations and species through direct release of wastewaters or effluents into surrounding freshwater systems, agricultural runoff to systems especially during high rainfall events, or atmospheric deposition, like fly ash settling from coal-fired power plants, or volatilized Se, which can settle in surrounding systems or in systems a considerable distance from the source (Maher et al., 2010). Aquatic systems with

higher productivity (i.e., biological activity) and longer residence times are expected to have greater accumulation of Se and potential for toxicity (Hillwalker et al., 2006; Young et al., 2010a). Lotic waters are characterized by flowing, less productive, and oxic conditions and tend to have Se more prevalent in the form of inorganic selenate, whereas lentic waters which are more productive and have more reducing conditions tend to have more Se in the form of inorganic selenite (Simmons and Wallschläger, 2005; Stewart et al., 2010). Selenate can be naturally reduced to selenite when in reducing conditions or by selenate-reducing bacteria. Laboratory experiments have demonstrated rapid reduction (< 96 hours) of selenate to selenite in static and static-renewal conditions when in the presence of the selenate-reducing bacterial family, Comamonadaceae (Conley et al., 2013). Oxidation of selenite to selenate in natural waters is unlikely due to the slow oxidation kinetics of dissolved oxygen and stability of selenite ions (Maher et al., 2010). Selenite is preferentially taken up over selenate by organisms at the base of food webs, and subsequent bioaccumulation and toxicity to higher trophic levels (e.g., fish) is seen to a greater extent in lentic systems than lotic systems (Simmons and Wallschläger, 2005; Orr et al., 2006; Stewart et al., 2010).

Biological and microbially mediated reactions drive Se cycling in aquatic systems (Young et al., 2010a). Aside from reducing selenate to selenite, microbial reactions can form organic Se^{2-}, which is even more bioavailable than selenite, or form insoluble Se^{0} which can accumulate in sediments or volatilize out of the system (Simmons and Wallschläger, 2005; Young et al., 2010a). In wetlands significant methylation and volatilization of Se occurs, and increased emissions have been linked with increasing temperature (Vriens et al., 2014). As mentioned previously, organisms at the base of the food web including bacteria, fungi and algae take up dissolved inorganic Se directly from the water and incorporate Se into the food web (Graham et al., 1992). In doing so, Se is biotransformed into highly bioavailable forms of organic Se, most commonly as SeMet and SeCys (Young et al., 2010a; Janz, 2011). Once Se is incorporated into the food web, it is passed to higher organisms through dietary pathways, where it can exhibit toxicity.

Ambient aqueous Se concentration is an important factor when considering Se uptake in various systems, as well as the natural species of Se found. A study performed by Fowler and Benayoun (1976) examining Se concentration over three orders of magnitude in marine

invertebrates found that Se uptake was highly dependent on variable ambient Se concentrations and that selenite was taken up to significantly greater extents than selenate in mussels.

1.4.2 Enrichment function and trophic transfer

Primary producers like algae and bacteria account for the most significant step of Se bioaccumulation and incorporation into aquatic food webs. These organisms bioconcentrate Se directly from the water column at a 10^2-10^6 fold increase from the water to their tissues (Stewart et al., 2010). The enrichment function (EF) represents this increase of Se concentration from water into these organisms and can be calculated by taking the concentration of Se in the tissue of the organism divided by the Se concentration in the water (Stewart et al., 2010). Once Se has been incorporated at the base of food webs, usually to a much greater extent than the concentration in the water, it can be passed on to higher trophic levels through dietary pathways (Graham et al., 1992; Stewart et al., 2010). Trophic transfer functions (TTF) represent the increase in Se concentration from lower trophic levels to higher trophic levels (e.g., from primary producers to invertebrates, and/or from invertebrates to fish) (Stewart et al., 2010).

In the Elk River Valley in British Columbia, Canada, waste rock from multiple open-pit coal mines leaches and drains into the river directly, or into the surrounding wetlands before entering the Elk River (Young et al., 2010b). Selenium is present in concentrations of over 300 µg/L in this drainage water and thus has accumulated significantly in various biotic compartments, as well as accumulated in nearby aquatic environments. A long-term monitoring site 60 km downstream from the coal mines experienced elevated levels of Se, and lentic systems nearby had significant accumulation of Se in biota. Periphyton had Se tissue concentrations of 5 µg/g dm, benthic invertebrates had concentrations of 26-96 µg/g dm compared to 2.7-9.6 µg/g dm in benthic invertebrates in lotic systems nearby, and fish tissue from lentic systems had up to 76 µg/g dm compared to 4-15 µg/g dm in fish from lotic systems (Young et al., 2010b). This case specifically demonstrates bioaccumulation and enrichment by organisms at the base of the food web and subsequent trophic transfer of Se up through food webs, as well as the complexity when dealing with Se mobilization and environmental fate for management at certain sites.

1.4.3 Current freshwater guidelines

Due to the ability of primary producers to bioaccumulate Se readily directly from the water column, looking at water concentrations alone is usually not sufficient in providing protection for fish and waterfowl populations exposed to Se contaminated waters (Stewart et al., 2010). In 2016, United States Environmental Protection Agency (US EPA) proposed a tissue-based guideline for Se and updated freshwater guidelines to help protect aquatic life. The guideline for fish tissue is as follows: Egg/ovary: 15.1 µg/g dm, whole body: 8.5 µg/g dm or fish muscle (boneless, skinless fillet): 11.3 µg/g dm. The previous freshwater Se guideline was set at 5 µg/L but has now been updated to 1.5 µg/L for lentic systems and 3.1 µg/L for lotic systems (US EPA, 2016).

The current Canadian guideline for Se in all freshwater systems is 1 µg/L, with no tissue-based guideline established (CCME, 2007). In British Columbia specifically, a guideline of 2 µg/L for both freshwater and marine ecosystems was established, with an alert guideline of 1 µg/L (BC MoE, 2014). Tissue-based guidelines have also been established and are as follows: 11 µg/g dm for egg/ovary, 4 µg/g dm whole body, 4 µg/g dm for muscle/muscle plug of fish, and an interim dietary guideline for invertebrate tissue of 4 µg/g dm. (BC MoE, 2014).

There is on-going debate regarding these guidelines, since the uptake of Se is highly site-specific (Simmons and Wallschläger, 2005). In certain systems, concentrations of aqueous Se of 1.5 µg/L is still enough to cause toxicity to higher organisms by dietary means through bioaccumulation of Se at the base of the food web (Janz, 2011). Aqueous Se concentrations below 0.7 µg/L have even been suggested due to the potential of Se toxicity, as this concentration can result in Se accumulation in fish gonads above recommended safe levels (Mailman, 2008).

1.5 Interactions of selenium and other molecules

1.5.1 Water chemistry variables

Water chemistry parameters such as light availability, pH, and humic substances can influence Se uptake from the water column into primary producers in freshwater systems. Salinity may have an influence on Se toxicity and accumulation, as some studies have found greater aqueous Se uptake in less saline waters, and lower mortality in fish in more saline waters

when exposed to organo-selenium compounds (Stewart et al., 2010). Selenite uptake has been correlated strongly with carbon uptake in relation to light availability and primary production, as well as to uptake in the dark, thus suggesting selenite uptake can be independent of light and primary production (Baines et al., 2004). This variation can be explained by uptake of Se in different environmental compartments, via phytoplankton and bacteria in this case (Baines et al., 2004).

Riedel and Sanders (1996) found that pH variation did not greatly influence selenate uptake, but strongly influenced selenite uptake at lower pH values. At pH values ≥7, selenite uptake by living phytoplankton *Chlamydomonas reinhardtii* was 17 x 10^{-18} g Se/cell per hour, but this uptake rate doubled (37 x 10^{-18} g Se/cell per hour) at pH 6. Furthermore, at pH 5 this uptake rate significantly increased to 167 x 10^{-18} g Se/cell per hour, demonstrating that lower pH values enhance selenite uptake in this phytoplankton species. Riedel and Sanders (1996) included heat-killed algal cells in this experiment to determine adsorption of selenite and found that except for at pH values of 5, selenite uptake in heat-killed cells was approximately half that of selenite uptake in living cells. In contrast, Ponton et al. (2018) reported that increasing pH increased selenite and selenomethionine accumulation in *C. reinhardtii*. Butler and Peterson (1967) performed a study using duckweed *Spirodela oligorrhiza* and found that duckweed cultured at a pH of 5 or 7.2 had no effect on selenate or selenite uptake, but that selenite was taken up three times more readily than selenate at both pH values.

Dissolved organic carbon (DOC) has also been documented to interact with Se. Pokrovsky et al. (2018) sampled approximately 70 lakes in the Western Siberia Lowland and found that Se exhibited a strong correlation/linear relationship with DOC during the summer and fall seasons when Se concentrations are highest, but not in the spring when Se concentrations are lowest. However, Roditi et al. (2000) found that DOC did not affect dissolved Se absorption by zebra mussels, but increased absorption of other trace metals when using tidal freshwater from the Hudson River. Gustafsson and Johnsson (1994) found that selenite readily complexed with humic substances when added to a Swedish brown-water lake with high humic-substance content. In the presence of iron, selenite retention increased, but significantly decreased in the presence of phosphate, suggesting that iron assists in selenite complexation, and that selenite

may behave similar to phosphate in how it binds to metal-organic complexes (Gustafsson and Johnsson, 1994).

An interesting study performed by Wang et al. (1995) examining Se in sediments from varying trophic statuses in Finland found positive relationships between Se and humic substances, as well as significant relationships between Se and both nitrogen and phosphorus concentrations in eutrophic lake sediments. They found that 52.2% of the total dissolved aqueous Se was bound to humic substances, which comprised 57% of the total organic carbon fraction. Wang et al. (1995) also found that perch (*Perca fluviatilis*) Se body burdens were significantly correlated with trophic status of these lakes. Although the Se concentrations did not vary significantly between these lakes, perch from the oligotrophic lakes had significantly higher Se accumulation in their tissues in comparison to perch from mesotrophic and eutrophic lakes, and perch from the mesotrophic lakes had significantly higher Se accumulation than those from the eutrophic lakes. Simmons and Wallschläger (2005) speculated that this difference (i.e., lowest Se accumulation in perch from eutrophic lakes) could be due to higher phosphate levels in these lakes, which may have an antagonistic interaction on the uptake of Se into food webs.

A study examining Se uptake in marine invertebrates found that temperature significantly influenced Se uptake in the mussel *Mytilus galloprovincialis* (Fowler and Benayoun, 1976). Selenium concentration factors were approximately doubled after 13 days of exposure when temperature was increased from 13°C to 24°C. This study also examined the benthic shrimp *Lysmata seticaudata*, which did not have the same response to increasing temperature and Se concentration, however, the shrimp kept at 24°C molted twice as often as those at 13°C. These molts contained 65% of the shrimp's whole body burden, which could potentially point to increased Se uptake at higher temperatures in the shrimp as well (Fowler and Benayoun, 1976).

1.5.2 Elements and other ions

In addition to interacting with water chemistry variables, Se has also been demonstrated to interact with various metals, including mercury, arsenic, copper, and manganese, generally antagonistically (Broyer et al., 1972; Janz, 2011), and ions such as phosphate, nitrate, and sulfate. Certain metals like mercury (Hg) and cadmium (Cd) can form complexes with Se, which can bind with very high affinity, decreasing their ability to be taken up by certain organisms, and thus reducing potential toxic effects from exposure to these compounds (Schiavon et al., 2017).

Increasing nitrate concentrations have been shown to decrease Se accumulation and intracellular distribution, along with decreasing selenite uptake rates when increased from 5 to 200 µM nitrate in a freshwater green algae species (Yu and Wang, 2004b).

Several laboratory studies have demonstrated an antagonistic interaction between sulfate and selenate (Williams et al., 1994; Riedel and Sanders, 1996; Hopper and Parker, 1999). Ponton et al. (2018) found that the green algae *C. reinhardtii* preferentially took up selenate in comparison to selenite in the presence of low sulfate levels but switched in the presence of high sulfate levels to favoring selenite uptake. A study examining selenate uptake in the presence of sulphate among two primary producers found that while tissue Se concentrations increased with increasing aqueous Se concentrations, increased sulfate concentrations significantly reduced Se uptake (Lo et al., 2015). This study also found differences in Se uptake among the primary producers used, *Lemna minor* (duckweed) and *Pseudokirchneriella subcapitata* (green algae), which highlights the importance of species composition when considering Se uptake in natural systems, as well as ions present (Lo et al., 2015).

A less studied yet important inhibitory interaction also exists between selenite and phosphate. A study performed by Friesen et al. (2017) with selenite-exposed periphyton under different light and nutrient conditions found that the least Se accumulation occurred in the treatment that included phosphorus after 21 days of incubation. Their results suggest that Se uptake by periphyton is influenced by factors other than aqueous Se concentration, and that water chemistry variables such as phosphate and species present within periphyton assemblages likely contribute to differences seen in periphyton Se accumulation (Friesen et al., 2017).

Various experiments using the model freshwater green algae *C. reinhardtii* have demonstrated the direct interaction between selenite and phosphate. Vriens et al. (2016) found distinct competitive inhibition of selenite uptake in the presence of increasing phosphate concentrations after exposing *C. reinhardtii* for 24 hours. Selenite uptake decreased by 15% when phosphate concentrations were doubled, and decreased by 50% when phosphate concentrations were increased 10-fold (Vriens et al., 2016). Riedel and Sanders (1996) reported that selenite uptake was greatly enhanced in phosphate-limited conditions using *C. reinhardtii*. Yu and Wang (2004b) found that increasing phosphate concentrations significantly reduced selenite uptake rates by 92x in *C. reinhardtii*. They also found that algal cells in P-depleted

mediums had 76-91% of Se in intracellular pools, while P-enriched mediums had only 39-43% of Se in intracellular pools (Yu and Wang, 2004b). However, a study performed by Morlon et al. (2006) using *C. reinhardtii* found no interaction between phosphate and selenite, although their exposure periods (60 minutes) were relatively short in comparison to the other studies mentioned. Yu and Wang (2004a) found that increasing ambient phosphate concentrations from 0.5 µM to 50 µM decreased selenite accumulation by 126x in the freshwater green algae *Scenedesmus obliquus*. Wang and Dei (2001a) found that a phosphate addition of 7.2 µM P significantly inhibited selenite uptake in the marine green algae *Chlorella autotrophica*. A similar study reported that selenite accumulation in *C. autotrophica* and the marine diatom *Skeletonema costatum* was also significantly and inversely dependent on ambient phosphate concentrations, likely due to competition for uptake (Wang and Dei, 2001b).

Hopper and Parker (1999) demonstrated competitive inhibition of selenite uptake by phosphate in two plant species. In ryegrass, a phosphate concentration increase from 2 to 20 µM P resulted in a 49% decrease in root Se when grown in 5 µM selenite soil conditions (Hopper and Parker, 1999). Another study performed in plants (Broyer et al., 1972) found that increasing selenite concentrations in *Astragalus bisulcatus* increased plant yield and selenite concentrations, while simultaneously decreasing plant phosphate concentrations. A similar experiment performed with *A. canadensis* using increasing phosphate concentrations demonstrated that as phosphate concentrations increased, plant growth was unaffected and plant Se concentration decreased (Broyer et al.,1972). Understanding the influence of nutrients on Se uptake in aquatic systems is crucial because if Se uptake is enhanced in low P conditions, these systems could be at a greater risk of Se accumulation and toxicity even at low Se concentrations (Wang and Dei, 2001a).

1.6 Lentic systems and the Canadian boreal forest region

Freshwater lentic systems are vital habitats and resources for an extremely wide diversity of organisms. Lentic systems are defined as standing water bodies that are mixed by wind and heat (Kalff, 2002). The littoral zone in lentic systems is defined as sediments in the near-shore region within the photic zone, which is often dominated by photosynthetic organisms such as periphyton (Kalff, 2002). Lentic systems are characterized by longer retention times and flushing

rates that vary depending on lake morphometry, catchment size, climate and runoff sources (Kalff, 2002). Lentic systems are generally reducing environments because they are less oxic due to standing conditions, and usually have higher productivity rates compared to lotic (flowing) systems (Young et al., 2010a). Due to lower flushing rates and larger water volumes, pollutants generally reside longer in lentic systems than lotic systems (Kalff, 2002), which can enhance toxicity to resident species depending on the contaminant.

The Canadian Boreal Shield is the largest ecozone in Canada, comprising approximately 20% of land mass. This region is responsible for 43% of commercial forestland and 22% of Canada's freshwater surface area and provides over $50 billion in gross domestic product through services including hydroelectricity, forestry, and mining (Environment Canada, 2000). These services provide significant risk to water quality due to release of industrial effluents, altering water quality parameters (e.g., increased turbidity, organic matter content or addition of contaminants), shoreline erosion and habitat destruction (Environment Canada, 2000).

Boreal lake freshwater ecosystems are generally nutrient limited and considered pristine due to limited exposure to anthropogenic inputs of contaminants and major nutrients like phosphorus and nitrogen, which can have corresponding effects on Se uptake (Riedel and Sanders, 1996; Wells et al., 2010). Clear lakes that have low total nutrient (nitrogen and phosphorus) and algal concentration are classified by the trophic level index as 'oligotrophic' lakes (Pavluk and Bij de Vaate, 2013). These systems are often phosphorus limited, and phosphorus plays an important role in lentic system dynamics. Phosphorus has no external mechanisms (i.e., no gaseous phase), and phytoplankton (algal) growth is generally proportional to phosphorus content (Schindler, 1977). Additions of phosphorus can also influence other nutrient dynamics in boreal lake systems (i.e., increase carbon and nitrogen content), and act as a leading contributor to eutrophication in these systems (Schindler, 1977; Schindler et al., 2008). Additional trophic level classifications exist for lentic systems, including 'mesotrophic' lakes which are defined as having moderate nutrient and algal concentration, and 'eutrophic' lakes which have higher nutrient and algal concentration (Pavluk and Bij de Vaate, 2013).

While lentic systems are crucial, they only make up a total of approximately 3% of the Earth's surface area and are continuously threatened by increasing anthropogenic activities and usage (Hoverman and Johnson, 2012). It is known that Se is widespread global contaminant that

can particularly decimate freshwater systems, as seen in the cases of Belews Lake, Kesterson Reservoir, and the Elk River Valley noted previously. Boreal lake systems are particularly vulnerable to Se toxicity due to the relatively low presence of ions known to interfere with Se uptake, such as sulfate and phosphate (Vriens et al., 2016; Gupta and Gupta, 2017; Ponton et al., 2020), low carbonate concentrations (Hecky and Hesslein, 1995), and proximity to invasive anthropogenic activities like mining (Environment Canada, 2000). These lentic systems are also generally at a greater risk of toxicity through Se bioaccumulation due to increased exposure duration from site-specific characteristics including lower flushing rates and longer residence times in comparison to flowing systems (Simmons and Wallschläger, 2005; Hillwalker et al., 2006; Orr et al., 2006; Stewart et al., 2010). Additionally, cold freshwater ecosystems like boreal lakes are also relatively understudied in comparison to marine and warm water systems when examining Se risk assessment (Janz et al., 2014).

1.6.1 Periphyton in lentic systems

'Periphyton', also known as "biofilm", is defined as a complex assemblage of benthic primary producers including algae, bacteria, and fungi, associated with shallow water sediments or vegetation in lentic systems and/or attached to substrate (Stevenson and Bahls, 1999; Kalff, 2002). Periphyton is an important bioindicator of overall aquatic ecosystem health and can be used to monitor potential environmental stressors by observing community shifts (Stevenson and Bahls, 1999). Periphyton in the littoral zone play a key role in energy cycling in lentic systems, serving as significant energy and carbon sources for many consumers, ranging from lower trophic levels including zooplankton, invertebrates, and some larval amphibians, to the highest trophic levels including fish (Stockner and Armstrong, 1971; Cattaneo, 1987; Hecky and Hesslein, 1995).

Periphyton is less frequently evaluated than phytoplankton due to the difficulties in obtaining representative samples for systems, as they grow in comparatively more heterogenous environments than phytoplankton and have more diverse littoral and pelagic consumers (Hecky and Hesslein, 1995). Despite of this, periphyton are extremely important to consider when understanding aquatic systems. Specifically, Hecky and Hesslein (1995) examined benthic algae and phytoplankton consumption in various predators using carbon isotopes in two of the experimental lakes at the IISD-ELA (International Institute for Sustainable Development –

Experimental Lakes Area) and reported that benthic algal carbon contributed similar proportions to phytoplankton carbon to the growth of omnivorous consumers. Due to the key role periphyton play in energy cycling and contaminant incorporation into food webs along with their relatively rapid colonization time, periphyton assemblages are distinctly important model organisms and increased research using these complex biofilms is warranted.

The littoral zone in boreal forest lakes provides ideal growing conditions for periphyton due to high light penetration, sloping shorelines, and an abundance of available substrate for growth (i.e., large portion of rocky bottoms of lakes). Since boreal lakes are generally nutrient limited, productivity and biomass are generally lower in these systems than others, as these nutrients influence periphyton growth (Stockner and Armstrong, 1971; McDowell et al., 2020). Temperature, light availability, flow rates and other water quality variables including carbon in some aquatic systems are additional factors that influence periphyton growth and potentially result in community shifts (Hill, 1996; He, 2010; McDowell et al., 2020). Generally, increasing nutrient levels and temperature correspond to increases in periphyton growth within various ranges. Different periphyton algal species can also vary seasonally in lentic systems (Stockner and Armstrong, 1971; Cattaneo, 1987). Factors like trophic status can influence periphyton community assemblages, with more eutrophic lakes favoring larger-celled filamentous algae in some systems (Cattaneo, 1987).

1.6.1.1 Algae

While periphyton is a complex assemblage of many organisms, algae made up a key fraction of periphyton in boreal lake systems. At the IISD-ELA, diatoms, filamentous blue-green and green algae, and desmids have been found to comprise a large fraction of periphyton algal groups found in the littoral zone (Stockner and Armstrong, 1971). These algae belong to four major algal phyla commonly found in freshwater lentic systems, including the bacillariophytes (diatoms), cyanophytes or cyanobacteria (blue-green algae), chlorophytes (green algae), and charophytes (desmids).

The bacillariophytes are a diverse phylum of eukaryotic algae that are found in all freshwater habitats, and as such are important bioindicators of ecosystem health. Diatoms are characterized by strong silicon dioxide cell walls and contain the carotenoid fucoxanthin in their plastids which creates a golden-brown colored appearance (Brinkmann et al., 2011; Kociolek et

al., 2015). Diatoms can be divided into “centric” diatoms which are radially symmetric, and “pennate” diatoms which are bilaterally symmetric, but not all diatoms are symmetrical (Kociolek et al., 2015). Diatoms often dominate in freshwater systems and are important sediment stabilizers in non-marine systems due to their resistant siliceous cell walls (Brinkmann et al., 2011).

The cyanophytes or cyanobacteria are an interesting group of prokaryotic organisms that are capable of photosynthesis and in some taxa, nitrogen fixation (Mohr et al., 2011). Cyanophytes technically belong to Domain Bacteria, but due to their very similar lifecycles to eukaryotic algae they are often considered as prokaryotic algae under the name “blue-green algae” (Mohr et al., 2011). Cyanophytes are found over an extensively wide range of habitats, including freshwater habitats to extreme environments like saline lakes, hot springs and polar regions, and can often outcompete eukaryotic algae in freshwater systems due to their high tolerance of changing conditions (Mohr et al., 2011; Sheath and Wehr, 2015). Cyanophyte ‘algal surface blooms’ are common in freshwater systems with higher nutrient levels which can be dangerous to organisms both within and near the impacted system, as cyanophytes produce cyanotoxins that can act as hepatotoxins and/or neurotoxins (Mohr et al., 2011; Sheath and Wehr, 2015).

Chlorophytes, or “green algae” are a diverse group of eukaryotic algae that can be found in a variety of freshwater and marine environments that can be grouped by structure, including flagellate, coccoid, colonial, and filamentous groups (John and Rindi, 2015). Chlorophytes are generally green coloured as they possess chlorophyll *a* and *b* pigments, starch, and cell walls comprised of cellulose (John and Rindi, 2015). The charophytes are another group of green algae, however they are distinct from chlorophytes by their evolutionary history. Charophytes are the ancestors of modern terrestrial plants and as such share unique characteristics, including that they are the only group of macroalgae known to possess rhizoids capable of nutrient uptake (Burkholder, 1996; Domozych et al., 2016). Charophytes are becoming increasingly important model organisms for examining plant molecular development and stress physiology due to their similarities to terrestrial plants regarding biosynthetic pathways for various growth regulators and cell wall polymers (Domozych et al., 2016).

1.6.1.2 Bacteria and other components of periphyton

While algae comprise a significant component of periphyton assemblages, other organisms like bacteria, fungi, protozoa, and detritus are additional important components to periphyton community composition. The mix of eukaryotic algae with these other organisms creates distinct habitats in aquatic systems and their metabolic activities play a crucial role in biogeochemical cycles on a global scale (Reitner, 2011). Bacteria are unique prokaryotic organisms that are found in an extremely wide range of environments and have the most diverse domain, including over 80 phyla (Hoppert, 2011). Many bacteria are heterotrophic, but some groups have developed photosynthetic abilities, including the cyanobacteria (Hoppert, 2011). Bacteria possess many complex metabolic pathways, which eukaryotes depend on for carrying out their own metabolic processes (Hoppert, 2011). Fungi are eukaryotic heterotrophs who can be parasitic, mutualistic, or saprophytic feeding on non-living organic material (i.e., detritus) (Weber and Büdel, 2011). Fungi are found in a wide variety of habitats and are characterized by containing cell walls made of chitin or other compounds apart from cellulose (Weber and Büdel, 2011). Some fungi produce harmful compounds known as mycotoxins which can have significant negative implications for a wide range of organisms from plants to humans (Singh et al., 2014).

1.7 Selenium and periphyton

It is known that Se is required for many of the organisms comprising periphyton, however many knowledge gaps remain in the essentiality, toxicity, uptake mechanisms, and metabolism of Se in these organisms at the base of the food web. Major nutrients like nitrogen and phosphorus are additionally required for basic algal physiological requirements, however algae will often take up more of these nutrients than necessary. This is known as ‘luxury consumption’ and does not correlate with an increase in algal growth, rather an increase in uptake of these nutrients as their concentrations rise (Gerloff and Skoog, 1954; Stockner and Armstrong, 1971). The same phenomenon has been seen with Se, where algae will take up more Se than required in the presence of Se, while their growth rates remain unaltered regardless of fluctuating Se concentrations (Baines and Fisher, 2001).

Growth rates and resulting Se concentrations vary among algal species and in the literature. A study by Abdel-Hamid and Skulberg (1995) examining Se effects on the growth of various green and blue-green algae reported that increasing external Se led to different degrees of growth in some algal species and significant inhibition in others. In some algal species, increasing growth and thus biomass sometimes correlates with less internal Se, in a concept described as 'growth dilution'. A study examining the trophic transfer of Se from periphyton to the mayfly *C. triangulifer* found that mayflies fed increased levels of Se-exposed periphyton demonstrated less tissue Se, likely attributed to growth dilution (Conley et al., 2011). Contrastingly, a study examining Se accumulation in the green alga *Chlorella vulgaris* found that internal Se concentrations increased with increasing external Se concentration, along with an increase in growth and biomass (Sun et al., 2014). These differences in how Se can seemingly influence (or not influence) growth in variable algal taxa suggests that further research regarding Se mechanisms be performed in different species.

1.7.1 Uptake and metabolism in algae

Selenium is required by algae in various quantities, but uptake rates do not seem to differ based on algal requirements alone (Baines and Fisher, 2001). Instead, the species of Se present, site-specificities, algal species and community composition differences appear to make more of a difference regarding Se uptake (Baines and Fisher, 2001; Stewart et al., 2010). For example, selenate is taken up by algae actively through the sulfate transporter, and can thus be inhibited if high amounts of sulfate are present (Stewart et al., 2010; Vriens et al., 2016). Differences in Se uptake by various marine algal species have also been observed (Bottino et al., 1984; Wang and Dei, 2001a; Wang and Dei, 2001b). Organic Se species are generally taken up more readily than inorganic Se, but are not the dominant Se species present in natural water columns (Graham et al., 1992; Simmons and Wallschläger, 2005). Differences exist in the uptake of inorganic Se by algae and periphyton, which is usually the dominant form of Se in the water, where selenite is taken up and bioconcentrated more preferentially than selenate (Riedel et al., 1991; Simmons and Wallschläger, 2005; Conley et al., 2013; Vriens et al., 2016).

There is some debate in the literature regarding whether biologically active or passive mechanisms are more important for Se uptake into algal cells. If Se is taken up actively, it will be biotransformed into organo-selenium compounds, which can be toxic to higher trophic levels

when passed through the food web (Bottino et al., 1984; Stewart et al., 2010). If Se is adsorbed to external surfaces of algae, it can still be passed through the food web via dietary means, but as an inorganic form (i.e., not biotransformed), and therefore less toxic to sensitive species like oviparous vertebrates. Regarding selenite specifically, there is evidence that uptake appears to occur both biologically (active, carrier-mediated uptake) and non-biologically (passive, adsorption). Active uptake of Se by an Se-specific transporter has not yet been identified, and so Se uptake is thought to be competitive with other similar ions like nutrients like sulfate, phosphate, and silicate (Schiavon et al., 2017). Vriens et al. (2016) saw different patterns of selenite uptake in *C. reinhardtii* under different water chemistry conditions; in a sulfate enriched medium selenite uptake was sigmoidal, but in a phosphate enriched medium uptake was competitively inhibited, suggesting a different (likely carrier-mediated) mechanism of uptake. A review by Winkel et al. (2015) reported that many algae and bacteria incorporate inorganic and organic Se actively via membrane transport systems, specifically where selenite is taken up via phosphate transporters and/or monocarboxylate transporters in certain species.

Riedel and Sanders (1996) reported that uptake rates of selenite in heat-killed algal cells varied from 10-50% that of living cells, excluding a silicate treatment in which heat-killed uptake was almost the same as living uptake (88% of the living uptake rate). An earlier study performed by Riedel et al. (1991) found that three species of heat-killed algal cells exhibited fairly similar rates of selenite uptake in comparison to living cells (78% in *Anabaena*, 76% in *Chlamydomonas*, and 63% in *Cyclotella* after 12 hrs exposure). This study also found selenite uptake to be a rapid process, reaching maximum uptake rates after only six hours of selenite exposure, and uptake was linear across a range of selenite concentrations (1, 2, 5, 10, 20 and 50 µg Se/L). Fournier et al. (2006) exposed *C. reinhardtii* to selenite, selenate and SeMet at increasing concentrations up to 2,000 µg Se/L for 1 hour in artificial freshwater and found differences in uptake processes. Selenate and SeMet uptake decreased with increasing concentrations, while selenite uptake was linear with no evidence of saturation. Markwart et al., (2019) found no significant differences in selenite uptake by periphyton under different treatments (unaltered, heat-killed, and excluding light), suggesting that non-biological processes like adsorption account for the majority of selenite uptake. Markwart et al. (2019) also found that Se uptake by cyanophytes (blue-green algae) was greater in comparison to bacillariophytes (diatoms) and chlorophytes. Mane et al. (2011) examined Se uptake in the charophyte *Spirogyra*

sp and the blue-green alga *Nostoc commune* and reported that pretreated algae (heat-treated, autoclaved, and chemically treated) adsorbed more Se than non-pretreated algae.

Fisher and Wente (1993) reported that Se uptake by marine phytoplankton was an active process. Using radiotracer experiments with ^{75}Se added as selenite, they found that Se concentrations were 6-10x greater in living cells than dead cells, with maximum uptake rates reaching 40x and 16x more in living cells than dead cells in certain phytoplankton species. Further, in these two species with maximum uptake rates, 99% of total Se added remained in the water column as dissolved Se in the exposures with the dead cells, confirming that dead cell uptake was negligible. Fisher and Wente (1993) also stated that selenite uptake is an active process due to the steady increase of Se uptake over time in the living cell exposures. Baines and Fisher (2001) found significant differences in selenite uptake by different algal species exhibiting a wide range of Se cell concentrations, suggesting that strong biological control exists in selenite uptake. This is also because cell growth rates were independent of extracellular selenite concentrations (ie. cell growth did not slow/stop as Se became depleted), suggesting that selenite uptake is a biological process that is enzymatically mediated (Baines and Fisher, 2001). The authors suggest that their results contrast with other trace metals that are primarily adsorbed because these elements generally demonstrate similar uptake rates per unit surface area. Morlon et al. (2006) found that selenite adsorption was negligible in comparison to the absorbed fraction in *C. reinhardtii* and suggested that adsorption is generally unlikely due to the negative nature of Se oxyanions and negatively charged functional groups on cell membranes, thus limiting attraction and therefore adsorption. Morlon et al. (2006) also found selenite uptake saturation, suggesting that facilitated (mediated) ion transport is a mechanism of selenite uptake. Baines et al. (2004) suggested that uptake of selenite is a regulated mechanism because of a strong relationship they found between selenite and carbon uptake. They suggest that since Se accumulation is so closely related to carbon fixation, thus the fixation of organic matter and cell growth, Se uptake is likely regulated because these processes are fundamental to cell function. Since the mechanism of Se uptake can influence potential toxicity to higher vertebrates in the food web, it is important to better understand the factors that influence Se uptake at the base of the food web.

In a study by Araie and Shiraiwa (2009), the marine algae *Emiliania huxleyi*, selenite at nanomolar concentrations (reflecting actual Se levels found in natural seawater) was found to be taken up actively through an ATP-dependent transport process with a high-affinity for selenite, as well as through a passive transport process with a low affinity for selenite. Their results suggest that selenite is taken up actively by *E. huxleyi* cells at the ocean surface where nanomolar concentrations of selenite are found. Additionally, active selenite uptake processes were not inhibited by the presence of selenate, sulfate or sulfite ions, and selenite at lower concentrations was concentrated more rapidly than selenate, and greater growth-stimulating effects in comparison to selenate in *E. huxleyi* (Araie and Shiraiwa, 2009). Using radiotracers, Araie and Shiraiwa (2009) also found that bioconcentrated selenite was rapidly metabolized to non-toxic Se intermediates, as ^{75}Se-labelled compounds selenite, selenocysteine and SeMet were not detected in their analyses. This further demonstrates species-specific uptake and metabolism of Se.

A review examining Se in different chlorophyte species reported that algal species differences impact bioconcentration of Se, as green algae species accumulate variable extents of Se (Gojkovic et al., 2015). This review also found dose-dependent effects of Se, and evidence of both active Se uptake via saturable transporters and passive transport mechanisms (Gojkovic et al., 2015). In natural periphyton assemblages obtained from a lotic system primarily composed of diatoms, Se exposure at concentrations of 2.4 – 13.9 µg/L resulted in periphyton Se concentrations of 2.2 – 25.5 µg/g (Conley et al., 2009). Mayflies exposed to periphyton of approximately 11 µg/g Se experienced significant negative impacts due to increased Se body burdens through dietary Se exposure, resulting in decreased fecundity, as well as reduced growth in adults (Conley et al., 2009). In an experiment with other groups of algae, chlorophytes were documented to take up the least amount of selenite compared to other phytoplankton species belonging to the prymnesiophytes, dinoflagellates, prasinophytes, diatoms and cryptophytes (Baines and Fisher, 2001).

A study by Ponton et al., (2018) compared Se accumulation in the green alga *C. reinhardtii* and field-collected microplankton and found that plankton accumulated significantly more Se than *C. reinhardtii*. Plankton samples were dominated by chrysophytes, dinophytes, euglenophytes and cryptophytes, and contained only 10% green alga taxa, so the differences in

Se accumulation observed are likely due to taxonomical differences (Ponton et al., 2018). Interestingly, the field-collected plankton samples contained bacteria, whereas the lab cultures of *C. reinhardtii* did not, which could also potentially explain some differences in Se accumulation. Currently, there are relatively few studies summarizing the effects of Se in many charophyte species. This is potentially due to a lack of distinction in the literature among green algae groups, or because they are only more recently becoming important model organisms (Domozych et al., 2016). Another knowledge gap identified from the algal studies presented is the lack of Se concentrations tested at environmentally relevant levels.

1.7.2 Uptake and metabolism in other components of periphyton

Bacteria are generally less studied than algae but represent an important contribution to Se uptake at the base of food webs (Stewart et al., 2010). Baines et al. (2004) found that bacterial uptake of selenite comprised a significant amount of total selenite uptake in both light and dark conditions. Bacteria took up 34 ±6% and 49±11% of total selenite at two river delta sites in California, with 42% and 67% of uptake occurring in dark conditions, respectively. Bacterial accumulation of Se has also been demonstrated in freshwater. Sanders and Gilmour (1994) found that *Pasteurella* spp. accumulated selenite to a greater extent than selenate, and potentially demonstrated both passive and active mechanisms of selenite uptake. In the first 2 hours of exposure, selenite uptake was rapid which is generally indicative of abiotic sorptive processes, but after 2 hours uptake was much slower, which is generally indicative of active uptake mechanisms. Sulfate transporters that are known to actively transport Se have been identified in various bacterial species, including *E.coli*, *Salmonella typhimurium*, *Mycobacterium tuberculosis* and *Cupriavidus metallidurans* (Staicu et al., 2017).

Orr et al. (2006) performed a study demonstrating that Se bioaccumulation in the food web more readily occurred in lentic systems than in lotic systems, due to the hydrological characteristics in the Elk River area in BC, Canada. They also found that the majority of accumulation was found in benthic detritivores, suggesting that the uptake of Se by benthic organisms from the detrital food chain is a key factor in Se cycling in aquatic systems, and play a very important role in subsequent accumulation and toxicity to fish and birds within that system. Fine textured sediments were also an important sink of Se, further contributing to the sediment-detrital cycle of Se uptake in these systems (Orr et al., 2006). Due to evidence of Se

incorporation into the food web through organisms other than algae, it is important to examine natural periphyton assemblages when determining selenite uptake into aquatic food webs instead of examining a single species.

A study examining a range of Se levels and subsequent uptake, accumulation and biotransformation in lactic acid bacteria found that concentrations of 1 mg/L sodium selenite resulted in the most biomass growth over a range of concentrations (Kousha et al., 2017). Lower and higher selenite concentrations did not negatively impact growth, but instead resulted in lower plateaued growth rates. Over an exposure range of 0.5 – 4 mg Se/L, total bacterial Se concentrations ranged from 0.17 – 1.89 mg/g, with each treatment being significantly different than the other (Kousha et al., 2017). Along with the significant concentration-dependent differences in Se uptake observed, the formation of different Se-containing amino acids, including SeCys, methylselenocysteine and SeMet, generally increased proportionally with external Se concentrations (Kousha et al., 2017). These concentration-dependent differences and evidence of biotransformation to more toxic organic forms of Se clearly indicates the importance of examining the bacterial component of periphyton in regard to Se risk assessment.

An interesting study by Luo et al. (2019) found that inoculation of wheat crops with arbuscular mycorrhizal fungi significantly increased Se uptake in the forms of selenite and selenate, but not SeMet. This symbiotic relationship can benefit certain Se-deficient populations through enrichment of Se into food crops, as the presence of this fungi led to higher internal Se concentrations in wheat (Luo et al., 2019). This example also highlights the importance of considering fungi when considering Se uptake in different ecosystems and organisms.

While considerable research articles examining Se incorporation into various organisms in periphyton exist, many research gaps remain involving Se uptake into aquatic food webs by organisms at the base of the food web. Specifically, knowledge gaps remaining include examining Se uptake mechanisms in specific algal phyla and water chemistry variables, the impacts of low environmentally relevant doses of Se, and use of natural periphyton to incorporate other important organisms found in periphyton communities that are often overlooked when examining Se risk assessment.

1.8 Proposed Research

1.8.1 Objectives and hypotheses

To address the identified knowledge gaps, I performed a large-scale experiment consisting of five individual exposures to examine Se uptake at the base of the food web, using inorganic Se as selenite and natural periphyton community assemblages grown in representative cold-water lentic systems. I used selenite because it is generally more prevalent than selenate in lentic systems and is preferentially taken up by organisms at the base of the food web. Low environmentally relevant levels of Se were also used that reflect a range of concentrations surrounding the current guidelines in freshwater systems in North America (CCME, 2007; BC MoE, 2014; US EPA, 2016). I used naturally grown periphyton because it is more complex and representative of natural lentic systems, and a knowledge gap exists regarding Se uptake into periphyton under variable macronutrient conditions (Morlon et al., 2006; Conley et al., 2013). Continued research is also needed in cold freshwater systems regarding the ecological risk assessment of Se (Janz et al., 2014), which is why multiple boreal lakes based on differences in water chemistry parameters were selected. Such insights of Se assimilation at the base of cold lentic food webs will help inform ecological risk assessment in boreal forest regions of Canada through better understanding the potential for Se toxicity in these systems.

The main objectives of Chapter 2 and their hypotheses are:

1) To characterize periphyton Se uptake curves for each lake, and determine if differential Se uptake exists among the periphyton from each of the five boreal lakes examined

H_0: Uptake of Se by periphyton from each of the five lakes will follow the same trend, and there will be no difference in periphyton tissue Se concentrations at the end of the experiment among the five Se exposure concentrations used.

H_1: Uptake of Se by periphyton from each lake will not be the same, due to the natural variation among the lakes including variable water chemistry and periphyton community composition, and the site-specificity that influences Se uptake. I predict that there will be a difference in Se uptake among periphyton from the five lakes for these reasons.

2) Determine which, if any, water chemistry and/or periphyton community composition variables explain the most variation if differential uptake among periphyton from the five lakes is found

H_0: Water chemistry and periphyton community composition will have no influence on periphyton uptake or enrichment functions of Se.
H_1: Water chemistry and/or periphyton community composition will have an influence on selenite uptake by periphyton. I predict that systems with greater nutrient levels will have lesser uptake of Se into periphyton due to competing ions in the water such as phosphate. Therefore, I predict that more oligotrophic systems will have higher uptake, due to less competition from other ions. I also predict that lakes with high levels of DOC will have more selenite uptake due to potential adsorption factors.

The main objectives of Chapter 3 are to integrate the research findings from Chapter 2 demonstrating how knowledge gaps were filled, provide feedback for improving experimental design if the experiment was to be repeated, present a developed experiment unable to be executed in this thesis, and highlight future research ideas identified from the current study and other ideas to continue to diminish the research gaps remaining in predicting Se risk assessment.

CHAPTER 2

DIFFERENTIAL SELENIUM UPTAKE BY PERIPHYTON IN BOREAL LAKE ECOSYSTEMS

Preface

The overall goal of this chapter is to better understand Se assimilation into various cold freshwater lentic food webs through examining the uptake of Se as selenite into naturally grown periphyton from multiple boreal lake ecosystems. This chapter was prepared for publication for submission to the journal *Environmental Pollution* and is displayed here with minor modifications to adhere to University of Saskatchewan thesis formatting guidelines. The corresponding supplementary information for this chapter can be found in appendix A. The full anticipated citation is:

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The author contributions are as follows:

Mikayla D. Oldach wrote the manuscript, performed statistical analysis, performed sample analyses, assisted with experimental design, and performed experiments.

Stephanie D. Graves provided scientific input, guidance for statistical analyses, and editorial assistance through manuscript revision.

David M. Janz conceived experiments, reviewed the manuscript, and provided scientific input, guidance, and editorial assistance through manuscript revision.

2.1 Abstract

The largest and most variable step of selenium (Se) assimilation into aquatic ecosystems is the rapid uptake of aqueous Se by primary producers. These organisms can transfer more harmful forms of Se to higher trophic levels via dietary pathways, although much uncertainty remains around this step of Se assimilation due to site-specific differences in water chemistry, hydrological and biogeochemical characteristics, and community composition. Thus, predictions of Se accumulation are difficult, and cold, freshwater systems are relatively understudied. To address these knowledge gaps, five static-renewal experiments were performed to examine the bioaccumulation of low, environmentally relevant concentrations of Se, as selenite, by naturally grown periphyton from multiple boreal lakes. Periphyton rapidly accumulated Se at low aqueous Se concentrations, with tissue Se concentrations ranging from 8.0 – 24.9 µg/g dm in the 1 – 2 µg Se/L treatments. Enrichment functions ranged from 2870 – 12 536 L/kg dm in the 4 µg Se/L treatment, to 11 867 – 22 653 L/kg dm in the 0.5 µg Se/L treatment among lakes. Periphyton Se uptake differed among the five study lakes, with periphyton from mesotrophic lakes generally accumulating more Se than periphyton from oligotrophic lakes. Higher proportions of charophytes and greater dissolved inorganic carbon in more oligotrophic lakes corresponded to less periphyton Se uptake. Conversely, increased proportions of bacillariophytes and total dissolved phosphorus in more mesotrophic lakes corresponded to greater periphyton Se uptake. Periphyton community composition and water chemistry variables were correlated, limiting interpretation of differences in periphyton Se accumulation among lakes. The results of this research provide insight on the biodynamics of Se assimilation at the base of boreal lake food webs at environmentally relevant concentrations, which can potentially inform ecological risk assessments in cold, freshwater ecosystems in North America.

2.2 Introduction

Selenium (Se) is a globally distributed trace element with a narrow margin between essentiality and toxicity. Anthropogenic activities including crude oil refinement, agricultural irrigation of seleniferous soils, coal, uranium, phosphate, and various other mining activities release excess levels of Se into aquatic environments where it can be efficiently incorporated into aquatic food webs (Maher et al., 2010; Janz, 2011). Organisms at the base of food webs

including algae, bacteria and fungi rapidly and variably accumulate dissolved Se directly from the water column 10^2 to 10^6-fold depending on the concentration and species of Se present, organism community composition, and site-specific water chemistry parameters (Graham et al., 1992; Baines and Fisher, 2001; Stewart et al., 2010). Inorganic Se oxyanions (selenate and selenite) are the most abundant forms of Se in water. These inorganic forms are incorporated by primary producers and rapidly biotransformed into highly bioavailable forms of organic Se such as selenomethionine (SeMet) and selenocysteine (SeCys), which are transferred to higher trophic levels through dietary pathways (Bottino et al., 1984; Maher et al., 2010; Young et al., 2010; Janz, 2011). While Se is essential in most organisms to maintain certain metabolic processes, oviparous (egg-laying) vertebrates are particularly sensitive to chronic Se toxicity via excess organic Se in their diet (Lemly, 2002; Janz et al., 2010). Selenium can act as a teratogen, which can lead to severe embryo-larval deformities from maternal transfer of Se to developing embryos. This is problematic because Se accumulated through dietary pathways from organisms at the base of the food web to higher trophic levels can lead to population declines in severe cases through impaired survivability and reproduction in certain oviparous vertebrate populations (Spallholz and Hoffman, 2002; Janz et al., 2010; Maher et al., 2010; Young et al., 2010; Janz, 2011). Therefore, a better understanding of the initial step of Se uptake from the water column into organisms at the base of food webs is crucial to helping predict the Se toxicity hazard to populations in different ecosystems (Presser and Luoma, 2010).

Lentic (lake) systems are generally at a greater risk of Se toxicity through food web bioaccumulation compared to lotic (flowing) systems because of their longer retention times, lower flushing rates, higher productivity, and large water volumes in standing conditions which create reducing environments (Kalff, 2002; Simmons and Wallschläger, 2005; Hillwalker et al., 2006; Orr et al., 2006; Stewart et al., 2010; Young et al., 2010). Boreal lake ecosystems specifically can be at greater risk to Se toxicity due to the generally low presence of ions known to interfere with Se uptake, such as sulfate and phosphate (Vriens et al., 2016; Gupta and Gupta, 2017; Ponton et al., 2020). The Canadian Boreal Shield is the largest ecozone in Canada and of great economic importance providing ~$50 billion in gross domestic product through services including forestry and mining (Environment Canada, 2000). These industrial services pose significant risk to pristine boreal lake ecosystems, which are important freshwater resources and diverse aquatic habitats (Environment Canada, 2000). Cold freshwater ecosystems like boreal

lakes are also relatively understudied in comparison to marine and warm water systems regarding Se contamination (Janz et al., 2014). Organisms at the base of the food web including periphyton from lentic systems are also relatively understudied regarding Se contamination in comparison to higher trophic levels.

Periphyton is defined as complex assemblages of algae, bacteria, detritus, and fungi associated with shallow water sediments or vegetation. Periphyton is an important food source for invertebrates and plays a key role in energy cycling, as well as in Se incorporation and biotransformation in the food webs of lentic systems (Stockner and Armstrong, 1971; Cattaneo, 1987; Graham et al., 1992; Kalff, 2002). Bioaccumulation of Se from the water column by organisms at the base of the food web including periphyton is the most significant and variable step of Se cycling in aquatic food webs, and yet many knowledge gaps remain regarding how these different organisms accumulate Se (Stewart et al., 2010; Conley et al., 2013). In addition to less available Se accumulation research in boreal lakes, there is debate regarding the protectivity of current freshwater Se guidelines for aquatic life due to the site-specificity of Se risk assessment (Simmons and Wallschläger, 2005), and relatively few studies examining a range of low environmentally relevant Se concentrations in natural systems.

To address the knowledge gaps surrounding Se accumulation at low levels in organisms at the base of the food web in cold-water systems, field experiments were performed to examine uptake of Se as selenite in naturally grown periphyton from five boreal lakes. The Se concentrations used in the experiment represent a range of low, environmentally relevant concentrations of Se (0.5, 1, 2, 4 µg Se/L) and were chosen to reflect the current range of Se guidelines in North America: 1 µg Se/L in Canadian freshwater systems (CCME, 2007), 1.5 µg Se/L in US lentic systems (US EPA, 2016), and 2 µg Se/L in freshwater systems in British Columbia specifically (BC MoE, 2014). Selenite was used because it is preferentially taken up by organisms at the base of food webs over selenate, and it is generally the dominant form of Se found in lentic systems due to their reducing conditions (Simmons and Wallschläger, 2005; Orr et al., 2006; Stewart et al., 2010; Vriens et al., 2016). The objectives of the study were to 1) characterize Se uptake by periphyton at a range of low Se concentrations, and 2) determine if differences exist in Se uptake by periphyton from multiple boreal lake systems.

2.3 Materials and Methods

2.3.1 Site selection

All field work was performed at the IISD-ELA in Ontario, Canada. IISD-ELA is a unique 'natural laboratory', located in a remote region in northern Ontario in the Kenora district. The IISD-ELA was established in 1968 and consists of 58 experimental lakes removed from human activity and industrial processes (Blanchfield et al., 2009). The IISD-ELA is also unique in the sense that it also includes a fully equipped on-site water quality laboratory, a team of experts, as well as several visiting researchers across Canada performing various projects. Over 50 large-scale ecosystem experiments have been conducted at IISD-ELA which have produced ground-breaking research results that in turn have significantly influenced regulatory decisions throughout Canada and worldwide (Blanchfield et al., 2009).

Five relatively distinct boreal shield lakes were selected based on various factors including differences in water chemistry variables including nutrient status and dissolved organic carbon (DOC) levels (Table 2.1), frequency of water quality monitoring/sampling by the IISD-ELA Chemistry lab, accessibility to camp, and differences in general lake parameters including depth and area (Table 2.2). The lakes selected included Lake 114, a shallow mesotrophic lake; Lake 227, a small and artificially 'eutrophic' lake; Lake 239, a larger oligotrophic lake; Lake 224, an ultra-oligotrophic lake; and Lake 470, a small and shallow meso-eutrophic pond. Lake 227 was the first lake to be used in a whole-ecosystem experiment at the IISD-ELA to study nutrient cycling and food web responses to nutrient levels (Blanchfield et al., 2009), whose phosphorus additions are still maintained regularly today. Variability among water chemistry parameters was observed over the experiment, and selenite was the dominant Se species in natural lake water from four of the five study lakes (Table 2.1). Selenate made up the other portions of aqueous Se, and no organic forms of Se were detected in any of the lakes (Graves et al., 2021).

Table 2.1: Mean values of water chemistry variables in study lakes including dissolved organic carbon, dissolved inorganic carbon, pH, total dissolved nitrogen, total dissolved phosphorus, chlorophyll *a*, ammonia, percentage of total Se as selenite, and measured aqueous Se from the study lakes taken in June 2018. Water samples were collected from lakes during the time/duration of the periphyton exposure experiments.

Lake	DOC (µM)	DIC (µM)	pH (SU)	TDN (µg/L)	TDP (µg/L)	Chl *a* (µg/L)	NH_3 (µg/L)	Selenite* (%)	Aqueous Se (µg/L)
L114	552	63.4	6.4	308	2.5	4.8	6.5	53	0.08
L224	286	121.4	7.1	177	1.8	0.9	6.0	58	0.05
L239	604	159.2	7.2	258	1.1	1.9	13.0	43	0.12
L227	690	27.3	8.8	405	6.3	21.9	18.5	60	0.05
L470	1000	65.4	6.2	492	5.1	1.8	36.0	83	0.06

Abbreviations: DOC = dissolved organic carbon, DIC = dissolved inorganic carbon, TDN = total dissolved nitrogen, TDP = total dissolved phosphorus, Chl *a* = chlorophyll *a*, NH_3 = ammonia.

*Selenite concentrations obtained from Graves et al., 2021 (supporting information). Samples were taken in August 2019.

Table 2.2: Maximum depths and surface areas of study lakes. Lakes 114, 224 and 239 are long-term ecological research (LTER) lakes that have been continually monitored by IISD-ELA and are not experimentally manipulated. Lake 227 is an artificially eutrophied lake subject to long-term phosphate additions.

Lake	Max Depth (m)*	Area (Ha)*
L114	5.0	12.1
L224	27.4	25.9
L239	30.4	54.3
L227	10.0	5.0
L470	1.7	4.2

*Values obtained from:https://www.iisd.org/ela/science-data/ourdata/interactive-map/

2.3.2 Experimental design

All materials were washed prior to use using the following protocol: tap water rinse, soap-wash/scrub, tap rinse, >30 minute bleach bath, tap rinse, >30 minute 5% nitric acid (Fisher Chemical, Ottawa, ON) bath, and rinsed thoroughly with nanopure water at the University of Saskatchewan, or reverse osmosis (RO) water at the IISD-ELA. Metal tools were sterilized with 70% ethanol (EtOH) prior to use. Individual periphyton samplers consisted of 5 buffed glass plates (20 cm x 20 cm x 5mm) to act as substrate for natural periphyton colonization and growth. Periphyton sampler frames and glass plates were constructed at the University of Saskatchewan as described previously (Markwart et al., 2019). Each study lake received five periphyton samplers, for a total of 25 plates per lake. Samplers were deployed in May 2018 in the littoral zone at a depth of approximately 1 m and allowed to colonize and grow naturally for at least seven weeks. As the littoral zone is often dominated by photosynthetic organisms and resides within the photic zone (Kalff, 2002), these areas are expected to be relatively oxygenated.

The nominal concentrations of Se used were 0.1-0.2 (control; no Se added), 0.5, 1, 2, 4 µg Se/L as selenite, with five replicates of each treatment per exposure. The selenite stock solution was made by adding 87.13 mg sodium selenite (Na_2SeO_3; Sigma-Aldrich, St. Louis, MO) to 1L of ultrapure water (Barnstead Nanopure 18.2 MΩ·cm, Thermo Fisher Scientific, Waltham, MA, USA), and stored at 4ºC. Appropriate volumes of stock solution were used to

spike appropriate Se levels. To make the proper Se concentrations in the 4L exposure vessels, 50 µL stock was added to make the 0.5 µg Se/L treatment, 100 µL to make 1.0 µg Se/L, 200 µL to make 2.0 µg Se/L, and 400 µL to make 4.0 µg Se/L.

A total of five experiments were performed, one for each study lake. Exposures were staggered due to logistical limitations and were performed from July 2 – August 15, 2018 in a static renewal system set up outdoors at the IISD-ELA research station laboratory (Table 2.3). The set up for each experiment consisted of 25 clear 4.2 L containers, or 'exposure vessels', that each housed a single colonized periphyton plate, with all vessels held in a large water bath to regulate temperature. Natural lake water was used from each lake in the corresponding experiment and transported to camp using multiple 10-L polyethylene containers. Lake water entering exposure vessels was filtered through a 53 µm plankton net to remove predatory zooplankton and prevent algae grazing. The duration of each exposure was eight days to attempt to reach pseudo-steady state of Se concentrations in periphyton while avoiding major community shifts due to altered growth conditions (Markwart et al., 2019). Water changes (100%) occurred every two days, and exposure vessels were re-spiked with appropriate Se concentrations after water changes and mixed with a clean plastic stir stick.

Table 2.3: Sampling dates for aqueous total Se analyses during summer 2018. Samples collected from exposure vessels after 48 hours/before water changes are denoted by "BW", and samples taken after re-spiking Se concentrations are denoted by "AS". A total of 6 samples were collected from one full replicate (one exposure vessel from each treatment) for each lake (n=150).

Lake	Day 0 AS	Day 2 BW	Day 4 AS	Day 6 BW	Day 6 AS	Day 8 BW
L114	Jul 2	Jul 4	Jul 6	Jul 8	Jul 8	Jul 10
L224	Jul 12	Jul 14	Jul 16	Jul 18	Jul 18	Jul 20
L239	Jul 15	Jul 17	Jul 19	Jul 21	Jul 21	Jul 23
L227	Jul 25	Jul 29*	Jul 29	Jul 31	Jul 31	Aug 2
L470	Aug7	Aug 9	Aug 11	Aug 13	Aug 13	Aug 15

*Samples for L227 Day 2 BW were collected on Day 4 BW.

Water quality parameters were monitored in exposure vessels every alternating day between water changes to ensure water quality and consistency using API Fishcare dropper tests for nitrate, general hardness and carbonate hardness, and a water quality probe to measure dissolved oxygen (DO) and temperature (YSI Environmental ProODO Handheld, Yellow Springs, OH). Water quality was consistent within exposure vessels over the duration of the experiment (Table 2.4). Water chemistry measurements used for statistical analyses were collected directly from each study lake and analyzed by the IISD-ELA Chemistry lab (Table 2.1). A multiparameter water quality meter (HI98194, Hanna Instruments Canada Inc, Laval, QC) was taken to the field to be used to measure pH, temperature, DO and conductivity inside individual exposure vessels, but broke upon arrival and was not fixed by Hanna Instruments until early August.

Table 2.4: Mean values of measured water quality parameters within exposure vessels. Vessel temperature, dissolved oxygen, nitrate, general and carbonate hardness data were collected every two days in each exposure vessel, and water bath temperature was measured continuously using temperature loggers.

Lake	Vessel Temp (°C)	WB Temp (°C)	DO (%)	GH/KH (mg/L)	NO_3 (mg/L)
L114	24.7 ± 5.7	23.1 ± 4.5	99.4 ± 7.7	18.4	0
L224	25.5 ± 3.8	22.3 ± 4.1	105.1 ± 3.6	19.7	0
L239	24.6 ± 3.5	21.7 ± 4.4	105.6 ± 7.0	17.9	0
L227	24.3 ± 3.5	19.1 ± 4.1	110.9 ± 12.1	17.9	0
L470	24.0 ± 2.6	22.2 ± 5.6	93.5 ± 6.6	17.9	0

Abbreviations: Temp = temperature, WB = water bath, DO = dissolved oxygen, GH/KH = general hardness/carbonate hardness, NO_3 = nitrate.

2.3.3 Sample collection

Aqueous dissolved Se samples (8 mL) were collected six times throughout each experiment (Table 2.3) from every exposure vessel to confirm aqueous target Se concentrations. Samples were collected using a 5 mL syringe and filtered through a 25 mm syringe filter with a 0.45 µm polyethersulfone membrane. Samples were filtered into acid-rinsed 8 mL high-density

polyethylene (HDPE) nalgene bottles, then acidified with 160 µL high purity nitric acid (HNO_3) (Fisher Scientific, Hampton, NH, USA) in the lab on-site. Samples were kept at 4ºC until analysis was performed at the University of Saskatchewan. Method blanks consisting of on-site RO water were taken in the field equivalent to 10% of the samples using the same materials and methods to ensure quality of aqueous dissolved Se sampling without external Se contamination.

Periphyton tissue samples for community composition and total Se analysis were collected on Day 0 and Day 8 for all replicates by scraping a known area (39 cm^2 for community composition; 78 cm^2 – 156 cm^2 for total Se analysis depending on the level of growth) on each periphyton plate using ceramic scrapers cleaned with 70% ethanol between each replicate. Periphyton for community composition analysis were then rinsed into 10mL falcon tubes containing RO water, preserved with 150 µL Lugol's iodine and wrapped in tinfoil to keep out light. Periphyton total Se samples from Day 0 were rinsed into 50 mL acid-rinsed falcon tubes with RO water, and immediately frozen upon collection to measure initial periphyton Se concentration. For Day 8 samples, all remaining algae was scraped from the plate into acid-rinsed 50 mL centrifuge tubes, and rinsed three times using RO water to measure final Se concentration. In between rinsing, samples were spun in a centrifuge at 1600 rpm and supernatant discarded to ensure any remaining Se spiked water was removed from the periphyton. Samples were stored at -20ºC until analysis at the University of Saskatchewan.

2.3.4 Total Se analysis

Inductively coupled plasma mass spectrometry (ICP-MS) operated in collision cell mode (8800 ICP-MS Triple Quad, Agilent Technologies, Santa Clara, CA) was performed to verify aqueous and periphyton total Se concentrations using previously validated in-house protocols (Graves et al., 2019). All ICP-MS analysis was performed at the University of Saskatchewan. Quality assurance/control procedures included instrumental certified reference material (1640a, trace elements in natural water, National Institute of Standards and Technology) and method blanks (ultrapure water) run with all samples, and method certified reference material (TORT-3, lobster hepatopancreas, National Research Council of Canada) run with periphyton samples analyzed for total Se.

Filtered and acidified aqueous samples (n=75) were measured directly for total dissolved Se using ICP-MS. Target nominal Se concentrations were confirmed in all five exposures across all time points as verified by ICP-MS with an instrumental minimum detection limit of 0.026 µg Se/L ± 0.01 (mean ± SD). Measured aqueous Se concentrations were not statistically significantly different from target (nominal) concentrations and no statistically significant differences were observed in aqueous Se concentrations among lakes (Table 2.5). Method blanks (n=14) consisted of on-site RO water and were below instrumental limits of detection or below sample Se measurements. The instrumental certified reference material 1640a (n=12) run with all water samples had a mean percent recovery of 99.67 ± 1.11%.

Table 2.5: Mean total aqueous Se concentrations determined in water samples collected from exposure vessels (n=75) in comparison to target (nominal) Se concentrations. No statistically significant differences were found among lakes or compared to nominal concentrations using Kruskal-Wallis analysis (p=0.95, KW = 0.71, Dunn's multiple comparisons test: p> 0.99; p=0.98, KW = 0.69, Dunn's multiple comparisons test: p > 0.99, respectively).

Lake	Control	0.5 µg Se/L	1 µg Se/L	2 µg Se/L	4 µg Se/L
L114	0.08 ± 0.0	0.6 ± 0.2	0.9 ± 0.3	1.9 ± 0.3	3.8 ± 0.3
L224	0.03 ± 0.0	0.5 ± 0.1	1.0 ± 0.0	1.9 ± 0.2	3.7 ± 0.0
L239	0.08 ± 0.0	0.6 ± 0.0	1.0 ± 0.0	1.9 ± 0.0	4.1 ± 0.3
L227	0.04 ± 0.0	0.5 ± 0.1	0.9 ± 0.1	1.8 ± 0.3	3.6 ± 0.5
L470	0.08 ± 0.0	0.5 ± 0.2	0.9 ± 0.3	1.7 ± 0.3	3.7 ± 0.6

Periphyton tissue samples (n=155) for total Se analysis required digestion before ICP-MS analysis could be performed. Samples were freeze-dried, weighed, and transferred to Teflon digestion vials. Samples were digested using 30% hydrogen peroxide (Fisher Chemical, Ottawa, ON) and 69% high purity HNO_3, and transferred into a MARS-5 microwave (CEM Corporation, Matthews, NC, USA) on the cycle 'Se BioXpress' ramping to 160ºC for 20 minutes. Samples in vials were allowed to cool fully, then transferred to pre-weighed 8 mL HDPE bottles. Samples were then diluted to 2% HNO_3 to complete preparation for ICP-MS analysis, which involved

filtering 1 mL of digested sample using a syringe with a 0.45 µm polyethersulfone membrane filter into a new acid-rinsed 8 mL HDPE bottle and diluting this sample with 4.5 mL Barnstead water. Samples were then refrigerated at 4ºC until submitted for ICP-MS analysis, or for long-term storage (non-diluted samples) for additional analysis if necessary. Method blanks (n=16) using ultrapure water and certified reference material samples (TORT-3) were included in all digestions accounting for 10% of total samples. The instrumental certified reference material 1640a (n=29) was run with all samples with a percent recovery of 98.35 ± 2.59%, and method certified reference material TORT-3 (n=18) had a mean percent recovery of 88.91 ± 9.62%. The instrumental minimum detection limit was 0.062 ± 0.08 µg Se/L.

2.3.5 Periphyton identification and additional analyses

Periphyton community composition was characterized by light microscopy at the University of Saskatchewan following the methodology in the US EPA Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Stevenson and Bahls, 1999). A Palmer counting cell was used to identify at least 300 cell units to the lowest taxonomic level possible using Freshwater Algae of North America (Wehr et al., 2015) and Phycokey (Baker et al., 2012) as resources. At least three cells (n=3-6) from each identified genus from each sample were measured using an ocular micrometer and used to determine algal biovolumes using the calculations described previously (Hillebrand et al., 1999; Sun and Liu, 2003). Relative abundances of each taxa were found by multiplying cell counts by determined biovolume, and then grouped by phylum for analyses. One sample from each treatment, for a total of five plate samples (n = 5 per lake, total n =25) from each study lake were analyzed to determine community composition for percent relative abundance for each lake. Only the algal component was identified and used for analyses. A complete list of identified algal genera and corresponding biovolume proportions (% relative abundance) are displayed below in Table 2.6.

Table 2.6: Percent relative abundance of algal genera grouped by phylum for each lake as identified by light microscopy. Values are mean ± SD of n=3-6 cell measurements of each genera per sample. Values showing 0.0 are < 0.04 but > 0.

Taxonomic Rank	Relative Abundance (%)

Phylum	Genus	Lake 114	Lake 224	Lake 239	Lake 227	Lake 470
Bacillariophyta	*Achnanthes*	0.1 ± 0.1	-	4.3 ± 1.7	6.9 ± 0.0	3.6 ± 3.9
	Actinella	0.1 ± 0.0	-	-	-	0.3 ± 0.5
	Biremis	0.0 ± 0.0	-	-	0.6 ± 1.0	0.0 ± 0.0
	Cyclotella	0.1 ± 0.2	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0.2
	Cymbella	0.1 ± 0.3	0.4 ± 0.2	0.4 ± 0.9	0.1 ± 0.0	0.1 ± 0.1
	Diatoma	0.4 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Eunotia	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.2	3.9 ± 0.0	7.8 ± 14.5
	Fragilaria	0.5 ± 0.5	0.2 ± 0.3	-	-	-
	Gomphonema	-	1.6 ± 1.4	1.7 ± 0.3	-	-
	Gyrosigma	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	-	0.1 ± 0.2
	Melosira	-	-	-	-	0.2 ± 0.0
	Navicula	1.8 ± 1.5	8.4 ± 0.8	5.6 ± 0.3	17.7 ± 1.3	17.7 ± 6.8
	Nitzschia	0.0 ± 0.1	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Pinnularia	0.3 ± 0.3	0.4 ± 0.0	1.4 ± 4.3	-	1.1 ± 2.1
	Rhopalodia	0.1 ± 0.0	0.3 ± 0.0	2.3 ± 1.6	-	0.3 ± 0.3
	Skeletonema	-	-	-	-	0.2 ± 0.0
	Synedra	0.4 ± 0.8	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 2.1
	Tabellaria	23.3 ± 6.6	3.6 ± 4.3	8.6 ± 2.0	0.6 ± 0.7	4.3 ± 2.8
	Total	**27.2 ± 10.9**	**15.7 ± 7.2**	**25.0 ± 11.6**	**30.2 ± 3.3**	**36.3 ± 33.4**
Cyanophyta (Cyanobacteria)	*Anabaenopsis*	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.1	-	0.2 ± 0.1
	Aphanothece	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
	Coelosphaerium	7.3 ± 16.9	-	2.1 ± 2.1	-	0.4 ± 0.0
	Eucapsis	0.3 ± 0.0	-	0.3 ± 0.3	0.1 ± 0.0	0.3 ± 1.0
	Gloeocapsa	1.2 ± 1.6	0.0 ± 0.0	-	2.1 ± 10.6	0.2 ± 0.3
	Gloeothece	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 1.5	0.2 ± 0.1	0.1 ± 0.2
	Gloeotrichia	0.1 ± 0.0	-	0.1 ± 0.0	-	0.5 ± 1.1
	Lyngbya	0.2 ± 0.2	0.1 ± 0.0	0.1 ± 0.0	7.5 ± 1.6	0.1 ± 0.1
	Merismopedia	1.5 ± 4.6	-	0.1 ± 0.0	-	0.1 ± 0.0
	Microcystis	2.2 ± 0.9	1.5 ± 0.7	0.0 ± 0.0	-	-
	Oscillatoria	0.2 ± 0.0	-	0.8 ± 0.0	2.3 ± 0.0	-
	Rhabdoderma	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	Spirulina	-	-	0.0 ± 0.0	-	0.0 ± 0.0
	Total	**13.2 ± 24.3**	**1.9 ± 0.9**	**4.5 ± 4.0**	**12.3 ± 12.3**	**1.9 ± 2.8**
Chlorophyta	*Ankistrodesmus*	-	-	-	0.0 ± 0.1	0.0 ± 0.0
	Ankyra	-	-	-	-	0.2 ± 0.0
	Apiocystis	0.1 ± 0.6	0.01 ± 0.0	0.1 ± 0.0	-	0.3 ± 0.7
	Bulbochaete	11.4 ± 23.3	29.5 ± 48.5	7.5 ± 11.2	4.8 ± 11.7	16.0 ± 15.1
	Chaetophora	-	3.1 ± 0.1	3.7 ± 8.7	-	2.8 ± 0.0
	Chlorella	0.6 ± 0.2	1.1 ± 0.5	0.2 ± 0.1	0.6 ± 0.3	0.3 ± 0.1

	Chlorococcum	1.4 ± 0.1	0.8 ± 0.0	0.1 ± 0.0	0.4 ± 0.1	0.1 ± 0.0
	Coccobotrys	-	-	0.1 ± 0.0	-	0.2 ± 0.0
	Coelastrum	-	-	-	-	0.1 ± 0.0
	Crucugenia	-	-	-	0.1 ± 0.1	-
	Oedogonium	11.7 ± 18.9	0.6 ± 0.6	-	-	10.2 ± 19.0
	Pediastrum	-	-	0.1 ± 0.0	0.5 ± 0.0	0.2 ± 1.1
	Scenedesmus	0.0 ± 0.0	-	0.0 ± 0.0	0.1 ± 0.2	0.0 ± 0.0
	Selenastrum	-	-	-	13.2 ± 6.3	0.0 ± 0.0
	Total	**25.1 ± 43.1**	**35.0 ± 49.7**	**11.7 ± 20.0**	**20.8 ± 18.9**	**30.3 ± 36.0**
Charophyta	*Bambusina*	-	-	-	-	0.3 ± 0.0
	Closterium	-	-	-	-	1.9 ± 0.0
	Coelochaete	-	13.6 ± 0.0	0.3 ± 0.0	-	-
	Cosmarium	0.1 ± 0.1	1.4 ± 1.6	0.6 ± 0.6	1.9 ± 0.0	1.0 ± 2.1
	Cylindocystis	0.0 ± 0.0	0.0 ± 0.0	-	-	0.2 ± 0.0
	Euastrum	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	-	0.5 ± 0.4
	Mougeotia	27.7 ± 15.9	25.4 ± 21.7	31.9 ± 14.4	22.5 ± 51.7	17.0 ± 14.7
	Netrium	-	-	-	-	3.8 ± 0.0
	Spirogyra	2.0 ± 0.0	-	21.7 ± 43.8	-	2.4 ± 0.0
	Spondylosium	0.2 ± 0.0	0.1 ± 0.0	-	0.0 ± 0.0	0.0 ± 0.1
	Staurastrum	2.4 ± 1.4	1.2 ± 5.0	1.3 ± 4.1	-	1.0 ± 0.6
	Staurodesmus	0.3 ± 0.6	0.1 ± 0.0	0.7 ± 0.7	-	0.5 ± 0.6
	Tetmemorus	0.1 ± 0.0	-	1.1 ± 0.0	8.8 ± 0.0	1.3 ± 5.5
	Xanthidium	-	-	0.7 ± 0.7	-	1.2 ± 3.0
	Zygnema	1.4 ± 3.6	5.2 ± 13.8	-	-	-
	Total	**34.3 ± 21.7**	**47.2 ± 42.2**	**58.4 ± 64.3**	**33.3 ± 51.7**	**31.0 ± 27.0**
Other						
Chrysophyta	*Chrysosphaerella*	-	-	0.0 ± 0.0	0.1 ± 0.0	-
Cryptophyta	*Cryptomonas*	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	3.3 ± 13.8	0.2 ± 0.8
Ochrophyta	*Dinobryon*	-	-	-	-	0.1 ± 0.0
	Goniochloris	-	0.1 ± 0.0	0.2 ± 0.0	-	-
Euglenozoa	*Menoidium*	-	0.1 ± 0.0	0.0 ± 0.0	-	0.2 ± 0.0
	Total	**0.0 ± 0.0**	**0.2 ± 0.0**	**0.3 ± 0.1**	**3.3 ± 13.8**	**0.5 ± 0.8**

Periphyton enrichment functions (L/kg dry mass (dm)) of Se were calculated using the formula: periphyton total Se ([TSe])/aqueous [TSe]. For statistical analyses, algae genera were grouped by phylum into five major groups: diatoms (Bacillariophyta or ‘bacillariophytes’), blue-green algae (Cyanophyta/Cyanobacteria or ‘cyanophytes’), green algae (Chlorophyta or ‘chlorophytes’), evolutionarily distinct green algae (Charophyta or ‘charophytes’), and those that did not fit in any of the above groups (‘other’). The ‘other’ group was omitted from statistical

analyses due to the small fraction of relative abundance (generally <1%) this group made up for each lake and high zero counts.

Periphyton tissue weights (µg dry mass (dm)) weighed on an analytical balance were used for calculating percent change in biomass per unit area (% increase (growth) in biomass per cm^2) (Table 2.7). To calculate the percent change in biomass per unit area (% increase (growth) in biomass per cm^2), periphyton tissue mass (µg dm) were taken from total Se analysis sample mass for each plate for Day 0 and Day 8. Day 0 mass was obtained at initiation of the experiment, by scraping a known area on the periphyton plate according to the level of growth available (78 cm^2 – 156 cm^2). Day 8 mass were obtained by scraping the remaining biomass at the end of the experiment from the whole plate area and adding the Day 0 scrape mass to account for the initial loss of biomass. Day 0 mass was then extrapolated to an initial biomass value for the whole plate area (800 cm^2) and then subtracted from Day 8 total tissue mass to obtain the increase value. The increase value was then divided by the extrapolated Day 0 mass and multiplied by 100 to obtain the percent increase (growth change) in biomass per cm^2. Samples that had negative values were removed (n=15 across all lakes) as these were due to sampling error during collection (i.e., periphyton tissue lost in the process of sampling). A sample calculation can be found in the supplementary information. Due to the logistics of performing five experiments, periphyton had variable lengths of colonization time, so total periphyton biomass among the different lakes was not compared. Biomass increase within each lake, however, was included in analyses.

Table 2.7: Periphyton percent biomass growth for all lakes over the duration of experiments displayed as mean ± SD of n = 9-16 samples. Biomass was calculated as percent increase (growth change) in biomass per unit area, or % increase in biomass per cm^2.

Lake	% Increase per cm^2
L114	308 ± 130
L224	119 ± 71
L239	53 ± 37
L227	54 ± 50
L470	29 ± 23

2.3.6 Statistical analyses

All statistical analyses were performed in RStudio integrated development environment (RStudio Team, 2020) using base package software and added software packages lme4 (Bates et al., 2015), tidyverse (Wickham et al., 2019), vegan (Oksanen et al., 2019), corrplot (Wei and Simko, 2017), and drc (Ritz et al., 2015), and in GraphPad Prism 8.4.3. Alpha was set at 0.05 for all statistical tests, and data are displayed as mean ± SD unless otherwise stated. Selenium concentrations were not normally distributed, so non-linear analyses were used. Measured aqueous Se concentrations were not normally distributed and therefore were compared to nominal concentrations and among each other to test for differences using a Kruskal-Wallis test with a Dunn's post-hoc multiple comparisons test.

To determine the best model for predicting Se accumulation across lakes as a function of aqueous Se, generalized linear mixed models (GLMMs) were used. Data were analyzed using a gamma distribution and inverse link function, with aqueous Se set as the fixed variable and lake as a random factor varied by intercept or intercept and slope (Table S7). Study lake was not of primary interest in the present study and therefore was included as a random factor. The following models were compared:1) intercepts vary among lakes, 2) slopes and intercepts vary among lakes, 3) slopes nor intercepts vary among lakes (no random effect), and 4) null model. Akaike Information Criterion (AIC) method was used to select the model that best predicted periphyton Se accumulation (i.e., the model with the lowest AIC value).

Curve-fitting techniques were used to characterize the relationship between Se uptake in periphyton and aqueous selenium concentrations for each lake (Graves et al., 2021). Rectangular hyperbolas (Michaelis-Menten type curves indicating saturation was reached), linear regressions (indicating constant rates of uptake), and power curves (indicating non-linear saturable relationships) were fit to periphyton Se data for each lake. The model that best explained periphyton Se uptake was selected using the AIC method for each lake (Table S8). When rectangular hyperbolas had the lowest AIC value, models were examined to determine if full saturation of Se was reached by periphyton. This was done through obtaining predicted maximum saturation values (V_{max}) from the model and comparing actual uptake maximums. Models were no longer considered appropriate if full saturation was not reached (i.e., actual

uptake maximums were lower than predicted saturation maximums), and the model with the next best (lowest) AIC value was selected.

Principal component analysis (PCA) was used to determine which environmental variables or algal genera may have influenced differences in periphyton Se uptake among lakes. Correlation matrices with a Kendall rank correlation coefficient were used to determine if any significant correlations between water chemistry and periphyton community variables existed. Data for PCA were log-transformed to account for skew in variability among datasets. Relative abundances (%) of major algal groups in periphyton were normally distributed and compared among lakes using a one-way analysis of variance (ANOVA) and Tukey post-hoc test for multiple comparisons.

Generalized linear models (GLMs) were used to determine which variables explained the most variance regarding differential Se uptake by periphyton among the lakes and were assessed with a gamma distribution and inverse link. The AIC method was used to determine the best model with comparison to null models (Table S9). Representative water chemistry parameters were included in model selection along with periphyton biomass change and periphyton taxa groups (bacillariophytes, cyanophytes, chlorophytes, and charophytes). The pseudo R^2 values (pR^2) were used to determine how much variance was explained by the model, using the formula: pR^2 = (null deviance – residual deviance)/null deviance (Graves et al., 2017).

2.4 Results and Discussion

2.4.1 Characterizing Se uptake by periphyton

Differences in Se accumulation by periphyton among the different lakes were observed (Figure 2.1). While similar patterns existed in Se uptake into periphyton among all five lakes, total Se accumulation by periphyton differed (GLMM; Figure 2.1; Table 2.8). Generally, greater uptake of Se was observed in lakes with higher nutrient status in comparison to more oligotrophic lakes. Accumulation of Se by periphyton increased in a concentration-dependent manner, meaning that as aqueous Se concentration increased, periphyton Se concentration increased correspondingly, which is a common trend found in the literature (Gojkovic et al., 2015; Kousha et al., 2017; Graves et al., 2021). Additionally, the enrichment functions for all

study lakes and treatments generally followed a trend of greater enrichment at lower Se concentrations (Figure 2.2), which is typical of Se enrichment in the literature (Ponton et al., 2020). The range of enrichment functions among lakes were 11 867 – 22 653 L/kg dm in the 0.5 µg Se/L treatment, 7983 – 15 725 L/kg dm in the 1 µg Se/L treatment, 4885 – 12 447 L/kg dm in the 2 µg Se/L treatment, and 2870 – 12 536 L/kg dm in the 4 µg Se/L treatment (Figure 2.2).

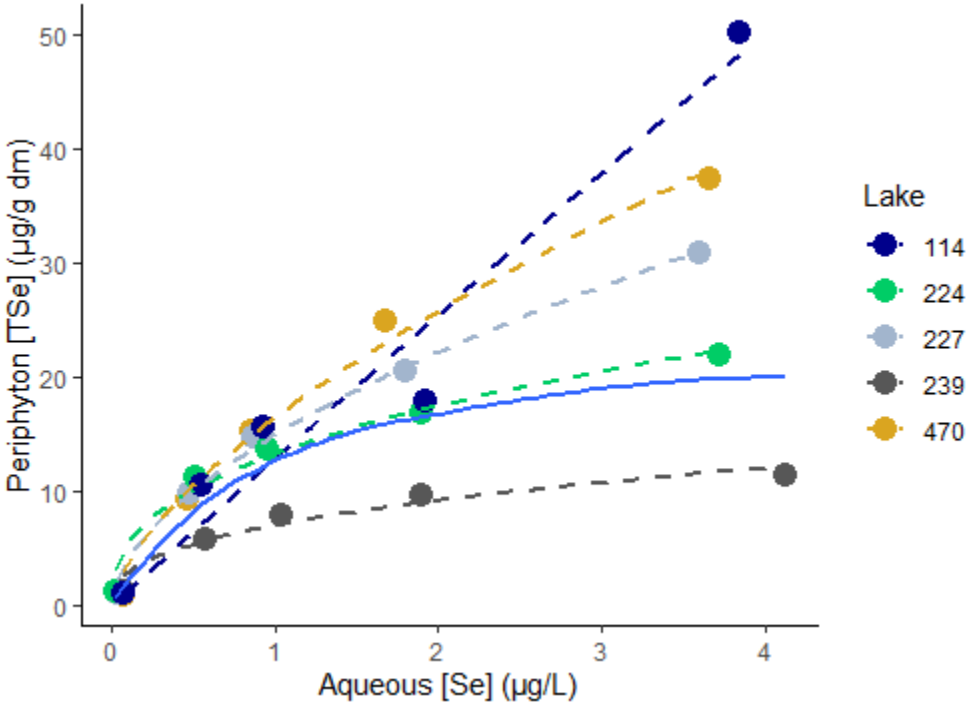

Figure 2.1: Mean periphyton total Se as a function of measured aqueous Se treatments for each of the five study lakes. Dashed lines represent the line of fit in Se uptake for each of the lakes, and the solid blue line represents line of fit for the best GLMM, Periphyton Se ~ (1/Aqueous Se) + (1|Lake).

Table 2.8: Model selection details for determining the best GLMM including the null model used for the best model. Other family distributions were examined but did not properly represent the fit of the data, and therefore only gamma distributions have been included.

Formula	Random effect	Family	Link function	Intercept	Slope AqSe	AIC	pR^2
Periphyton Se ~ 1	1|Lake	gamma	inverse	0.07 ± 0.01	-	191.3	0
Periphyton Se ~ AqSe	1|Lake	gamma	log	1.71 ± 0.22	0.53 ± 0.12	176.6	0.51
Periphyton Se ~ AqSe	1|Lake	gamma	inverse	0.13 ± 0.21	-0.02 ± 0.01	181.3	0.51
Periphyton Se ~ AqSe	1|Lake	gamma	identity	0.75 ± 0.29	11.52 ± 1.25	150.8	0.62
Periphyton Se ~ AqSe	1+AqSe|Lake	gamma	log	1.71 ± 0.22	0.52 ± 0.13	180.5	0.60
Periphyton Se ~ AqSe	1+AqSe|Lake	gamma	inverse	0.13 ± 0.03	-0.03 ± 0.01	185.3	0.51
Periphyton Se ~ AqSe	1+AqSe|Lake	gamma	identity	0.68 ± 0.41	12.05 ± 2.58	147.2	0.86
*Periphyton Se ~ (1/AqSe)	1|Lake	gamma	inverse	0.04 ± 0.02	0.04 ± 0.00	137.8	0.91
Periphyton Se ~ (1/AqSe)	1+AqSe|Lake	gamma	inverse	0.04 ± 0.01	0.04 ± 0.00	143.5	0.95

Intercepts and slopes of fixed effects are shown as ± standard error.
Abbreviations: AqSe = aqueous selenium, AIC = Akaike Information Criterion, pR^2 = pseudo R^2
*Indicates best model as per AIC method.

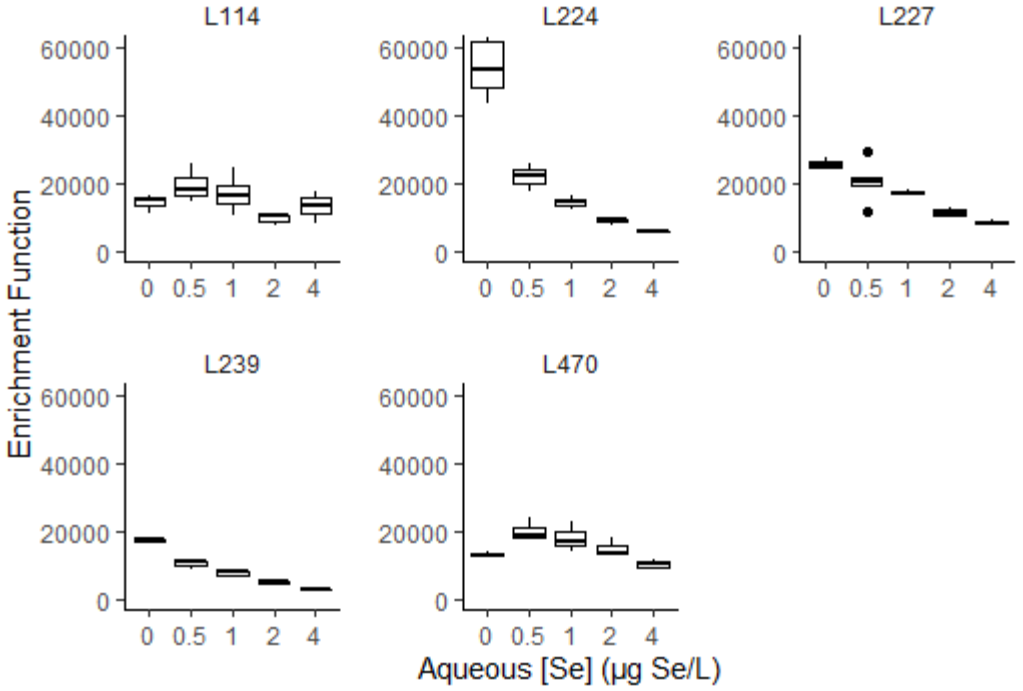

Figure 2.2: Boxplots of periphyton enrichment functions (EFs) for all lakes by aqueous Se concentrations for each of the lakes. Enrichment functions are calculated as periphyton total Se [TSe]/aqueous [TSe]. Box represents interquartile range, whiskers as minimum and maximum, horizontal bold line as median and dots as outliers.

Large variation in periphyton Se accumulation at a given aqueous Se level was observed among lakes, highlighting the site-specificity of Se accumulation. Differences in Se accumulation by periphyton varied 1.9-fold in the lowest Se treatment, to 4.4-fold in the highest Se treatment among lakes. The current freshwater Se guidelines are between 1 – 2 µg Se/L in North America, and periphyton exposed to these aqueous Se concentrations in the present study had a wide range of tissue Se concentrations, ranging from 8.0 – 24.9 µg/g dm. This is an important finding, as instances of Se toxicity at aqueous Se levels surrounding the current guidelines in higher trophic levels have been reported. In certain systems, concentrations of aqueous Se of 1.5 µg/L is enough to cause toxicity to higher organisms by dietary means through bioaccumulation of Se at the base of the food web (Janz, 2011). Aqueous Se concentrations below 0.7 µg/L have even been suggested due to the potential of Se toxicity, as this concentration can result in Se accumulation in fish gonads above recommended safe levels (Mailman, 2008). In the highest Se treatment (4 µg Se/L), periphyton Se concentrations reached

values of 30.9 – 50.2 µg/g dm in some lakes (Figure 2.1). A study examining Se uptake into periphyton and subsequent dietary exposure to the mayfly *Centroptilum triangulifer* at Se concentrations of 10 µg Se/L and 30 µg Se/L reported periphyton Se concentrations of 12.8 µg/g dm and 36 µg/g dm respectively, which corresponded to adverse effects in mayfly secondary production and in survival and time to emergence at the two respective Se concentrations (Conley et al., 2013). These periphyton Se concentrations were similar to those found in the present study even though higher Se treatment concentrations were used, further emphasizing the importance of considering site-specificities for Se risk assessment. This finding could also potentially indicate that adverse effects to higher trophic levels could occur in the systems in the present study when exposed to higher dietary Se concentrations bioaccumulated by organisms at the base of the food web from relatively low aqueous Se concentrations.

Power curves, which represent nonlinear, saturable relationships best described Se accumulation in all lakes (non-linear regression (NLR), $p<0.001$ to 0.01) (Figure 2.3; Table 2.9). There was no significant difference in the fit of linear regression versus a power curve for lake 114 specifically (Table 2.9). Selenium uptake was greatest in periphyton from lakes 114, 470, and 227, which are more nutrient-rich lakes in comparison to oligotrophic lakes 224 and 239, where periphyton accumulated significantly less Se. Power curves are typical in describing Se uptake by organisms at the base of the food web and have been reported previously (Ponton et al., 2020; Graves et al., 2021). It is not surprising that full saturation was not reached in any of the lakes over the duration of the exposure given that low levels of aqueous Se were used.

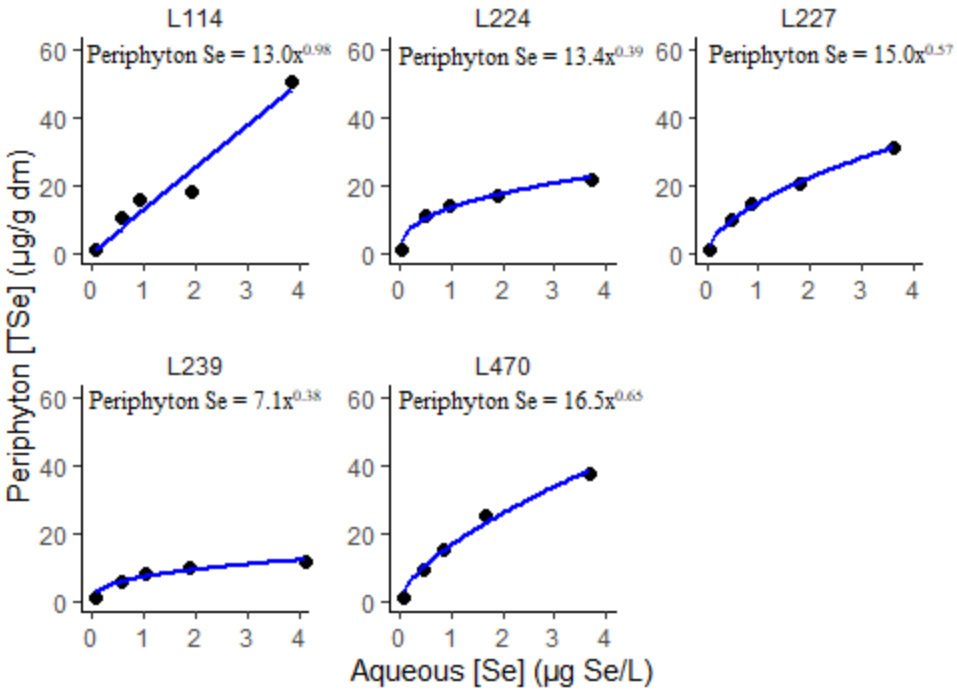

Figure 2.3: Uptake curves for Se in periphyton as a function of mean measured aqueous Se for five boreal lakes. Power curves were the best fit for all lakes.

Table 2.9: Model selection details for determining best fits of Se uptake curves for each lake. Model equations shown are for the best fitting (power curve) models. Periphyton Se was set as the Y variable and aqueous Se as the X variable for each model, except for null models where the X variable was set as 1.

Lake	AIC Power	Null Model AIC	Null Model Estimate	AIC RH*	AIC LR	Model Equation
L114	33.4	46.3	19.1	33.8	33.1	Periphyton Se = $13.0x^{0.98}$
L224	19.9	37.4	13.1	18.7	31.4	Periphyton Se = $13.4x^{0.39}$
L239	18.2	30.7	7.3	-16.1	26.0	Periphyton Se = $7.1x^{0.38}$
L227	17.9	41.3	15.5	19.4	30.1	Periphyton Se = $15.0x^{0.57}$
L470	23.0	43.5	17.6	9.2	31.5	Periphyton Se = $16.5x^{0.65}$

Abbreviations: AIC = Akaike Information Criterion, RH = rectangular hyperbola, LR = linear regression.
*Saturation was not reached in any lake at the tested Se concentrations

2.4.2 Variation in Se accumulation among lakes

Periphyton community composition varied among the five study lakes (Figure 2.4) in addition to water chemistry parameters (Table 2.1). Statistically significant differences were observed among the proportion of charophytes (lake 114 vs. 239; lake 239 vs. 470), chlorophytes (lake 224 vs. 239; lake 239 vs. 470), cyanophytes (lake 114 vs. 224; lake 114 vs. 239; lake 114 vs. 470; lake 224 vs. 227; lake 227 vs. 470; lake 239 vs. 227), and bacillariophytes (lake 114 vs. 224; lake 224 vs. 227; lake 224 vs. 470; lake 239 vs. 470; lake 114 vs. 470; lake 224 vs. 239) (one-way ANOVAs with Tukey's post-hoc tests; $0.03 < p < 0.0001$). There were no differences in relative abundances of algal taxa between Day 0 and Day 8 for all lakes or among treatment within lakes (i.e., steady communities). In all experiments, an increase in periphyton biomass was observed but to differing extents, with the greatest increase in growth in lake 114 and the least in lake 470 (Table 2.7).

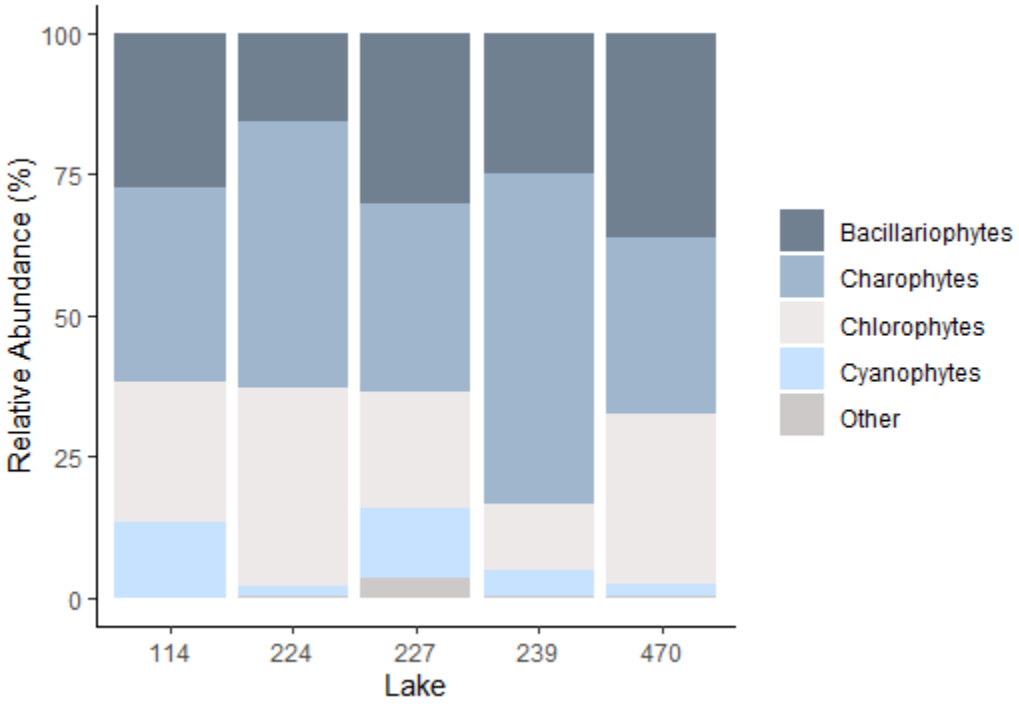

Figure 2.4: Relative abundances of dominant periphyton algal phyla in each study lake.

Principal component analysis and correlation matrices of water chemistry and periphyton community variables revealed several positive and negative correlations of various strengths

among the variables (Figure 2.5; Figure 2.6). This is not necessarily surprising, as water chemistry inherently influences periphyton growth. Nutrients such as nitrogen and phosphorus influence periphyton growth and are generally limited in boreal lake systems, often resulting in lower productivity and biomass (Stockner and Armstrong, 1971; McDowell et al., 2020). Lake trophic status can also influence periphyton community assemblages, with more eutrophic lakes favoring larger-celled filamentous algae in some systems (Cattaneo, 1987). Other factors including temperature, light, and inorganic carbon can influence periphyton growth and potentially result in community shifts in some aquatic systems (Hill, 1996; He, 2010; McDowell et al., 2020).

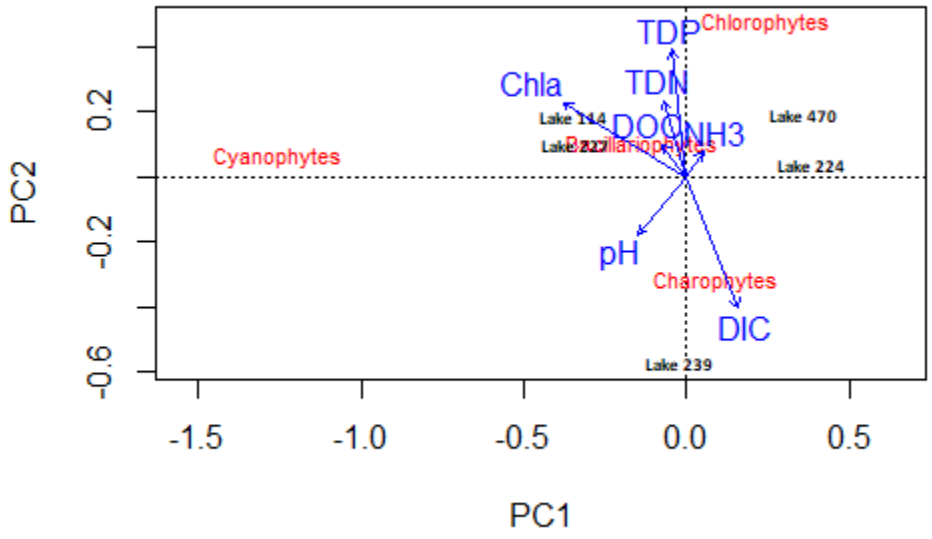

Figure 2.5: Principal component analysis (PCA) triplot of log-transformed water chemistry and periphyton community (% relative abundance) variables. PC1 and PC2 account for 75.8% and 15.6% of variance, respectively. Significant water chemistry variables included dissolved inorganic carbon (DIC), chlorophyll a (Chl *a*) and total dissolved phosphorus (TDP) (p=0.001). Total dissolved nitrogen (TDN) and pH approached significance (p=0.06).

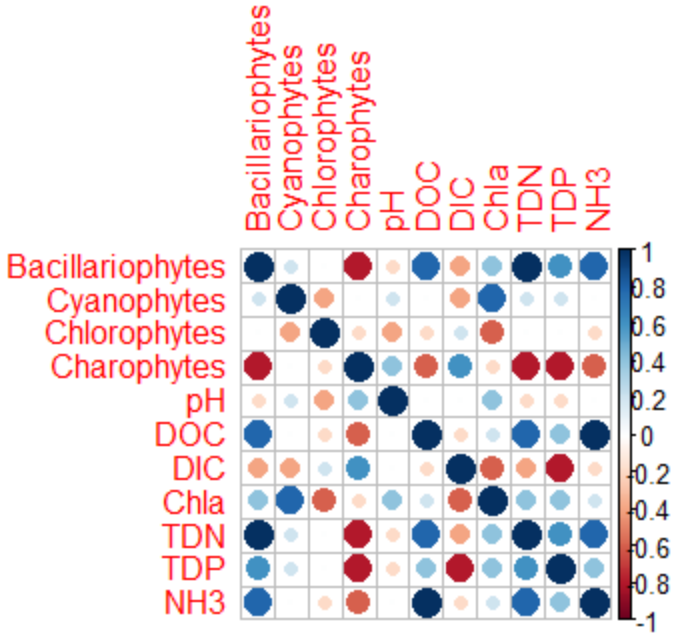

Figure 2.6: Correlation matrix of water chemistry and periphyton major phyla. Blue dots represent positive correlations and red dots represent negative correlations. The strength of the correlation is indicated by the size and intensity of the colour shown according to the scale on the right. Large dark circles indicate strong correlations and correspond to larger numbers, small light circles indicate weak correlations and correspond to smaller numbers, and blank spaces indicate no correlation.

Aqueous Se concentration alone explained 82% ($pR^2 = 0.82$) of the variance in Se bioconcentration by periphyton (GLM; Table A.1). The addition charophyte abundance explained an additional 8% of the variance and was the most parsimonious model explaining the most variance, however, this model was not significantly different from other models (GLM; Table A.1). Generally, increasing charophyte proportions and dissolved inorganic carbon (DIC) concentrations corresponded with decreasing Se accumulation by periphyton, whereas increasing total dissolved phosphorus (TDP) and bacillariophyte (diatom) proportions corresponded to increasing Se accumulation by periphyton (Figure 2.7). Charophyte abundance was significantly positively correlated with DIC, and negatively correlated TDP and diatom abundance, limiting the interpretation of these results. Periphyton community and water chemistry variables are important to consider regarding differences in Se accumulation by periphyton, however,

combinations of these variables explained no more than an additional 10% of variance in Se accumulation in these models (GLM; Table A.1). The general trends of other select water chemistry and periphyton community parameters on Se bioconcentration by periphyton are shown in Figure 2.8.

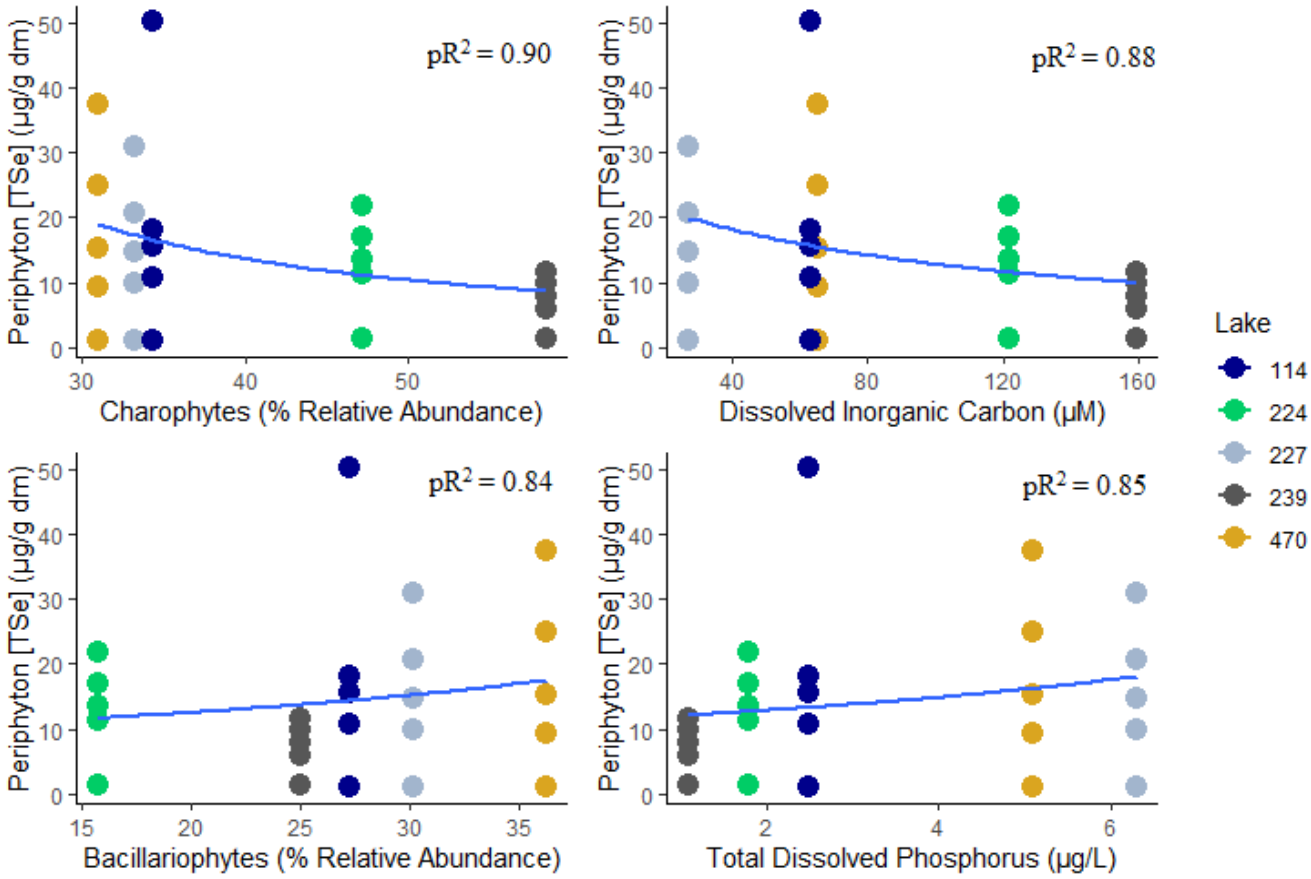

Figure 2.7: Periphyton total selenium versus water chemistry and periphyton community variables used in determining the best GLMs. The solid blue line represents the overall trendline of the relationship of the variable with periphyton Se accumulation by each lake. The pseudo R^2 (pR^2) values are shown for each variable.

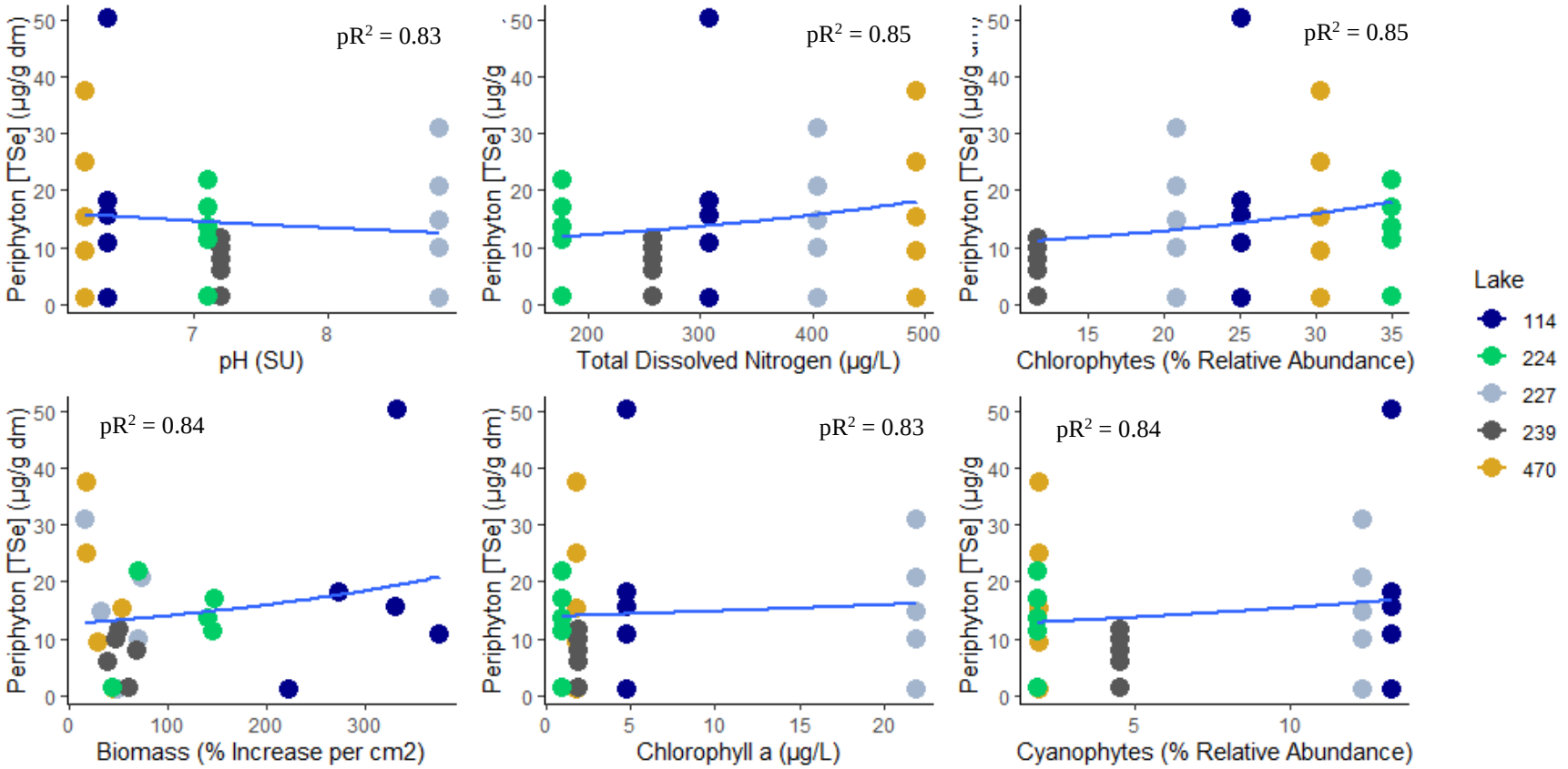

Figure 2.8: Periphyton total selenium versus water chemistry and periphyton community (% relative abundance) variables used in determining the best GLMs. The solid blue line represents the overall trendline of the relationship of the variable with periphyton Se accumulation by each lake. Water chemistry and periphyton community variables needed to be pooled by each lake due to the nature of the sampling data available. There were no differences seen among treatments in regard to periphyton community variables, and water chemistry variables used were obtained directly from the lake water used in the experiments. The pseudo R^2 (pR^2) values are shown for each variable.

Reports of the influence of algal community composition on Se bioconcentration vary among the literature. A recent study examining Se uptake in naturally grown periphyton reported differential Se uptake among major algal groups, specifically that Se uptake by cyanobacteria was greater in comparison to diatoms and chlorophytes (Markwart et al., 2019). A study reported wide variation among diatom species in Se bioconcentration, including the observation that some species accumulated significantly more Se than physiologically required (Baines and Fisher, 2001). A review examining Se concentration in different chlorophyte species found that algal species alone can influence bioconcentration of Se, as green algae species accumulate variable extents of Se (Gojkovic et al., 2015). It is therefore possible that certain species differences among periphyton groups may explain the differential Se accumulation observed. Charophytes are the ancestors to modern land plants and the only group of macroalgae known to possess rhizoids capable of limited nutrient uptake (Burkholder, 1996; Domozych et al., 2016), which may influence their ability to regulate Se uptake, potentially explaining the trend of decreasing Se uptake by periphyton with increasing charophyte abundance. Alternatively, bacillariophytes may possess the ability to accumulate great amounts of Se, potentially explaining the trend of increasing Se uptake by periphyton with increasing diatom abundance. Further, while species differences among major algal groups may exist regarding basic Se requirements and therefore accumulation potential, more research is needed regarding algal Se uptake and requirements (Baines and Fisher, 2001). Additional research is also recommended regarding Se accumulation within and among specific algal groups, in addition to other organisms comprising periphyton. A limitation of this study was only being able to identify the algal component of periphyton, as bacteria and fungi are also important components of periphyton and play roles in Se uptake and incorporation into aquatic food webs (Baines et al., 2004; Conley et al., 2013; Luo et al., 2019).

In addition to the influences water chemistry variables have on periphyton growth and community composition, water chemistry variables are known to influence Se uptake. A study examining selenite uptake in plankton communities suggested that Se uptake is likely a regulated process due to the strong relationship found between Se accumulation and inorganic carbon uptake, as both processes are fundamental to cell function (Baines et al., 2004). A study examining Se uptake in the freshwater green algae *Chlamydomonas reinhardtii* reported that selenate uptake was inhibited in the presence of high sulfate concentrations, and selenite uptake inhibited in the presence of high phosphate concentrations (Vriens et al., 2016). A study

performed in plants conversely found that increasing selenite concentrations in *Astragalus bisulcatus* increased plant yield and selenite concentrations, while simultaneously decreasing plant phosphate concentrations (Broyer et al., 1972). While certain ions including sulfate and phosphate are known to inhibit Se uptake, competition among these ions in the present study was unlikely due to boreal lakes generally having low levels of these ions (Gupta and Gupta, 2017; Ponton et al., 2020). Sulfate, however, was not quantified in this experiment. Higher nutrient levels corresponding to increased Se uptake by periphyton could be attributed to increased growth, however, while periphyton from lakes with higher nutrient status did accumulate more Se, there was no relationship between higher nutrients and biomass increase in the present study (Table 2.7). A limitation of this study is the inability to separate the effects of community composition and water chemistry variables on Se uptake by periphyton. Future research should examine individual water chemistry variables to examine the impacts of these specifically on Se accumulation, as well as incorporating a mix of Se treatments of selenate and selenite. Selenite was generally the dominant form of Se in the study lakes used, however, 100% selenite additions can represent a "worst case scenario" in uptake due to preferential uptake for some organisms at the base of the food web.

2.4.3 Conclusions

The present study highlights the variability in Se bioconcentration by periphyton among different boreal lakes, emphasizing the importance of site-specific differences and the importance of incorporation of Se into food webs by periphyton. Due to the correlations observed in the present study it cannot be concluded which specific water chemistry and/or community variables alone were potentially driving differential Se concentration by periphyton, however, charophyte abundance, dissolved inorganic carbon, diatom abundance and total dissolved phosphorus are likely important factors in combination in determining the variation seen in the present study. These variables could be used as representative predictive factors when assessing the risk of Se in different aquatic systems as important site-specific variables to consider. The present study also highlights the need for future Se accumulation research focusing on various organisms comprising periphyton community assemblages, which may provide further insight relevant to ecological risk assessments of Se in boreal lake ecosystems.

2.5 Acknowledgements

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CHAPTER 3

RESEARCH INTEGRATION INTO EXISTING PERIPHYTON SELENIUM BIOCONCENTRATION LITERATURE AND FUTURE RESEARCH DIRECTIONS

Preface

The overall goals of this chapter are to integrate experimental results from Chapter 2 with the existing literature, outline ideas for improving experimental design, discuss limitations and advantages of field-based research, and to highlight future research directions to further reduce the knowledge gaps associated with Se uptake by organisms at the base of cold freshwater food webs. Additionally, Chapter 3 outlines a proposed experiment examining the interactions of phosphorus and selenium which was first attempted in summer 2019 but was unable to be completed due to various unforeseen circumstances outlined in section 3.2.3. A lab-modified version of this experiment was then planned to be executed in the lab in March 2020 but was unable to be performed due to the COVID-19 pandemic and subsequent restrictions. The author contributions are as follows:

Mikayla D. Oldach wrote the chapter and conceptualized the proposed experiment.

David M. Janz provided scientific input and conceptualized the proposed experiment.

3.1 Relevance of present study among the current literature

The motivation for performing the present research was to diminish the knowledge gaps surrounding the incorporation of Se into the base of coldwater aquatic food webs by primary producers. Currently, there remains much uncertainty regarding the variability in Se uptake in different aquatic systems and the potential factors that may lead to more or less than expected Se uptake and movement through the food web. To my knowledge, there has not been any other studies examining Se uptake at a range of very low environmentally relevant concentrations, while simultaneously comparing naturally grown periphyton assemblages from multiple distinct boreal lakes. The present research found significant differences in Se uptake among periphyton from different boreal lakes with variable water chemistry and community structures in their response to Se at low environmentally relevant levels. Significant concentration-dependent uptake of Se was demonstrated both within and among the five lakes tested. Emerging trends include further investigation into the Charophyta and Bacillariophyta algal phyla, and dissolved inorganic carbon and total dissolved phosphorus water chemistry parameters as potential drivers of the differential Se uptake among the periphyton communities from the five lakes studied. While the generalized linear modelling results were not statistically significantly different, these results are important findings biologically, as they demonstrate clear trends in their influence on Se uptake and could be representative variables to consider in the future when considering Se risk assessment.

Different algal species, thus periphyton community composition may have a significant impact on Se uptake in different systems, as well as the ability to influence whole lentic systems. Because periphyton play a key role in energy cycling in boreal lake systems and are significant energy and carbon sources for a wide range of secondary consumers (Stockner and Armstrong, 1971; Cattaneo, 1987; Hecky and Hesslein, 1995), community shifts due to Se exposure could have significant implications for lentic systems with Se toxicity. Abdel-Hamid and Skulberg (1995) found that increasing Se concentrations and different Se species had significantly different effects among different green and blue-green algal species. In the green algae, increasing Se concentrations resulted in detrimental effects in *Selenastrum capricornutum*, marginal growth effects in *Scenedesmus obliquus* at low concentrations and inhibited growth at higher concentrations, marginal growth in the green algae *Chlorella sp*., inhibition of growth of

Monoraphidium contortum at low concentrations and stimulation at higher Se concentrations, and the opposite occurring for *Monoraphidium griffithii*. In addition to this diverse response to Se among the green algal taxa, the blue-green algae demonstrated significantly enhanced growth of *Anabaena flos-aquae,* significantly increased the biomass of *Microcystis aeruginosa,* but inhibited *Oscillatoria agardhii* when exposed to higher Se concentrations. These are relatively common algae species in lentic systems, and similar genera were found in the present study. If a system were to be exposed to excess Se, there is a strong possibility that certain algal species would be favoured in their growth due to differences among taxa, and a system could become dominated by less ideal taxa like blue-green algae. This shift in community could be problematic as cyanophytes are known to produce harmful cyanotoxins that can detriment aquatic organisms (i.e., potential consumers) and organisms near the impacted system, as well as causing surface blooms from excess growth that can detriment the lentic system itself (Mohr et al., 2011; Sheath and Wehr, 2015). Differential uptake among different periphyton assemblages is another important factor to consider when predicting Se risk in different systems. In natural periphyton communities from a lotic system primarily composed of diatoms, Se exposure at concentrations of 2.4 – 13.9 µg/L resulted in periphyton Se concentrations of 2.2 – 25.5 µg/g dm (Conley et al., 2009), which is similar to the periphyton Se found in the present study. However, the maximum concentration used in the present study was 4 µg Se/L in comparison to 13.9 µg Se/L, which is significantly lower, yet periphyton concentrations at the highest treatments were comparable in many lakes (lakes 224 (21.9 µg/g dm), 227 (30.9 µg/g dm), and 470 (37.4 µg/g dm)), significantly lower in lake 239 (11.5 µg/g dm), and significantly higher in lake 114 (50.2 µg/g dm). The differences in Se accumulation are potentially due to different algal taxa found between lotic and lentic systems, or other exposure differences involving laboratory versus field experiments. Another study using concentrations of selenite and selenate at low (10 µg/L) and high (30 µg/L) concentrations in lotic periphyton exposed for 196 hours (~ eight days) had resulting periphyton Se concentrations of 12.8 µg/g dm (low) and 36 µg/g dm (high) (Conley et al., 2013), which also contrasts significantly from the present study. These results highlight the importance of site-specific differences in Se bioconcentration among different algal taxa and stress the importance of considering periphyton community differences and site-specificities when determining potential ecotoxicological risks of Se in different ecosystems. Another important factor to consider is that water chemistry parameters also inherently influence the

periphyton within those systems. Nutrients, like phosphorus which is often limited in boreal lakes, and carbon can influence periphyton growth and productivity in lentic systems and can potentially influence community shifts (Stockner and Armstrong, 1971; Hill, 1996; He, 2010; McDowell et al; 2020). The trends seen in the experiment regarding Se uptake by periphyton among lakes with higher nutrient status than more oligotrophic lakes could be potentially explained by the presence of increased nutrient levels leading to an increase in growth, corresponding to an increase in Se uptake, even though the trends in biomass were not quite representative of this.

Interestingly, charophytes are the ancestors to modern land plants and therefore share unique characteristics with these organisms (Domozych et al., 2016), which may influence their ability to concentrate Se. Terrestrial plants accumulate Se uptake through specific transport systems in their root cell membranes, where selenite is taken up by phosphate transport systems and selenate by sulfate transporters (Gupta and Gupta, 2017). It has been demonstrated that Se uptake in plants varies depending on external concentrations of Se, other ions such as sulfate and phosphate, and Se species present, as some plant species more efficiently incorporate selenate over all other Se species (Gupta and Gupta, 2017). Charophytes are the only group of macroalgae that are known to possess rhizoids that are capable of nutrient uptake (Burkholder, 1996). It is possible therefore that periphyton from the present study with greater abundances of charophyte species accumulated less Se by potentially preferring for selenate over selenite for uptake or possessing greater ability to exert more control of Se uptake through active saturable transporters rather than through passive extracellular adsorption. Further, species differences among major periphyton groups may exist regarding basic Se requirements and therefore accumulation potential, but more research is needed regarding algal Se uptake and requirements (Baines and Fisher, 2001). Additional research is also recommended regarding Se accumulation in and among specific algal groups, in addition to other organisms comprising periphyton.

Additionally, the way in which Se is removed from the water column and incorporated into algal species also varies among different taxa. There is evidence in the literature that the majority of Se uptake in algae appears to occur actively (Fisher and Wente, 1993; Baines and Fisher, 2001; Baines et al., 2004; Morlon et al., 2006; Araie and Shiraiwa, 2009; Vriens et al., 2016), passively (Riedel et al., 1991; Mane et al., 2011; Markwart et al., 2019), or a more equal

combination of both pathways (Riedel et al., 1996; Gojkovic et al., 2015). There are clear differences among different algal taxa in regard to uptake mechanisms, which can have implications when considering the risk of Se toxicity in different systems. If Se is taken up actively, it will be biotransformed into organo-selenium compounds, which can be toxic to higher trophic levels when passed through the food web (Bottino et al., 1984; Stewart et al., 2010). If Se is adsorbed to external surfaces of algae, it can still be passed through the food web via dietary means, but as an inorganic form (i.e., not biotransformed), and therefore potentially less toxic to sensitive species like oviparous vertebrates. In the present study, it is unclear what mechanisms contributed to the uptake of selenite into periphyton. It can be speculated that uptake is active instead of passive because biomass was not correlated to Se uptake. Due to trends seen in Conley et al. (2011) with growth dilution resulting in less overall Se and in Sun et al. (2014) with proportionally increasing biomass with increasing Se, it is somewhat surprising that no trends were seen regarding biomass in the present study. This lack of trend could potentially be explained by active mechanisms controlling Se uptake, rather than a correlation with biomass that may correlate with adsorption, that is also associated with variable taxa present in different periphyton assemblages.

The present study demonstrated the rapid integration of Se into the algal component of periphyton at environmentally relevant levels which has significant relevance among the current literature and contributes to the overall knowledge of Se assimilation in cold freshwater food webs. In general, the knowledge gaps the present study contributed to are increasing the body of knowledge regarding Se exposure at low-environmentally relevant levels at a range reflecting the current guidelines in North America. Additionally, the present study contributed knowledge regarding Se risk assessment in more vulnerable cold freshwater systems, specifically boreal lake systems. The present study also examined the impacts of Se on naturally derived complex periphyton community assemblages that were completely unaltered, reflecting environmentally relevant authentic responses to the addition of a range of Se from these organisms. The responses of the periphyton from different lakes to the same Se additions were markedly different, revealing that more research regarding Se risk assessment at the base of boreal food webs should be performed. An additional research area to investigate includes the potential role of charophytes, bacillariophytes, dissolved inorganic carbon and total dissolved phosphorus as representative factors that can potentially predict Se incorporation in periphyton community

assemblages. The results of this research also demonstrated the importance of considering organisms at the base of the food web when determining the risks of Se in different ecosystems. Future Se risk assessment in lentic systems should include sampling of periphyton, phytoplankton, sediment, and water for total Se concentrations to better characterize potential risks to higher trophic levels on a site-specific basis.

3.1.1 Advantages of field-based research in the present study

Field-based research provides accumulation of knowledge very relevant to a wide variety of real ecosystem dynamics which can be extremely beneficial in certain contamination scenarios. An advantage of the present study was having the IISD-ELA as the study location. The IISD-ELA is a unique 'natural laboratory', located in a remote region in northern Ontario in the Kenora district. IISD-ELA was established in 1968 and consists of 58 experimental lakes removed from human activity and industrial processes (Blanchfield et al., 2009). IISD-ELA is also unique in the sense that it also includes a fully equipped on-site water quality laboratory, a team of experts, as well as several visiting researchers across Canada performing various projects. Over 50 large-scale ecosystem experiments have been conducted at IISD-ELA which have produced ground-breaking research results that in turn have significantly influenced regulatory decisions throughout Canada and worldwide (Blanchfield et al., 2009).

A long-term ecological research (LTER) program has existed at IISD-ELA since 1968, in which five lakes have been continuously monitored and not manipulated in any way from other experiments, and therefore have a very large corresponding data set to monitor subtle changes due to changes in the environment. Lakes 114, 224, and 239 in the present study are LTER lakes. Lake 227 used in the present study was the first lake to be used in a whole-ecosystem experiment at IISD-ELA to study nutrient cycling and food web responses to nutrient levels (Blanchfield et al., 2009), whose phosphorus additions are still maintained regularly today. Additional ecosystem level studies performed at IISD-ELA that have made crucial findings and enhanced full scale knowledge in aquatic ecosystems have included the investigation of synthetic estrogens, acid rain, algal blooms, nanosilver, mercury and diluted bitumen.

Another advantage of field research in the present study included using naturally grown periphyton communities and unmodified lake water directly from the ecosystems being studied. Using natural periphyton assemblages from boreal lakes provided extremely environmentally

relevant results, especially combined with using very low relevant levels of Se. Periphyton communities are very complex and diverse communities that play a key role in nutrient incorporation into aquatic food webs. Synthetically grown cultures have the potential to miss out on key organisms in periphyton and could potentially bias results when examining Se uptake into these communities, including less dominant algal species present, and smaller organisms like bacteria and fungi. Using natural lake water in addition to natural periphyton further increases the environmental relevance of the results in the present study. Aquatic microorganisms are sensitive to the composition of water and using other modified or artificial water sources may result unforeseen changes in the test organisms used unrelated to the interest of the study. Therefore by using natural lake water, the impacts of Se alone are highlighted, and unnecessary stress to the natural periphyton used is less likely.

The exposure to natural ambient outdoor conditions when designing field research studies comes with various advantages and obvious disadvantages. An advantage is that the conditions experienced are relevant to what periphyton would be experiencing in a real-life scenario. Specifically in this experiment, exposure to natural sunlight instead of synthetic light in the lab provides an extremely realistic response of periphyton to these natural conditions. Blanken et al. (2013) recommends that algae be grown in natural sunlight instead of artificial light on a large-scale, due to the increased cost of using artificial light and energy losses into algal biomass during energy fixation.

3.1.2 Limitations of present study

While using natural periphyton community assemblages provided realistic information on how these communities assimilate selenium, a limitation of this study was only being able to characterize the algal component of the natural periphyton community used. Bacteria and fungi can also play a key role regarding Se uptake and assimilation (Staicu et al., 2017; Luo et al., 2019), but it was not possible to characterize the present bacterial or fungal communities in the present study. Metagenomic analysis may be helpful in future studies for characterizing these organisms, along with confirming light microscopy taxa identification results.

Field work is important for obtaining realistic environmentally relevant information to better understand natural systems, however, challenges come with field-based research. In the case of the present study, it was not logistically possible to run all five exposures at the same

time so there were temporal differences among the times of experiments performed. While the time frames were still relatively similar, there were likely some differences in ambient conditions for the five exposures. Photosynthetically active radiation (PAR) was also likely to have fluctuated over the duration of the five exposures but was unable to be quantified for each container for each exposure over the entire eight day duration in the present study. An obvious disadvantage of exposure to ambient conditions is that they are not constant (light, temperature, etc.) and is harder to regulate, if not impossible. Temperature and light fluctuations may have potentially contributed to some of the variation seen, as temperature and light can influence periphyton growth, which could have possibly influenced Se uptake. There was no correlation demonstrated in the present study between Se uptake variability and biomass increase, however.

While selenite is generally dominant in lentic systems and was confirmed to be more dominant in most lakes used in the present experiment confirmed in 2019 via ion chromatography inductively coupled plasma mass spectrometry (IC-ICP-MS) (Graves et al., 2021), using 100% selenite in experiments generally represents a 'worst-case' scenario in Se contamination in a natural system. Additionally, potential presence of phytoplankton from lake water collection used in the experiment that would not be filtered out by 53 µm plankton net could have potentially contributed to competition for uptake of Se with the periphyton communities. It is known that phytoplankton can regulate periphyton productivity through competition for uptake of limiting nutrients in the water column, as well as by influencing light availability (Schindler and Scheuerell, 2002). It has also been reported that phytoplankton can accumulate more Se than periphyton in some instances in boreal lake systems (Graves et al., 2021). Phytoplankton, however, was not quantified in the present study.

3.1.3 Recommendations for improving experimental design

The experiment presented in Chapter 2 provided challenges to overcome through being a fully outdoor-conducted study using multiple remote study lakes. The process of transporting 110L of natural lake water for water changes every second day and initial retrieval of periphyton plates to set up exposures was very labour intensive, as some of the lakes used in the study were only accessible by boat and trail hiking, and ATV access not possible for access to some lakes. To overcome these challenges if this study were to be repeated, I would recommend increasing the persons available for field help if choosing a remote lake due to unique characteristics to help

with physical work, find lakes that are less hard to access but still removed from most human activities, design a similar experiment but modified to a lab, or perform the experiment at the lake of interest using a microcosm design in the littoral zone.

The Se speciation results using IC-ICP-MS (Graves et al., 2021) from the experiment presented in Chapter 2 demonstrated that Se in natural lake water from the boreal study lakes used was dominantly in the form of selenite, but also existed as selenate, although no organic forms of Se were detected. If this experiment were to be repeated, adding a mix of selenite and selenate in proportions similar to those found in the present experiment (Table 2.1) would be beneficial to examine differences in uptake among the Se species in various algal species. The use of radiolabelled (^{75}Se) selenite and selenate and subsequent fractionation methodology (Besser et al., 1994) could potentially provide more insight on the quantity and species of Se these different algal species accumulate. Additional analysis examining proportions of organic Se species like SeMet could also potentially provide more insight on uptake kinetics (passive vs. active uptake) in algae, as passively incorporated Se species would likely not be biotransformed to organic species of Se. These results may provide a more environmentally realistic scenario of Se uptake at the base of the food web by algae, as it is a more relevant mix of Se forms found naturally, in comparison to using 100% selenite which can represent a worst case Se uptake scenario.

Phytoplankton abundance and community characterization should be included if this research is to be repeated. It is known that phytoplankton can rapidly assimilate Se, sometimes to a higher degree than periphyton (Graves et al., 2021). It is possible that phytoplankton, because it was likely not removed when lake water used in the experiment was filtered through a 53 µm plankton net to remove predatory zooplankton because it is too small, could have contributed to some Se uptake and therefore less Se potentially available for periphyton. In addition to adding phytoplankton components to this study, future studies should include all components of periphyton that are feasibly possible when examining Se uptake into periphyton. It is known that bacteria and fungi can play key roles regarding Se uptake (Staicu et al., 2017; Luo et al., 2019), and these organisms could potentially explain the remaining variation seen regarding differential Se uptake among the periphyton from the various study lakes. While it was not possible to quantify bacteria and fungi in this experiment, future studies could attempt to quantify the

bacterial and fungal (heterotrophic) portion of periphyton using flow cytometry or metagenomics (Sgier et al., 2018).

Additional measurements that should be added if this experiment is repeated is the quantification of sulfate levels in the water, as sulfate is known to compete for active uptake with some Se species (Lo et al., 2015; Ponton et al., 2018). Regulating the amount of light received by the algae in the form of photosynthetically active radiation (PAR) in a lab setting may also be a good measurement/adjustment to make, as light availability influences photosynthesis and growth in benthic algae (Hill, 1996), which could in-turn potentially impact variation in Se uptake. This was unable to be regulated or measured using the current experimental design, as the experiment was performed outside, so this could be modified in the lab in the future if repeated. Repetition of this experiment with synthetically grown periphyton communities could be of great value to better study the direct effects of Se on certain known algal species without the presence of unknown species. However, natural periphyton communities are complex and diverse in nature, and a synthetically grown community would not likely have the same dynamics as a naturally grown community. Attempting to bridge the gap between field and lab-based research is a difficult balancing act between creating environmentally relevant science and controlled factored results.

3.2 Proposed phosphorus-selenium experiment

3.2.1 Objectives and hypotheses

There are conflicting reports in the current literature regarding the influence of phosphate on selenite uptake in algae. It has been reported that phosphate has no effect on selenite uptake (Morlon et al., 2006) and that the majority of this uptake is through passive adsorption (Markwart et al., 2019), and that phosphate significantly impacts selenite uptake in various algal species and that the majority of this uptake is via active transport pathways (Wang and Dei, 2001b; Vriens et al., 2016).

To further examine if phosphorus (as phosphate) affects selenium (as selenite) uptake into periphyton through an inhibitory mechanism, and to better understand if the more important

mechanism of Se uptake is biologically active (carrier-mediated) or passive (adsorption), the following objectives will be performed through the following experiments:

1) To determine if phosphate additions influence selenite uptake by natural periphyton assemblages

H_0: Phosphate additions will not influence selenite uptake by periphyton.

H_1: Phosphate additions will influence selenite uptake by periphyton. As phosphate concentrations increase, selenite uptake is predicted to decrease due to competition for anionic transporters in the cell-membranes of primary producers (i.e., an inhibitory interaction).

2) To determine if selenite uptake by periphyton is more importantly a biologically active (i.e., carrier-mediated) or passive (i.e., adsorptive) process

H_0: There will be no difference observed in selenite uptake in living versus heat-killed periphyton in the presence of various phosphate concentrations.

H_1: There will be a difference observed in selenite uptake in living versus heat-killed periphyton. I predict that heat-killed periphyton will have less selenite uptake than living periphyton due to the biologically active mechanisms of selenite uptake, and consequent inhibition by increasing phosphate concentrations. I predict that some adsorption will occur, but to a lesser extent than active uptake. I also predict that phosphate additions to the heat-killed periphyton will not influence any adsorptive processes.

3.2.2 Proposed experimental design

3.2.2.1 Site selection

Martins Lake is located in a provincial park 112 km north of Saskatoon, SK. Martins Lake is considered a meso-eutrophic lake, with total phosphorus concentrations measured at approximately 30 µg/L in May 2018 (Hudson laboratory, University of Saskatchewan). Martins Lake was chosen as the primary study site because it is an uncharacteristically deep prairie lake (Figure 3.1), water monitoring data is currently available from the Hudson lab for this lake, and other logistical reasons (i.e., close proximity to Saskatoon, etc.). While it is a provincial park,

there are various areas of the lake that are not accessible by motorized boats and therefore relatively undisturbed by the general public.

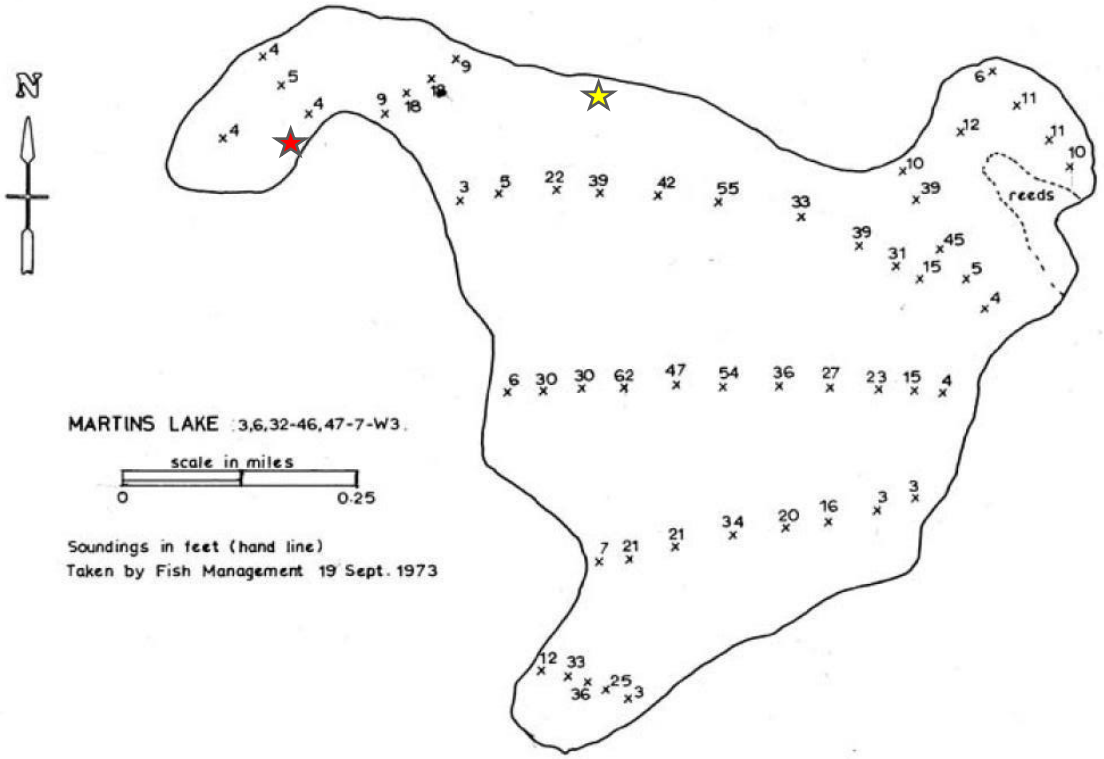

Figure 3.1: Bathymetric map of Martins Lake, SK measured in 1973. Depths are noted in feet. The red star marks where periphyton samplers were deployed in May 2019, and the yellow star marks where additional samplers were deployed in July 2019. Map was provided by Anglers Atlas (https://www.anglersatlas.com/place/112773/martins-lake).

3.2.2.2 Methods

Periphyton sampler frames were developed at the University of Saskatchewan by the Janz lab based on a modified design initially outlined in Markwart et al., (2019). The modified design consisted of polyethylene pipe to hold 10 small glass plates (5 cm x 5 cm x 5mm) that were originally buffed by Blue Markwart. All glass plates and sampler pieces were washed, bleached, and acid-rinsed before use. Six small periphyton samplers (60 periphyton plates) were deployed

in Martins Lake, SK at the end of May 2019 in the ‘springs” area, which is an area of the lake that receives fresh groundwater intake. This area of the lake is slightly colder, and less accessible to the public than the rest of the lake. An additional five large periphyton samplers (25 periphyton plates) used in the first experiment were deployed in a different area away from the springs area in Martins Lake in July 2019. This was done to attempt to ensure enough periphyton tissue was available to conduct the experiment, as growth in the springs area was slow-going. All samplers were deployed in the littoral zone of these areas at a depth of 1 m (as measured by a meter stick) and allowed to grow naturally for at least seven weeks.

A static-renewal system was planned to be set up in the Aquatic Toxicology Research Facility (ATRF) at the University of Saskatchewan using small clear plastic containers as exposure vessels, a water-table, fluorescent lights, an aeration system, and facility water to complete an eight day exposure, and is as follows. Periphyton plates will be exposed to 1 or 5 ug Se/L as selenite, and increasing phosphate concentrations (6, 18, 54 µg P/L) simulating oligotrophic, mesotrophic, or eutrophic conditions (Pavluk and Bij de Vaate, 2013). Similar phosphate exposure methods are found in Yu and Wang (2004b). The treatments (five replicates for each) for living periphyton plates are as follows: control [0.1-0.2 µg Se/L], 1 µg Se/L + 6 µg P/L, 1 µg Se/L + 18 µg P/L, 1 µg Se/L + 54 µg P/L, 5 µg Se/L + 6 µg P/L, 5 µg Se/L + 18 µg P/L, 5 µg Se/L + 54 µg P/L, for a total of 35 living replicate containers. Additionally, heat-killed periphyton (five replicates per treatment) will be exposed to 1 µg Se/L, along with treatments of 6 µg P/L (low phosphate) and 54 µg P/L (high phosphate). Including heat-killed controls (five replicates), there will be a total of 15 heat-killed replicates that will be run simultaneously with living replicates, for a total of 50 containers. Periphyton will be heat-killed using the protocol outlined in Markwart et al., (2019). Periphyton plates will be submerged in 80-85ºC water for 8-10 minutes to ensure a complete stop of all biological activity, while still maintaining periphyton structure. A small sub-sample will be examined using light microscopy to verify the efficacy of heat-killing treatment to ensure cells are not living.

The exposure period will be performed similarly to the method outlined in the experiment in Chapter 2 (i.e., experiment #1). Water changes (100%) will be performed every two days, using 50% ATRF facility water and 50% RO water stored in a clean carboy. Exposure vessels will be re-spiked with appropriate selenite and phosphate levels after each water change. Water

quality will be performed every other day using a YSI probe (HI98194, Hanna Instruments Canada Inc) to measure pH, dissolved oxygen, temperature and conductivity. Dropper tests (API® Freshwater test kits) measuring phosphate, nitrate, general hardness and carbonate hardness will also be used. These same water quality measurements will be performed on ATRF facility water that is to be used for water changes before use.

The selenite stock solution will be prepared as in experiment #1. The phosphate stock solution will be made according to the sodium phosphate protocol outlined by Cold Spring Harbor Protocols (2006). Water samples and periphyton tissue collection will be taken as per the same protocol as in the experiment #1.

3.2.2.3 Endpoint analysis

Periphyton tissue and water samples for [Se] analysis will be analyzed using ICP-MS as per the protocol outlined as in experiment #1. Aqueous P concentrations will be verified by determining total phosphorus (TP) using a spectrophotometer (photometric mode, wavelength 895 nm, 10 cm cuvette) according to the protocol outlined by the Hudson Lab at University of Saskatchewan. Periphyton community composition samples will also be analyzed through light microscopy as outlined as in experiment #1.

3.2.2.4 Statistical analysis

If the data collected is parametric and meets the appropriate assumptions, a two-way ANOVA and Tukey post-hoc tests will be used to determine the effects of phosphate concentrations and selenite concentrations on periphyton enrichment functions. Live versus heat-killed [Se] will be compared using a one-way ANOVA or independent t-tests. All alpha vales will be set at 0.05 (α=0.05). SPSS Statistics 25 (SPSS Inc, IBM) and GraphPad Prism 8.0.2 will be used to perform all statistical analyses.

3.2.2.5 Environmental relevance

Anthropogenic loading of excess Se into freshwater ecosystems is an issue of increasing concern, particularly in coldwater lakes of the boreal ecoregion, because of its extreme hazard and potential adverse effects on oviparous vertebrates. My research will help reduce the uncertainty regarding the rapid uptake of Se at the base of coldwater food webs which represents the most significant step of bioaccumulation of Se, thus influencing more sensitive higher trophic levels through dietary exposure. My research will also help identify certain water quality parameters that can influence Se uptake in coldwater systems, which can increase predictive accuracy when assessing the risk of Se loading. The proposed research goals will contribute to Se risk assessment in Canada through increasing our understanding of Se assimilation into coldwater food webs, and to better predict the effects of Se loading in systems with variable water chemistry.

3.2.2.6 Limitations of study

Periphyton communities are complex, variable, and difficult to quantify manually. Species identification via light microscopy will therefore be largely focused on algal components, while quantification of bacteria (excluding *Cyanobacteria*) fungi and detritus will be limited due to limits in identification abilities. Additionally, other nutrients (ex. nitrogen) play a pivotal role in determining whether natural lentic system classification of being oligotrophic, mesotrophic, or eutrophic. Nutrient status inherently influences periphyton growth as well, so this must be taken into account when assessing Se uptake in natural systems. This is experiment is attempting to elucidate the mechanism of phosphate interaction in regards to Se uptake. Selenite, while often the dominant form of Se in lentic systems, represents a “worst-case scenario” when added at 100% concentrations, as mentioned previously.

3.2.3 Unforeseen circumstances

This experiment was unable to be completed in summer 2019 due to various unforeseen circumstances. The periphyton plates in Martins Lake were unable to be used due to lack of

periphyton growth in both locations chosen. The springs area was chosen because it is more difficult to access and therefore has significantly less human activity, but the periphyton plates in this location likely did not grow well due to the influx of colder groundwater in this area. The periphyton plates deployed in July in another area of Martins Lake also surprisingly did not result in enough periphyton growth to conduct this experiment. This is potentially due to a higher presence of predation on the plates, as snails and zooplankton are abundant in this lake. The addition of predator mesh protecting the plates from potential predation could be helpful in future studies depending on the nature of the study lake. Predator mesh was not necessary in the boreal lakes studied in the first experiment.

Due to the slow growth observed at Martins Lake by mid-summer, two back-up lakes were chosen in Northern Saskatchewan if periphyton from Martins Lake was not usable. The first lake chosen was Cub Lake, where five large periphyton samplers (25 periphyton plates) had been deployed the summer previously by another Toxicology student and were unused for their experiment. Upon retrieval of these samplers, we discovered that a tree had fallen on these samplers and broke the glass plates as well as the sampler frames. Another five large periphyton samplers (25 periphyton plates) were also deployed in Summit Lake in August 2019, but also did not achieve enough growth to perform the experiment.

A total of 135 periphyton plates were deployed in 2019, but none were able to be used due to various unfortunate circumstances. To overcome this, a modified lab experiment incorporating potential effects of temperature changes in addition to phosphate levels in algal selenite uptake was designed and planned to be performed in April 2020 using *Chlamydomonas reinhardtii* cultures obtained from the Canadian Phycological Culture Centre (CPCC) at the University of Waterloo. This experiment was unable to go forward, however, as the coronavirus (COVID-19) pandemic onset occurred in March 2020. This caused closures and restrictions to access the University of Saskatchewan, as well as restricting the activities of the CPCC. Because of these intense restrictions and the general uncertainty of when/if this experiment could proceed, it was decided that this second experiment would not be included in this thesis.

3.3 Large-scale questions remaining in Se bioconcentration

3.3.1 Integration of results as predictive variables

The present study in Chapter 2 offers various insights into how periphyton from different boreal lake systems accumulate Se. Along with the existing literature, some predictions can be made in other systems when examining the risk of Se. It is known that lentic systems are generally more vulnerable to Se toxicity due to various characteristics including lower flushing rates and higher productivity in comparison to lotic systems (Hillwalker et al., 2006; Young et al., 2010). It is important to consider factors like residence time, oxygen and productivity levels when assessing lentic systems for Se risk assessment, as lake morphology and communities present can play a significant role in determining potential for toxicity. Organisms at the base of the food web are not the only organisms capable of accumulating and biotransforming inorganic Se to more toxic organic forms, as seen in the case of invertebrates (Stewart et al., 2010). Additionally, the species of Se in the system is an important factor when considering Se risk assessment. Selenite is more commonly found in reducing environments like lentic systems, and selenate is more commonly found in oxic lotic environments. Selenite is preferentially taken up over selenate by organisms at the base of food webs, and subsequent bioaccumulation and toxicity to higher trophic levels (e.g., fish) is seen to a greater extent in lentic systems than lotic systems (Simmons and Wallschläger, 2005; Orr et al., 2006; Stewart et al., 2010). Interestingly, laboratory experiments have demonstrated rapid reduction (< 96 hours) of selenate to selenite in static and static-renewal conditions when in the presence of the selenate-reducing bacterial family Comamonadaceae (Conley et al., 2013), so ongoing Se speciation sampling should be performed when examining an at risk lentic system. The oxidation of selenite to selenate in the presence of dissolved oxygen is unlikely due to slow oxidation kinetics (Maher et al., 2010).

In addition to assessing Se species and general aquatic system type, it is known that certain ions like phosphate and sulfate can influence Se uptake into periphyton (Riedel et al., 1996; Yu and Wang, 2004a; Yu and Wang, 2004b; Lo et al., 2015; Vriens et al., 2016; Ponton et al., 2018), so measuring these concentrations in a system could be helpful for risk prediction. It can be predicted that systems with higher levels of phosphate and sulfate in the water column may have less Se uptake into periphyton due to known inhibitory interactions regarding active uptake of Se at sulfate and phosphate transporters. Nitrogen concentrations could be another

predictive variable, as nitrogen influences periphyton growth, which may influence Se concentrations in periphyton via growth dilution (Conley et al., 2011) or increased Se concentrations through increased growth (Sun et al., 2014).

While it might seem straightforward in predicting that lakes with higher nutrient status take up generally less Se, that was not the case in the present study. Lakes that were more oligotrophic took up less Se than lakes that were more mesotrophic. This could be due to the lack of buffering capacity in the soft-water oligotrophic lakes chosen in the experiment or due to the taxa present in the various periphyton assemblages. Periphyton community composition likely plays an important role in the level of Se accumulation in aquatic systems. In the literature however, there does not appear to be any clear trends of specific species accumulating great amounts of Se among various studies. In the present study, the clearest trends were periphyton communities with higher proportions of charophytes accumulating less Se than others, and communities with higher proportions of bacillariophytes accumulating more Se. These trends were correlated strongly with higher presence of dissolved inorganic carbon and total dissolved phosphorus levels, respectively. When predicting Se toxicity in a system, characterizing the periphyton community could provide some information regarding potential risk along with categorizing certain water chemistry variables. If examining boreal lake systems, a higher proportion of charophytes in periphyton could predict less Se uptake, whereas low proportions of charophytes could predict higher Se accumulation. This trend specifically has not yet been reported in the literature, but it is important to note that charophyte abundance was highly correlated with other water chemistry variables. While predicting the risk of Se is possible, there are often many other site-specific variables that influence Se incorporation into food webs unique to different systems that should be considered and evaluated.

An interesting aspect of excess Se in aquatic systems is the potential of bioremediation of Se by organisms found in periphyton including algae and bacteria. Because algae and bacteria can incorporate Se rapidly from the water column directly and general tolerance over other organisms, they have been proposed as bioremediators in Se contaminated systems. Specifically, the green alga *Chlorella zofingiensis* has one of the highest tolerable limits of selenite (100 mg/L) where growth was similar in Se treated cells and control cells, whereas the green alga *Scenedesmus quadricauda* had completely inhibited growth at Se concentrations at 100 mg/L,

but only slowed growth at 50 mg/L (Vítová et al., 2015). Either of these algae could make excellent bioremediators of Se, depending on the level of contamination in the system. Bioremediation through these organisms also offers a more cost-effective method to reducing Se contamination in various impacted systems (Eswayah et al., 2016). In addition to remediating contaminated systems, enriched algae and bacteria could be used as potential nutritional supplements for humans and animals deficient in Se (Vítová et al., 2015).

3.3.2 Identified research gaps and future research

In addition to the research suggestions outlined in section 3.1.3 regarding improving experimental design of the present study and the experiment outlined in section 3.2 regarding Se-PO_4^{3-} interactions, there are other research directions that should be explored to further the existing knowledge of Se assimilation at the base of lentic food webs. In the experiments going forward, it is recommended that a relevant mix of selenite and selenate at low environmentally relevant concentrations are used to represent more naturally occurring Se distributions in freshwater systems and fill in research gaps remaining at low Se levels.

Examining the assimilation of Se in various algal phyla specifically is an area of research that should be explored using low levels of Se. A review by Gojkovic et al. (2015) found differences among different chlorophyte species regarding Se uptake, which means there is potential differences in many other alga species from various phyla in how they incorporate Se. From the results of the present study, an experiment examining Se uptake in several different charophyte species commonly found in freshwater systems (i.e., desmid species, and/or filamentous species including *Spirogyra* and *Mougeotia*) could better understand the impact charophyte abundance, and which charophytes specifically, may influence Se assimilation into food webs. Examining these species both in monoculture and mixed would be interesting to see if there is any differential uptake of Se exhibited. Additionally, performing the same experiment examining multiple common diatom species (i.e., *Navicula*, *Tabellaria*, *Achnanthes*) would also better the understanding of the role diatoms play in Se incorporation into food webs. To further understand the role different algal phyla play in Se assimilation, creating artificial biofilms including known proportions of charophytes, chlorophytes, diatoms and cyanophytes in varied replicates could reveal potential differences in Se uptake if water chemistry variables are kept constant, and the duration of the experiment is relatively short to prevent community shifts. In

addition to further exploring the impacts of periphyton community variables, it is important to further investigate the impacts of certain water chemistry variables to determine the roles they may play in influencing Se uptake into organisms at the base of the food web. It is known that carbon can influence periphyton growth and that it was a potentially key factor in driving differential uptake in the present study, therefore an experiment examining the impacts of DIC and Se uptake in periphyton should be explored. Variable levels of DIC with a range of Se concentrations surrounding current water quality guidelines, along with characterized artificial assemblages of periphyton could help narrow down the potential impact of DIC on Se uptake.

Another important aspect that should receive attention in future research is further investigating how Se is incorporated into algae from different periphyton assemblages, as well as examining the actual Se requirements of different algal species (Baines and Fisher, 2001). There are many different reports of both active and passive uptake of Se in the literature among many different freshwater base-level species, but this should continue to be explored. It is an important factor to consider because if inorganic Se is incorporated actively, it will be biotransformed to forms of organic Se which is more toxic to higher trophic levels receiving this Se through their diet. Markwart et al. (2019) used heat-killing methods to determine if active or passive uptake occurred in different periphyton groups, which could be employed with other isolated periphytic organisms like single algal species, bacteria or fungi, as bacteria have also demonstrated both passive and active uptake in the literature (Sanders and Gilmour, 1994). Another way of examining Se uptake mechanisms could be to use high and low concentrations of known inhibitory ions like sulfate and phosphate with natural periphyton assemblages to see if the presence of these ions influences Se uptake. If so, it is likely that Se in those periphytic organisms is an active process.

While artificial periphyton assemblages are important to help attempt to tease out the effects of other important variables influencing Se uptake, it is important to note that natural periphyton assemblages should be used as often as possible, as artificial biofilms can miss key components of periphyton that can play significant roles in Se uptake, including bacteria. An experiment isolating the ability of different common freshwater bacteria to incorporate low levels of aqueous Se would be very beneficial, but this may be challenging due to the small size of bacteria, where the potential for contamination by other species might be higher, and counting

methods (i.e., flow cytometry) may be more difficult. However, an experiment examining other components of periphyton specifically (i.e., fungi, detritus, bacteria) with a range of Se concentrations and/or variable water chemistry would be highly valuable in better understanding how natural complex assemblages of periphyton accumulate Se.

Another potentially important aspect to explore regarding Se incorporation into periphyton is differences in temperature. Fowler and Benayoun (1976) found that increasing temperature increased Se concentration in some marine invertebrates, and He (2010) found that temperature influences biomass and periphyton community shifts, however there is little research available on the effects that temperature has on Se uptake by periphyton. Therefore, an experiment examining the effects of variable temperatures reflecting realistic temperatures periphyton may experience depending on their depth in the water column (~10 – 30 ºC) with a range of low Se exposure concentrations in naturally grown periphyton should be explored. This could provide further insight on how natural temperature fluctuations may influence the risk of Se in various aquatic systems, as temperature can vary between different lakes depending on lake morphometry or other factors like seasonality. Fowler and Benayoun (1976) also saw differences in the influence of temperature on Se uptake between mussels and shrimps, so exploring taxa differences in freshwater systems (i.e., algae, bacteria, fungi, invertebrates, etc.) may also be helpful in determining the potential impacts temperature differences has in Se incorporation in freshwater food webs.

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APPENDIX A

SUPPLEMENTARY INFORMATION FOR CHAPTER 2

A.1 Periphyton biomass sample calculation

A sample calculation is as follows where Day 0 tissue mass = 4.04 mg dm, Day 8 tissue mass = 65.85 + 4.04 (Day 0 mass) = 69.89 mg dm:

4.04 mg dm/117cm^2 = x/800cm^2

x= 27.62 mg dm (Day 0 mass extrapolation)

Increase value: 69.89 – 27.62 = 42.27 mg dm

% Increase: (42.27/27.62) * 100 = 153% growth change in biomass per cm^2

Table A.1: Model selection details for determining the best GLM, including null model details. All models are fitted with a gamma distribution and inverse link function. The model formula for all models was Periphyton Se ~ 1/Aqueous Se + Parameter(s) P_1 + P_2 + P_3, where the parameters are water chemistry and periphyton community composition parameters reported in order of appearance in the model. Other family distributions were examined but did not properly represent the data and were therefore not included in model selection and not included.

Model Parameters	Intercept	Slope AqSe	Slope P_1	Slope P_2	Slope P_3	AIC	pR^2
Periphyton Se ~ 1	$6.89E^{-02} \pm 1.13E^{-02}$					187.3	0.00
Periphyton Se ~ (1/AqSe)	$2.70E^{-02} \pm 6.40E^{-03}$	$3.98E^{-02} \pm 6.61E^{-03}$				142.8	0.82
DIC	$4.24E^{-03} \pm 8.64E^{-03}$	$3.93E^{-02} \pm 5.90E^{-03}$	$3.05E^{-04} \pm 1.08E^{-04}$			135.0	0.88
TDP	$4.32E^{-02} \pm 1.22E^{-02}$	$3.97E^{-02} \pm 6.72\ E^{-03}$	$-4.37E^{-03} \pm 2.48E^{-03}$			140.8	0.85
Chl*a*	$2.99E^{-02} \pm 7.99E^{-03}$	$3.98E^{-02} \pm 6.81E^{-03}$	$-4.22E^{-04} \pm 6.01E^{-04}$			144.2	0.83
TDN	$5.34E^{-02} \pm 1.78E^{-02}$	$3.97E^{-02} \pm 6.59E^{-03}$	$-7.65E^{-05} \pm 4.51E^{-05}$			141.2	0.85
pH	$-3.27E^{-03} \pm 4.17E^{-02}$	$3.98E^{-02} \pm 6.60E^{-03}$	$4.29E^{-03} \pm 5.91E^{-03}$			144.1	0.83
Bacillario	$5.83E^{-02} \pm 2.35E^{-02}$	$3.98E^{-02} \pm 6.54E^{-03}$	$-1.13E^{-03} \pm 7.82E^{-04}$			142.2	0.84
Cyano	$3.58E^{-02} \pm 1.03E^{-02}$	$3.96E^{-02} \pm 6.58E^{-03}$	$-1.17E^{-03} \pm 9.88E^{-04}$			143.1	0.84
Chloro	$5.78E^{-02} \pm 1.93E^{-02}$	$3.95E^{-02} \pm 6.51E^{-03}$	$-1.18E^{-03} \pm 6.57E^{-04}$			140.6	0.85
*Charo	$-4.34E^{-02} \pm 1.99E^{-02}$	$3.92E^{-02} \pm 5.36E^{-03}$	$1.87E^{-03} \pm 5.48E^{-04}$			129.3	0.90
Biomass	$3.33E^{-02} \pm 8.47E^{-03}$	$3.99E^{-02} \pm 6.54E^{-03}$	$-5.03E^{-05} \pm 3.87E^{-05}$			143.0	0.84
DIC + TDP	$-2.64E^{-02} \pm 2.67E^{-02}$	$3.92E^{-02} \pm 5.77E^{-03}$	$4.94E^{-04} \pm 1.94E^{-04}$	$4.48E^{-03} \pm 3.77E^{-03}$		135.1	0.89

DIC + Chl*a*	$-2.56E^{-02} \pm 1.50E^{-02}$	$3.91E^{-02} \pm 5.31E^{-03}$	$5.66E^{-04} \pm 1.61E^{-04}$	$1.51E^{-03} \pm 6.73E^{-04}$	130.2	0.91
DIC + TDN	$1.18E^{-03} \pm 2.97E^{-02}$	$3.93E^{-02} \pm 6.03E^{-03}$	$3.16E^{-04} \pm 1.47E^{-04}$	$6.62E^{-06} \pm 6.13E^{-05}$	136.9	0.88
DIC + pH	$-6.38E^{-02} \pm 3.33E^{-02}$	$3.91E^{-02} \pm 5.41E^{-03}$	$4.19E^{-04} \pm 1.28E^{-04}$	$8.44E^{-03} \pm 4.15E^{-03}$	131.3	0.90
DIC + Bacillario	$5.88E^{-04} \pm 3.34E^{-02}$	$3.93E^{-02} \pm 6.04E^{-03}$	$3.14E^{-04} \pm 1.35E^{-04}$	$1.08E^{-04} \pm 9.56E^{-04}$	136.9	0.88
DIC + Cyano	$-6.78E^{-03} \pm 1.73E^{-02}$	$3.94E^{-02} \pm 6.10E^{-03}$	$3.71E^{-04} \pm 1.46E^{-04}$	$8.12E^{-04} \pm 1.11E^{-03}$	136.2	0.88
DIC + Chloro	$4.25E^{-02} \pm 1.85E^{-02}$	$3.91E^{-02} \pm 5.37E^{-03}$	$3.71E^{-04} \pm 1.20E^{-04}$	$-1.66E^{-03} \pm 7.23E^{-04}$	129.2	0.91
DIC + Charo	$-4.99E^{-02} \pm 2.60E^{-02}$	$3.92E^{-02} \pm 5.46E^{-03}$	$-8.58E^{-05} \pm 2.24E^{-04}$	$2.21E^{-03} \pm 1.05E^{-03}$	131.1	0.91
DIC + Biomass	$8.39E^{-03} \pm 9.85E^{-03}$	$3.94E^{-02} \pm 5.78E^{-03}$	$3.18E^{-04} \pm 1.13E^{-04}$	$-4.12E^{-05} \pm 3.18E^{-05}$	134.8	0.89
TDP + Chl*a*	$4.64E^{-02} \pm 1.33E^{-02}$	$3.97E^{-02} \pm 6.85E^{-03}$	$-6.46E^{-03} \pm 3.47E^{-03}$	$6.65E^{-04} \pm 7.81E^{-04}$	141.7	0.86
TDP + TDN	$4.90E^{-02} \pm 1.98E^{-02}$	$3.97E^{-02} \pm 6.83E^{-03}$	$-3.03E^{-03} \pm 4.36E^{-03}$	$-3.14E^{-05} \pm 8.15E^{-05}$	142.5	0.85
TDP + pH	$-5.75E^{-03} \pm 3.34E^{-02}$	$3.95E^{-02} \pm 6.40E^{-03}$	$-6.16E^{-03} \pm 2.71E^{-03}$	$7.88E^{-03} \pm 4.97E^{-03}$	139.2	0.87
TDP + Bacillario	$4.93E^{-02} \pm 2.57E^{-02}$	$3.97E^{-02} \pm 6.83E^{-03}$	$-3.72E^{-03} \pm 3.48E^{-03}$	$-3.06E^{-04} \pm 1.12E^{-03}$	142.7	0.85
TDP + Cyano	$4.59E^{-02} \pm 1.29E^{-02}$	$3.96E^{-02} \pm 6.64E^{-03}$	$-3.80E^{-03} \pm 2.59E^{-03}$	$-6.44E^{-04} \pm 1.06E^{-03}$	142.2	0.85
TDP + Chloro	$8.03E^{-02} \pm 2.56E^{-02}$	$3.94E^{-02} \pm 6.55E^{-03}$	$-4.80E^{-03} \pm 2.62E^{-03}$	$-1.36E^{-03} \pm 7.52E^{-04}$	137.5	0.88
TDP + Charo	$-7.46E^{-02} \pm 3.40E^{-02}$	$3.90E^{-02} \pm 5.22E^{-03}$	$3.13E^{-03} \pm 2.86E^{-03}$	$2.39E^{-03} \pm 7.16E^{-04}$	129.6	0.91
TDP + Biomass	$6.99E^{-02} \pm 1.67E^{-02}$	$3.99E^{-02} \pm 5.85E^{-03}$	$-7.99E^{-03} \pm 2.85E^{-03}$	$-1.08E^{-04} \pm 4.10E^{-05}$	134.6	0.89
Chl*a* + TDN	$5.36E^{-02} \pm 1.85E^{-02}$	$3.97E^{-02} \pm 6.76E^{-03}$	$-1.24E^{-04} \pm 6.09E^{-04}$	$-7.46E^{-05} \pm 4.78E^{-05}$	143.2	0.85
Chl*a* + pH	$-1.52E^{-01} \pm 6.25E^{-02}$	$3.93E^{-02} \pm 5.81E^{-03}$	$-3.61E^{-03} \pm 1.32E^{-03}$	$2.89E^{-02} \pm 1.02E^{-02}$	136.2	0.88
Chl*a* + Bacillario	$5.87E^{-02} \pm 2.46E^{-02}$	$3.98E^{-02} \pm 6.72E^{-03}$	$-2.32E^{-04} \pm 6.04E^{-04}$	$-1.09E^{-03} \pm 8.32E^{-04}$	144.0	0.84

Chl*a* + Cyano	$3.58E^{-02} \pm 1.06E^{-02}$	$3.96E^{-02} \pm 6.72E^{-03}$	$5.40E^{-05} \pm 7.75E^{-04}$	$-1.22E^{-03} \pm 1.27E^{-03}$	145.1	0.84
Chl*a* + Chloro	$8.11E^{-02} \pm 2.71E^{-02}$	$3.94E^{-02} \pm 6.50E^{-03}$	$-1.09E^{-03} \pm 6.84E^{-04}$	$-1.79E^{-03} \pm 8.49E^{-04}$	139.2	0.87
Chl*a* + Charo	$-4.75E^{-02} \pm 2.13E^{-02}$	$3.91E^{-02} \pm 5.42E^{-03}$	$2.61E^{-04} \pm 5.07E^{-04}$	$1.93E^{-03} \pm 5.61E^{-04}$	130.9	0.91
Chl*a* + Biomass	$3.87E^{-02} \pm 1.08E^{-02}$	$3.99E^{-02} \pm 6.73E^{-03}$	$-6.46E^{-04} \pm 6.54E^{-04}$	$-5.90E^{-05} \pm 4.09E^{-05}$	143.7	0.84
TDN + pH	$3.24E^{-02} \pm 4.39E^{-02}$	$3.97E^{-02} \pm 6.60E^{-03}$	$-7.21E^{-05} \pm 4.50E^{-05}$	$2.77E^{-03} \pm 5.30E^{-03}$	142.8	0.85
TDN + Bacillario	$3.07E^{-02} \pm 3.24E^{-02}$	$3.97E^{-02} \pm 6.97E^{-03}$	$-2.44E^{-04} \pm 2.17E-^{04}$	$2.90E^{-03} \pm 3.62E^{-03}$	142.2	0.85
TDN + Cyano	$6.34E^{-02} \pm 2.08E^{-02}$	$3.96E^{-02} \pm 6.43E^{-03}$	$-8.18E^{-05} \pm 4.84E-^{05}$	$-1.08E^{-03} \pm 9.47E^{-04}$	141.5	0.86
TDN + Chloro	$8.50E^{-02} \pm 2.55E^{-02}$	$3.94E^{-02} \pm 6.43E^{-03}$	$-6.77E^{-05} \pm 3.88E-^{05}$	$-1.32E^{-03} \pm 7.28E^{-04}$	138.3	0.87
TDN + Charo	$-1.06E^{-01} \pm 4.54E^{-02}$	$3.90E^{-02} \pm 5.07E^{-03}$	$8.36E^{-05} \pm 5.50E^{-05}$	$2.76E^{-03} \pm 8.06E^{-04}$	128.1	0.92
TDN + Biomass	$8.15E^{-02} \pm 2.28E^{-02}$	$3.99E^{-02} \pm 5.98E^{-03}$	$-1.26E^{-04} \pm 5.18E^{-05}$	$-8.77E^{-05} \pm 3.78E^{-05}$	137.2	0.88
pH + Bacillario	$3.83E^{-02} \pm 5.01E^{-02}$	$3.97E^{-02} \pm 6.59E^{-03}$	$2.49E^{-03} \pm 5.51E^{-03}$	$-1.04E^{-03} \pm 7.99E^{-04}$	143.9	0.84
pH + Cyano	$-4.18E^{-03} \pm 3.82E^{-02}$	$3.95E^{-02} \pm 6.42E^{-03}$	$5.92E^{-03} \pm 5.46E^{-03}$	$-1.40E^{-03} \pm 9.45E^{-04}$	143.6	0.84
pH + Chloro	$5.16E^{-02} \pm 5.41E^{-02}$	$3.95E^{-02} \pm 6.64E^{-03}$	$7.68E^{-04} \pm 6.30E^{-03}$	$-1.15E^{-03} \pm 7.11E^{-04}$	142.6	0.85
pH + Charo	$-5.45E^{-02} \pm 3.14E^{-02}$	$3.92E^{-02} \pm 5.43E^{-03}$	$1.74E^{-03} \pm 3.85E^{-03}$	$1.84E^{-03} \pm 5.56E^{-04}$	131.0	0.91
pH + Biomass	$2.16E^{-02} \pm 5.05E^{-02}$	$3.99E^{-02} \pm 6.65E^{-03}$	$1.57E^{-03} \pm 6.71E^{-03}$	$-4.51E^{-05} \pm 4.47E^{-05}$	144.9	0.84
Bacillario + Cyano	$6.71E^{-02} \pm 2.68E^{-02}$	$3.97E^{-02} \pm 6.45E^{-03}$	$-1.17E^{-03} \pm 8.59E^{-04}$	$-1.02E^{-03} \pm 9.57E^{-04}$	142.8	0.85
Bacillario + Chloro	$9.71E^{-02} \pm 2.91E^{-02}$	$3.94E^{-02} \pm 6.33E^{-03}$	$-1.13E^{-03} \pm 6.27E^{-04}$	$-1.48E^{-03} \pm 7.25E^{-04}$	138.1	0.87
Bacillario + Charo	$-1.21E^{-01} \pm 5.37E^{-02}$	$3.90E^{-02} \pm 5.06E^{-03}$	$1.50E^{-03} \pm 9.41E^{-04}$	$2.83E^{-03} \pm 8.44E^{-04}$	127.7	0.92

Bacillario + Biomass	$7.96E^{-02} \pm 2.76E^{-02}$	$3.98E^{-02} \pm 6.19E^{-03}$	$-1.59E^{-03} \pm 8.63E^{-04}$	$-6.68E^{-05} \pm 3.60E^{-05}$		140.5	0.86
Cyano + Chloro	$1.06E^{-01} \pm 2.93E^{-02}$	$3.90E^{-02} \pm 5.70E^{-03}$	$-2.52E^{-03} \pm 9.91E^{-04}$	$-2.29E^{-03} \pm 8.52E^{-04}$		134.7	0.89
Cyano + Charo	$-4.24E^{-02} \pm 2.26E^{-02}$	$3.92E^{-02} \pm 5.48E^{-03}$	$-7.61E^{-05} \pm 7.68E^{-04}$	$1.86E^{-03} \pm 5.70E^{-04}$		131.3	0.90
Cyano + Biomass	$3.63E^{-02} \pm 1.05E^{-02}$	$3.98E^{-02} \pm 6.68E^{-03}$	$-6.97E^{-04} \pm 1.26E^{-03}$	$-3.23E^{-05} \pm 4.99E^{-05}$		144.6	0.84
Chloro + Charo	$-1.53E^{-02} \pm 2.96E^{-02}$	$3.91E^{-02} \pm 5.29E^{-03}$	$-8.46E^{-04} \pm 6.92E^{-04}$	$1.71E^{-03} \pm 5.48E^{-04}$		129.1	0.91
Chloro + Biomass	$6.39E^{-02} \pm 2.05E^{-02}$	$3.97E^{-02} \pm 6.37E^{-03}$	$-1.19E^{-03} \pm 6.72E^{-04}$	$-4.72E^{-05} \pm 3.90E^{-05}$		140.9	0.86
Charo + Biomass	$-3.98E^{-02} \pm 2.07E^{-02}$	$3.92E^{-02} \pm 5.27E^{-03}$	$1.87E^{-03} \pm 5.56E^{-04}$	$-3.08E^{-05} \pm 2.83E^{-05}$		129.7	0.91
Charo + TDP + DIC	$-7.38E^{-02} \pm 3.48E^{-02}$	$3.90E^{-02} \pm 5.34E^{-03}$	$2.25E^{-03} \pm 1.10E^{-03}$	$3.40E^{-03} \pm 3.31E^{-03}$	$4.63E^{-05} \pm 2.71E^{-04}$	131.6	0.91
Charo + Bacillario + DIC	$-1.46E^{-01} \pm 6.16E^{-02}$	$3.89E^{-02} \pm 5.06E^{-03}$	$3.76E^{-03} \pm 1.40E^{-03}$	$1.66E^{-03} \pm 9.41E^{-04}$	$-1.92E^{-04} \pm 2.30E^{-04}$	128.6	0.92
Charo + Bacillario + TDP	$-1.25E^{-01} \pm 5.39E^{-02}$	$3.89E^{-02} \pm 5.12E^{-03}$	$2.93E^{-03} \pm 8.67E^{-04}$	$1.29E^{-03} \pm 1.04E^{-03}$	$1.45E^{-03} \pm 2.95E^{-03}$	129.3	0.92
Charo + Chloro + Bacillario	$-9.17E^{-02} \pm 6.65E^{-02}$	$3.90E^{-02} \pm 5.12E^{-03}$	$2.63E^{-03} \pm 9.18E^{-04}$	$-5.65E^{-04} \pm 7.08E^{-04}$	$1.23E^{-03} \pm 9.88E^{-04}$	128.7	0.92
Charo + Chloro + TDP	$-4.47E^{-02} \pm 4.73E^{-02}$	$3.90E^{-02} \pm 5.24E^{-03}$	$2.15E^{-03} \pm 7.82E^{-04}$	$-6.96E^{-04} \pm 7.25E^{-04}$	$2.42E^{-03} \pm 3.14E^{-03}$	130.3	0.92
Charo + Chloro + DIC	$1.10E^{-02} \pm 5.24E^{-02}$	$3.91E^{-02} \pm 5.40E^{-03}$	$9.17E^{-04} \pm 1.42E^{-03}$	$-1.25E^{-03} \pm 9.70E^{-04}$	$1.87E^{-04} \pm 3.08E^{-04}$	130.6	0.91
Bacillario + TDP + DIC	$-1.73E^{-02} \pm 3.60E^{-02}$	$3.92E^{-02} \pm 5.88E^{-03}$	$-4.00E^{-04} \pm 1.07E^{-03}$	$5.12E^{-03} \pm 4.19E^{-03}$	$4.88E^{-04} \pm 1.95E^{-04}$	136.9	0.89

Intercepts and slopes are shown as ± standard error.

Abbreviations: AqSe = aqueous selenium, DIC = dissolved inorganic carbon, TDP = total dissolved phosphorus, Chl*a* = chlorophyll *a*, TDN = total dissolved nitrogen, Bacillario = Bacillariophytes, Cyano = Cyanophytes, Chloro = Chlorophytes, Charo = Charophytes, AIC = Akaike Information Criterion, pR^2 = pseudo R^2

*Indicates best model as per AIC method and rule of parsimony.

APPENDIX B

ADDITIONAL RESULTS FROM CHAPTER 2

B.1 Concentration-dependent differences among Se treatments

An additional objective of this thesis was to investigate if there are concentration-dependent differences in bioconcentration of Se by periphyton among Se treatments compared to controls both within and among lakes. The corresponding hypotheses are:

H_0: There will be no concentration-dependent differences in periphyton bioconcentration of Se.
H_1: There will be concentration-dependent differences in selenite uptake by periphyton. I predict that since Se is an essential trace element, more uptake (i.e., higher enrichment functions) is expected at lower concentrations, as is typical of essential nutrients.

To determine if significant differences between control treatments and Se treatments existed both within lakes and among study lakes, a generalized linear mixed model (GLMMs) was employed. Statistical analysis was performed in RStudio integrated development environment (RStudio Team, 2020) using base package software and the added software package lme4 (Bates et al., 2015) and emmeans (Lenth, 2020). A gamma distribution and log link function were used in this model, with Se treatment set as the categorical fixed variable and lake as a random factor. A Tukey post-hoc test ($\alpha = 0.05$) for multiple comparisons was performed to determine where specific significant differences occurred among the Se treatments.

Significant concentration-dependent differences among Se treatments in Se uptake by periphyton were observed among all treatments compared to controls and among different treatments both within lakes and among lakes, as determined by GLMM (Table B.1). Generally, greater uptake of Se was observed in lakes with higher nutrient status in comparison to more oligotrophic lakes. With lake accounted for as a random factor, all selenite addition treatments were significantly different from controls for all five lakes when examining treatment as a categorical variable ($p < 0.0001$; Tukey post-hoc test). Additionally, all treatments were different from each other when run in a Tukey multiple comparisons post-hoc test for all lakes ($0.03 < p < 0.0001$), except for 0.5 vs 1 µg Se/L ($p = 0.16$) and 1 vs 2 µg Se/L ($p = 0.34$) (Table B.1). These concentration-dependent differences regarding Se uptake by organisms at the base of aquatic food webs are commonly found among the literature (Gojkovic et al., 2015; Kousha et al., 2017; Graves et al., 2021).

Table B.1: Tukey post-hoc test output from the select GLMM. The GLMM formula used was Periphyton Se ~ Se treatment + (1|Lake). Significant differences noted by '*'.

Contrast	Estimate	SE	df	z ratio	p value
Ctrl – 0.5	-8.00	1.37	Inf	-5.85	<0.0001 *
Ctrl – 1	-11.88	1.99	Inf	-5.98	<0.0001 *
Ctrl – 2	-16.17	2.67	Inf	-6.05	<0.0001 *
Ctrl – 4	-27.21	4.46	Inf	-6.11	<0.0001 *
0.5 – 1	-3.88	1.72	Inf	-2.25	0.1618
0.5 – 2	-8.17	2.27	Inf	-3.60	0.0030 *
0.5 – 4	-19.21	3.90	Inf	-4.92	<0.0001 *
1 – 2	-4.29	2.31	Inf	-1.86	0.3418
1 – 4	-15.33	3.76	Inf	-4.08	0.0004 *
2 – 4	-11.04	3.72	Inf	-2.96	0.0253 *