## Impacts of Invasive Spiny Water Flea (*Bythotrephes cederströmii*) on Walleye (*Sander vitreus*) Mercury Accumulation, Ecosystem Biomagnification, and Bioenergetics

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## Abstract

An evolving threat to freshwater ecosystems is invasive species. The spiny water flea (Bythotrephes) is an invasive zooplankter well known for its severe negative impacts on native freshwater zooplankton, but some knowledge gaps exist regarding their impacts to fishes, high-level trophic organisms, and food chains. Changes in trophic positions at all levels of the ecosystem can affect contaminant concentrations in large organisms. In this study I used Walleye (Sander vitreus) total muscle mercury concentration ([MeHg]) and size data, as well as stable isotope ratios and a bioenergetics model to evaluate the potential impacts of *Bythotrephes* on the build up of toxic metals in fishes. I expected to see an increase in Walleye mercury concentration and a systematic impact of Bythotrephes on Walleye growth efficiency. In my first data chapter (chapter 2) I found that the presence of *Bythotrephes* was associated with an increase in small (<50cm) Walleye [MeHg] in Quetico Provincial Park and in Rainy Lake. These results suggest that Bythotrephes have a greater impact on Hg accumulation in smaller fish and may alter Walleye growth trajectories. In my second data chapter (chapter 3) I found that ecosystem biomagnification and trophic position of Walleye from a set of 8 lakes differed among lakes but were not associated with Bythotrephes invasions. Similarly, prey consumption, activity, and Walleye biomagnification factors differed among these lakes but did not differ based on invasion status. However, I found significant correlations between Walleye prey consumption, Walleye biomagnification factor, and activity with lake surface area. My findings suggest that anglers are potentially at an elevated risk of excess Hg exposure when consuming Walleye less than 50 cm from large (i.e., > 5000 ha) lakes, particularly where data informing consumption advisories are based on old information

(prior to *Bythotrephes* invasion). Further sampling of lakes recently invaded or at risk of *Bythotrephes* invasions may be required to update consumption advisories to ensure safe consumption of Walleye by anglers.

## Lay Summary

This thesis addresses a gap in previous research regarding the build up of mercury in Walleye and food chain impacts brought on by spiny water flea, an invasive zooplankton. Spiny water flea invasions result in major declines of native zooplankton communities and are estimated to cause fish to take up and retain more mercury in their tissues, posing a risk to people who might eat those fish. In this thesis, I investigate these potential impacts, particularly in Walleve, which is an important sport fish in Ontario and is often consumed by anglers. Mercury is poisonous, and in high enough doses can cause health problems for humans who consume fish. I found that the presence of spiny water fleas was associated with increases in small Walleye (<50cm) mercury in Quetico Provincial Park and in Rainy Lake, with this impact being more severe in larger lakes. This is of concern as smaller Walleye are typically most frequently angled and consumed by humans. I also found that lake food chains were not impacted by spiny water flea invasions. Lasty, I found that spiny water flea did not have any obvious impact on Walleye prey consumption or foraging activity. Interestingly though, I found that increasing lake surface area was directly related to changes in Walleye feeding and foraging activity. This study provides new insight into the impacts of invasive species on Walleye mercury dynamics and potential implications for future resource management and consumption advisories.

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## **Chapter 1: General Introduction**

#### 1.1 Background

Mercury (Hg) is a toxic heavy metal that exists naturally in the environment but is also linked to industrial pollution (Selin 2009, Pirrone et al. 2010, Ajsuvakova et al. 2020). Industrial activity is a major source of mercury contamination in the environment including the combustion of fossil fuels (Pirrone et al. 2010, Lavoie et al. 2013, Ajsuvakova et al. 2020) which emits gaseous elemental Hg into the atmosphere (Díaz-Somoano et al. 2007). Due to its atmospheric persistence, Hg can be deposited thousands of kilometres away from its source, causing significant contamination in remote areas (Dastoor and Larocque 2004).

Once inorganic Hg is deposited in aquatic ecosystems, it may be converted to organic Hg through the process of methylation to produce methylmercury, the more toxic and bioavailable form of mercury (Lavoie et al. 2013, Bringham et al. 2021). Methylation of Hg is performed primarily by iron- and sulfate-reducing microbes in anaerobic sediments (Gilmour et al. 2013). In this organic form, methylmercury is rapidly incorporated into the food web (Lavoie et al. 2013) and can remain present in the environment for several decades (Lodenius 1991).

Bioaccumulation is the uptake and retention of contaminants in an organism (Rodgers and Beamish 1983, Mathers and Johansen 1985). Methylmercury bioaccumulates in aquatic organisms through the uptake of contaminated water across the gills, or through the consumption of contaminated food across the gastrointestinal tract (Mathers and Johansen 1985, Hill et al. 1996). Comparative studies have demonstrated that fish typically accumulate the majority of methylmercury through ingestion versus through waterborne uptake across the gills (Hill et al. 1996, Becker and Bingham 1996, Hall et al. 1997, Ajsuvakova et al. 2020). Methylmercury consumed by fish accumulates as the rate of assimilation via consumption greatly exceeds the rate of elimination (Mathers and Johansen 1985, Ajsuvakova et al. 2020). As methylmercury accumulates and is transferred through the food chain, organisms occupying higher trophic positions typically have greater methylmercury concentrations, often thousands of times greater than in the surrounding water (Gobas et al. 1999, Lavoie et al. 2013). This process is called "Biomagnification" and is a common phenomenon in aquatic food webs (Lavoie et al. 2013). Biomagnification of methylmercury can have significant health impacts on organisms that accumulate large quantities of methylmercury, including decreased reproductive success and neurological damage which have both been reported in predatory fish and humans (Yang et al. 2020).

Consumption rates and accumulation of Hg can be influenced by the feeding efficiency, activity, and growth rates of fish (Trudel et al. 2006). With increasing body size, [Hg] also increases, but this increase is linked to the rate at which fish grow; Hg concentration increases most rapidly with body size when growth efficiency decreases with body size (Simoneau et al. 2005, Trudel et al. 2006). This means that at a given feeding rate and prey mercury concentration, a decrease in activity or other metabolic costs would result in a faster growing fish, with greater growth efficiency (Simoneau et al. 2005, Trudel et al. 2006). Therefore, fish with a lower growth efficiency will have greater [Hg] at the same length than those of the same species that have more optimal growth efficiency (Simoneau et al. 2005) assuming similar environmental conditions. In addition to variation in growth efficiency, analyses of aquatic trophic interactions have shown that food chain length influences the trophic position of predatory fish, and by extension, their accumulation of contaminants (Cabana et al. 1994). Comparative studies have shown that concentrations of bioaccumulative contaminants (e.g. Hg, PCBs) in predatory pelagic fishes are directly correlated to increased trophic position and increasing food chain length (Rasmussen et al. 1990, Cabana et al. 1994, Vander Zanden and Rasmussen 1996). As such, the introduction of a zoo-planktivorous invasive species would be expected to extend the length of pelagic food chains and result in greater contaminant concentrations in predatory fishes (Rasmussen et al. 1990, Cabana et al. 1994).

An invasive zoo-planktivorous species that has been investigated for its impact on contaminant accumulation and fish growth efficiency is the spiny water flea (*Bythotrephes cederströemi*), hereafter referred to as *Bythotrephes. Bythotrephes* is a predatory zooplankton that was introduced to the Laurentian Great Lakes in the 1980s (Colautti et al. 2005) through discharged ballast water (Yan et al. 2011). Following its introduction to the Great Lakes watershed, the range of *Bythotrephes* has expanded to hundreds of inland lakes (Yan and Pawson 1997, Yan et al. 2002, Kerfoot et al. 2016), in large part due to their ability to reproduce parthenogenically, allowing them to rapidly increase in density (Kerfoot et al. 2011). This spread is primarily attributed to recreational fishing activities, during which adult *Bythotrephes* can become tangled on fishing gear, and resting eggs of *Bythotrephes* (i.e., ephippia, the product of sexual reproduction in cladocerans) which are capable of surviving when consumed and excreted by fish and

can be spread through water transported as ballast, in bilge water or in live wells (Kerfoot et al. 2011).

Bythotrephes have significant negative impacts on zooplankton communities. Following invasion, they can severely decrease the richness and abundance of crustacean Zooplankton (Yan and Pawson 1997, Kerfoot et al. 2011, Kerfoot et al. 2016). Comparative studies have reported that populations of *Bythotrephes* can consume significant quantities of zooplankton, disrupting the feeding of planktivorous fishes and greatly reducing zooplankton biodiversity (Kerfoot et al. 2011, Kerfoot et al. 2016). Zooplankton communities following *Bythotrephes* invasion are often characterized by significant declines in abundance and richness of smaller zooplankton species and increases in the abundance of larger or faster zooplankton species that are not consumed by *Bythotrephes* (Yan and Pawson 1997).

The significant damage *Bythotrephes* can impose on planktonic communities is well documented, but relatively little is known regarding how these changes to food webs impact contaminant accumulation through invaded ecosystems. Based on previous observational studies, the invasion of a mid-trophic organism would be predicted to result in lengthened aquatic food chains and increased contaminant accumulation in predatory fishes (Rasmussen et al. 1990, Cabana et al. 1994, Vander Zanden and Rasmussen 1996). Additionally, changes to the zooplankton community following *Bythotrephes* invasion have been also demonstrated to result in an increase in carnivorous or heterotrophic zooplankton, thus elevating the estimated trophic position of the zooplankton community as a whole, regardless as to whether *Bythotrephes* are directly consumed by fish or not (Rennie et al. 2011). This increase in the trophic position of the zooplankton community

might therefore be predicted to increase contaminant accumulation in zoo-planktivorous fishes and their consumers (Rennie et al. 2011). However, direct attempts to observe these impacts have not detected a strong effect of mid-trophic invasions with respect to contaminant accumulation in fishes (Rennie et al. 2010, Johnston et al. 2003). Some studies have reported no significant change as a result of mid-trophic invasion (Johnston et al. 2003), while others have reported reductions in contaminant accumulation that are potentially associated more with climate change than invasive species impacts (Rennie et al. 2010).

In addition to disruptions to food web complexity, *Bythotrephes* invasions have been shown to have negative impacts on Walleye growth rates (Hansen et al. 2020, Gartshore and Rennie 2023) but this is not always the case as some studies have found no effect on growth (Jordan et al. 2023). In lakes invaded by *Bythotrephes*, young-of-year (YOY) Walleye mean length was reduced by up to 13% in mid-summer (Hansen et al. 2020). In addition to decreased mean length, Walleye growth rates in invaded lakes declined by approximately 2mm per standardized degree day, which was 9.6% slower than those of Walleye in uninvaded waterbodies (Hansen et al. 2020). If Walleye growth rates decrease following *Bythotrephes* invasions, there is potential that this is associated with a decrease in their growth efficiency, which would lead to an increase in the accumulation of contaminants during their lifetime.

Bioenergetics models can be used to estimate food consumption and growth efficiency, and therefore provide insights into how invasive species impact these variables (Cerino et al. 2013, Rennie et al. 2012) as well as how the presence of invasive species might impact the accumulation of contaminants in consumers. My research

directly addresses the impacts of *Bythotrephes* invasions on contaminant accumulation and growth efficiency in the predatory sportfish Walleye (*Sander vitreus*). Firstly, I evaluate whether the presence of *Bythotrephes* alters size-dependent rates of mercury accumulation in Walleye. Second, I evaluate ecosystem biomagnification and food chain length through stable isotope analysis. Lastly a bioenergetic model is applied to determine potential mechanisms behind differences in Hg accumulation between invaded/noninvaded lakes (via estimates of consumption and growth efficiency). These investigations will examine Walleye populations found in northwestern Ontario, including several that occur primarily within the bounds of a remote provincial park, in ecosystems relatively free of anthropogenic disturbance.

# **Chapter 2: Impacts to Walleye Mercury Bioaccumulation Across Time and Space as a Result of an Alien Invader**

#### 2.1 Abstract

Eurasian spiny water flea (Bythotrephes) is an invasive zooplankton species introduced to the Laurentian Great Lakes and surrounding watershed in the 1980s. A predatory organism, Bythotrephes restructures planktonic communities, diminishing native zooplankton species richness, diversity, and biomass. Bythotrephes are also predicted to increase the accumulation of contaminants in higher trophic species. In this study, I tested this prediction using Walleye (Sander vitreus) as a model organism, collected from lakes in Quetico Provincial Park, the Boundary Waters region, and within the Rainy Lake complex in northwestern Ontario. I tested this hypothesis across both spatial and temporal scales, accounting for environmental factors also known to have influence on methylmercury [MeHg] where possible. At the spatial scale, the scaling of Walleye [MeHg] with body size depended on both the presence of Bythotrephes and lake size, such that Walleye sizes vulnerable to fishing had higher [MeHg] in invaded lakes compared to reference lakes, with the magnitude of the difference decreasing with declining lake size. At the temporal scale, concentrations of Hg in smaller Walleye (<500 mm FL) were higher during post-invasion years in Rainy Lake. In Quetico provincial park lakes, no significant difference was found between the bioaccumulation slopes in lakes after Bythotrephes invasion compared to before invasion, despite an apparent increase in elevation of Walleye [MeHg] across all sizes following Bythotrephes invasion. The sum of evidence in this study suggests that the presence of *Bythotrephes* can increase Hg accumulation in Walleye of harvestable sizes.

**Key Words:** Invasive Species; Methylmercury; Bioaccumulation; Biomagnification; Zooplankton

#### 2.2 Introduction

Mercury is a toxic heavy metal that naturally occurs in sediments and can be emitted through volcanoes or other geological activity (Gustin 2003). However, anthropogenic activities including mining and fossil fuel combustion can also release mercury (Hg) into the environment (Pirrone et al. 2010, Lavoie et al. 2013, Ajsuvakova et al. 2020). Anthropogenic Hg pollution has led to well-documented adverse health effects for both human and animal communities (Kudo et al. 1998, Yang et al. 2020). Anthropogenic Hg emissions have declined over the last few decades in North America through the application of wet scrubbers (Diaz-Somoano et al. 2007), which should result in decreased fish [Hg] in lakes (Blanchfield et al. 2022). However, Hg can persist in aquatic and terrestrial ecosystems given its long half-life (Lodenius 1991). Further, the persistence of Hg in the atmosphere allows it to be transported great distances from its source to remote regions around the globe (Dastoor and Larocque 2004).

When Hg enters the environment, it can deposit into lakes (either directly or via overland transport: Pirrone et al. 2010) and settle in anerobic sediments, where it is methylated by bacteria (Lavoie et al. 2013, Bringham et al. 2021). Methylation transforms inorganic Hg into organic methylmercury (MeHg), a bioavailable and potent neurotoxin (Gilmour et al. 2013). Because the rate of assimilation of MeHg exceeds its elimination rate, MeHg bioaccumulates in organisms (Rodgers and Beamish 1983). The primary route of MeHg assimilation in fish is through the consumption of prey contaminated with MeHg (Mathers and Johansen 1985, Hill et al. 1996, Hall et al. 1997), which vastly exceeds rates of waterborne uptake (Porcella 1994, Becker and Bigham 1995, Hill et al. 1996). As such, the total amount of MeHg in consumers will increase over time with prey consumption, even when prey MeHg is constant (Mathers and

Johansen 1985, Ajsuvakova et al. 2020). Because of this general pattern of accumulation over time, there is a strong correlation between tissue [MeHg] and fish size/age; the larger and older a fish is, the higher the concentration of MeHg tends to be (Storelli et al. 2007, Lescord et al. 2018). Additionally, larger fish have a greater gape and can consume larger prey (Dörner and Wagner 2003, Dörner et al. 2007), which often contain more MeHg than smaller prey (Kamman et al. 2005) which increases bioaccumulation in larger fish. For piscivorous fish, typically 95% or more of total body Hg burden is MeHg (Bloom 1992, Porcella 1994, Becker and Bingham 1995, Lescord et al. 2018).

Fish size is not the only factor that determines their MeHg concentrations; a large body of literature has demonstrated that both biotic and abiotic environmental variables can influence MeHg accumulation in fish. Abiotic factors known to influence accumulation of MeHg include waterbody type (river, lake, reservoir; Kamman et al. 2005). Additionally, water chemistry, waterbody surface area, and surrounding land use can all affect MeHg accumulation rates in fish (McMurtry et al. 1989, Kamman et al. 2005, Sumner et al. 2020). Changes in atmospheric deposition of MeHg can also induce changes in fish Hg burdens (Harris et al. 2007; Blanchfield et al. 2022). Common biotic factors that impact MeHg accumulation include prey availability, growth efficiency (Kaufman 2006, Swanson et al. 2006), the trophic position of the fish, and the food chain length of the ecosystem it was collected from (Cabana et al. 1994, Vander Zanden and Rasmussen 1996). Given the putative impact of food chain length on fish MeHg, species invasions that may lengthen food chains have been hypothesized to increase MeHg accumulation in predatory fishes (Swanson et al. 2003, Rennie et al. 2011). However, direct tests of mid-trophic invasions on the [MeHg] of predatory fishes have generally not

supported this hypothesis (Johnston et al. 2003, Hogan et al. 2007, Rennie et al. 2010, Rennie et al. 2023 in review).

One mid-trophic invasive species that has been investigated for its potential role in impacting accumulation of MeHg in fish is the Eurasian Spiny Water Flea (*Bythotrephes cederströmii*, hereafter referred to as *Bythotrephes*). *Bythotrephes* is a relatively large cladoceran zooplankton (~10 mm). Genetic evidence suggests they were introduced to the Laurentian Great Lakes in the 1980s through ballast water (Colautti et al. 2005) and have since spread actively through inland lakes (Kerfoot et al. 2011). The inland spread of *Bythotrephes* has been enabled largely by gut-viable eggs, baitfish use, and hitchhiking on improperly cleaned fishing gear and boating equipment (Jarnagin et al. 2000). *Bythotrephes* can significantly reduce native zooplankton abundance and richness (Yan and Pawson 1997, Kerfoot et al. 2011, Kerfoot et al. 2016), and impact early life growth rates of fishes (Hansen et al. 2020, Gartshore and Rennie 2023, Rennie et al. 2023 in review).

Despite predictions that *Bythotrephes* invasion should elevate bioaccumualtiom in fish based on changes in zooplankton diet composition (Rennie et al. 2011), this has not been supported by published field observations (Rennie et al. 2010). Only recently has evidence emerged suggesting that *Bythotrephes* may alter the MeHg bioaccumulation rates of planktivorous fishes (Rennie et al. 2023 in review). The impacts of *Bythotrephes* have yet to be directly tested on MeHg accumulation rates of predatory fish such as Walleye (*Sander vitreus*). Walleye prefer planktivorous fish species as prey (Sheppard et al. 2015), such as Cisco (*Coregonus artedii*). Cisco appear to prefer *Bythotrephes* as prey (Gatch et al. 2021, Martin et al. 2023), and changes in zooplankton community

composition following *Bythotrephes* invasion are expected to elevate [MeHg] in planktivorous fishes (Rennie et al. 2011). Thus, MeHg accumulation in predatory fishes might be expected to increase following *Bythotrephes* invasion.

Walleye naturally accumulate high [MeHg] relative to other common species (Kamman et al. 2005) and are of particular interest given their importance to recreational, commercial and subsistence fisheries. In 2015, Walleye accounted for more than a quarter of all fish caught by recreational anglers in Canada (Department of Fisheries and Oceans Canada 2019). Walleye are also an important economic commercial resource, accounting for over 5000 tonnes and over \$21 million of commercial landings during 2021 in Ontario alone (Department of Fisheries and Oceans 2021). Fish consumption is also important for Indigenous communities, accounting for up to 40% of average daily consumption of traditional foods for Ontario Indigenous communities (Islam and Berkes 2016, Marushka et al. 2021). Given their importance to humans, piscivorous diet, and naturally high trophic position, understanding further impacts of invasive *Bythotrephes* to Walleye MeHg accumulation is essential.

This study sought to measure the potential effect that invasive *Bythotrephes* have on the accumulation of MeHg in Walleye, examining accumulation as the linear relationship between [MeHg] and Walleye body length across lakes on the landscape (e.g. among lakes with and without *Bythotrephes* present) as well as in invaded lakes over time. Given the theorized impact that a mid-trophic invader like *Bythotrephes* would have on food chains and trophic positions, I expected to observe higher accumulation of MeHg in Walleye within invaded waterbodies compared to those where they were absent prior to invasion.

#### 2.2.1 Objectives

**Objective 1:** The first objective of this chapter was to measure the potential effects of invasive *Bythotrephes* on mercury accumulation across space in Quetico Provincial Park. Slopes of Walleye mercury accumulation (MeHg-length) were compared between lakes invaded with *Bythotrephes* and uninvaded lakes across the landscape, controlling for environmental factors known to influence fish MeHg.

**Hypothesis 1**: I hypothesize that the presence of invasive *Bythotrephes* will be associated with greater contaminant concentrations in Walleye at a given size compared to lakes that have not been invaded.

**Objective 2**: The second objective was to measure the potential effects of invasive *Bythotrephes* on mercury accumulation across time in Quetico Provincial Park. Slopes of Walleye mercury accumulation (MeHg-length) were compared between years before and after the invasion of *Bythotrephes*, comparing patterns observed to uninvaded lakes, controlling for factors known to affect fish MeHg.

**Hypothesis 2**: I hypothesize that MeHg accumulation will be greater in post-invasion years when compared to the accumulation relationship in pre-invasion years. Reference lakes (*Bythotrephes* not present) will either have no difference between the two time periods or will have decreased accumulation in contemporary years.

#### 2.3 Methods

2.3.1: Study area

Lakes within Quetico Provincial Park (QPP) as well as surrounding lakes in the Rainy River drainage were included in the study, several of which also had available long-term data on Walleye Hg and size (Tables 2.1, 2.2) available from Ontario Ministry of Environment, Conservation and Parks (OMECP; Gewurtz et al. 2011).

Given the remote location of QPP in Ontario and tight restrictions on use of motorized watercraft within in the park, Quetico lakes are subjected to lower anthropogenic pressures than most other waterbodies in Ontario. Lakes Namakan, Sandpoint, and Lac La Croix are not within Quetico Provincial Park, but are afforded some protections as they border the Boundary Waters Canoe Area (BWCA) and Superior National Forest to the South. The BWCA was established in 1964 and is directly adjacent to QPP. The BWCA has a specific wilderness management goal in place to reduce disturbances to the land, which includes required travel plans for all groups, a limit on group sizes, restricted motorized watercraft permissions, designated entry points, and managed campsites to prevent unauthorized clearing of new sites (Cole 2016). Additionally, Lac la Croix is a First Nation community (LLFN), and the fishery there is protected and maintained by traditional governance practices. Although Lac La Croix is not within the boundary of QPP, there is a partnership between QPP and LLFN, and Lac La Croix is included in the Quetico Fisheries Stewardship Plan (Sullivan 2006).

Rainy Lake supports a popular Walleye fishery in both Canada and the United States (Talmage and Staples 2011), and the presence of commercial fisheries in this system has caused significant historical declines in Walleye abundance (Chevalier 1977). Generally, commercial fishing has significant effects on fish populations. Size selectivity of commercial harvest can result in earlier age-of-maturity and decreased recruitment (Heino 1998) as well as reduced abundance (Hsieh et al. 2010). An ongoing effort exists to enforce strict management of angling and commercial harvest in the Rainy Lake fishery (Gangl and Pereria 2003). Intensive fishing can also reduce competition and results in improved growth efficiency resulting in lower rates of accumulation of Hg (Verta 1990). Rainy Lake is also not located within QPP or the BWCA, and therefore not afforded the same protections from human disturbance. Due to the large size and complex structure of the Rainy Lake complex, Red Gut Bay, North Arm, and South Arm of Rainy Lake were treated as separate waterbodies in the current analysis (Table 2.3).

#### 2.3.2 Tissue Collection and Mercury analysis

Historical fish samples were collected as raw muscle fillets as part of the OMECP Fish Contaminant Monitoring Program (Gewurtz et al. 2011). To supplement historical data, fish were sampled during 2021-22 following the Broad-scale Monitoring (BsM) procedures and protocols (Sandstrom et al. 2013) with several key modifications. To mitigate potential mortality to bycatch, four single-gang NA1 gillnets were used, which are designed for catching fish ≥20cm (Sandstorm et al. 2013). Each gang has 8 panels of mesh sizes increasing 13mm per panel from the smallest (38mm) to the largest mesh (127mm). Up to 4 nets were fished simultaneously for a duration of approximately 3 hours, up to 4 times daily. Between lakes, nets and gear were washed with chlorinated water and dried out between 5-10 days to prevent the spread of invasive species. The target sample size for each lake was 20 Walleye from across as wide a size range of fish as possible to attempt to describe the relationship most accurately between [MeHg] and

fish size (fork length) within each lake. Collection of Walleye tissue samples for mercury analysis was conducted in accordance with the Protocol for the Collection of Sport Fish for Contaminant Analyses section of the BsM procedures and protocols (Sandstrom et al. 2013). Briefly, tissues were collected as skinless boneless fillets from a region of the epaxial musculature on the left side of the fish, above the lateral line and near the insertion of the dorsal fin. Samples were labeled and kept on ice in an insulated cooler for a minimum of 1 and a maximum of 4 days until they could be placed in a -20°C freezer at Lakehead University. All samples were kept frozen until mercury analysis was conducted.

Fish tissue [MeHg] was measured using a Direct Mercury Analyzer (DMA-80 Milestone). The DMA does not differentiate between different inorganic Hg vs organic MeHg, however, >95% of mercury in predatory fish is composed of methylmercury (Bloom 1992, Porcella 1994, Becker and Bingham 1995, Lescord et al 2018). As such, Hg measured on the DMA was interpreted as a good estimate of Walleye MeHg. All samples were run following EPA method 7473 SW-846 (Environmental Protection Agency, 2003). Samples of fish muscle tissue were weighed and analyzed as wet samples (raw tissue). Duplicate samples were run every fifth sample to determine the repeatability of results. If duplicate samples within a run exceeded an average percent difference of greater than 10%, then the run was re-analyzed. The standard reference material (SRM) TORT-3 was analyzed approximately 6 times on each run to indicate the ongoing accuracy and precision of the DMA. The SRM has a certified range for [Hg] of 0.292  $\pm$ 0.022 ppm (~7.5%). If the SRM within a run showed evidence of drift (SRM [Hg] deviating in a consistent direction across a run of a magnitude greater than 5%), then all

samples on the run were reanalyzed. Across all runs, duplicate samples of Walleye tissue had a mean percent difference of 5.9% and were not significantly different (Paired *t*-test,  $t_{59} = -0.717$ , p = 0.48). The average of SRM concentrations for each run was within the acceptable range, with the overall average of 0.277 ppm (± 0.001).

# 2.3.3 Spatial Analysis: Comparison of [MeHg] scaling with body size between Uninvaded vs Invaded Lakes.

To determine if invasive *Bythotrephes* were associated with differences in Walleye tissue [Hg] between lakes with and without *Bythotrephes* present, I compared the relationship between Walleye [Hg] and size (Log10 fork length, mm) across lakes with different invasion status (invaded or non-invaded) using linear mixed effects models. Fish from invaded lakes were only those collected after *Bythotrephes* were established within each lake, while uninvaded lake data included only those data from sampling years after *Bythotrephes* were first detected in QPP (2003; Table 2.1). *Bythotrephes* invasion status was included as a fixed categorical predictor variable (invaded or not). Additionally, lake size (continuous variable) was included as a fixed effect as it was one of the only physical variables available to be tested for all the lakes in this study. Lake size and fish length were Z-score standardized prior to analysis. Each lake was included as a random effect to control for additional spatial variation not included in the model (e.g., effects of contiguous wetlands, forest fire history, and pH on fish Hg).

Several models were constructed, varying in complexity. The full model with all fixed effects (Eq 2.1) was first evaluated to optimize random effects as either random intercepts (Eq 2.2) or a random intercepts and slopes with fish length (Eq 2.3). Random

effects were optimized by comparing models with log likelihood ratio tests and determining which explained a greater proportion of variance.

$$Log_{10}[Hg] \sim 1 + Lake size + fork length*Invasion Eq 2.1$$

$$Log_{10}[Hg] \sim 1 + Lake size + fork length*Invasion, random = 1 | Lake Eq 2.2$$

$$Log_{10}[Hg] \sim 1 + Lake size + fork length*Invasion, random = 1 + fork length | Lake Eq 2.3$$

After optimizing random effects, models were compared using log likelihood ratio tests to determine the significance of fixed effects. The significance of a fixed effect was determined by comparing a model with a specific effect to another model with all the same interactions and effects, except for the effect of interest (e.g, Table 2.4). If the comparison was significant, then the fixed effect was retained in the model, but if the comparison was not significant then it was dropped from further comparisons.

# 2.3.4 Temporal Analysis: Comparisons between pre- and post-invasion size-[Hg] in *Walleye*.

To determine if the relationship between Walleye tissue [Hg] and body size changed in lakes after *Bythotrephes* invasion relative to uninvaded lakes, I selected lakes invaded by *Bythotrephes* with available data both prior to and after reported invasion (Table 2.2). Temporal changes in lakes not invaded by *Bythotrephes* were included as a reference to help evaluate/quantify temporal changes in fish [Hg] not associated with *Bythotrephes*. The year 2009, which was estimated as the median *Bythotrephes* invasion year of all invaded lakes in QPP, was selected as the cut-off to differentiate "historical" and "contemporary" dates for uninvaded reference lakes. Analysis was limited to lakes where sufficient data had been collected over a broad temporal range, or on either side of the estimated invasion year for that lake (or for 2009 in uninvaded lakes). "Time-Status" was used to differentiate between the "historical" and "contemporary" time periods for both invaded and uninvaded lakes (Table 2.2). The fixed effect "Time-Status" had 4 levels: 1) historical uninvaded, 2) contemporary uninvaded, 3) historical invaded, 4) contemporary invaded. The random effects in this analysis were Lake and year, which was concatenated into "LakeYear" to differentiate lakes with data collected in the same years. For example, data was collected in 1985 in both Agnes (Lake 1) and Robinson (Lake 8). The data was organized so the model treated those years as unique integers: 11985 (Agnes) and 81985 (Robinson) but still ordered them with respect to invasion status.

Three models of varying complexity were constructed (Eq 2.6-2.8) to evaluate temporal changes in Walleye Hg. The random effects were optimized by comparing random slopes (Eq 2.8) against random intercepts (Eq 2.7) and then random intercepts against fixed effects only (Eq 2.6) to determine which model explained a greater proportion of variance with log likelihood ratio tests.

$$Log_{10}[Hg] \sim 1 + fork length *Time-Status$$
 Eq 2.6

$$Log_{10}[Hg] \sim 1 + fork length *Time-Status, random = ~1 | LakeYear Eq 2.7$$

 $Log_{10}[Hg] \sim 1 + fork length*Time-Status, random = ~1 + fork length | LakeYear Eq 2.8$ 

Fixed effects were optimized through model comparisons made with log likelihood tests (Table 2.5). The significance of an effect was determined by excluding it from one model in the comparison; if the result was significant then the effect was retained, if the result was not significant, then the effect was dropped in further comparisons. Rainy Lake temporal analysis followed the same model selection criteria with each bay of the lake being treated individually as "Lakes" (Table 2.6) with the same tests for fixed and random effects as the QPP temporal analysis. I checked assumptions for all three linear analyses in this study and found that residuals were normally distributed, and variance was homogenous.

Differences in Walleye [MeHg] between invaded and uninvaded (spatial and temporal comparisons) were calculated with the formula for percent difference:

Percent Difference = 
$$\frac{(A-B)}{(A+B)/2}$$
\*100 Eq 2.13

Where A is the MeHg concentration of an uninvaded lake Walleye for a given size (mm) and B is the MeHg concentration of an invaded lake Walleye of the same size.

#### 2.4 Results

#### 2.4.1 Spatial Analysis of QPP and Boundary Water Lakes

I found that the inclusion of random slopes explained a significantly greater proportion of variance compared to the model with only random intercepts (LLR test, LLR = 22.601, df = 9,7, p < 0.0001). Further testing revealed the inclusion of 3-way interaction between lake surface area (Ha), Walleye size (fork length in mm), and *Bythotrephes* invasion explained more variance in Walleye [Hg] than a model with singular fixed effects and all possible 2-way interactions (LLR test, LLR = 7.576, df= 12,11, p = 0.003; Figure 2.2).

Across lake sizes, the slopes of Walleye Hg accumulation with body size differed significantly when comparing across *Bythotrephes* invasion classes. To better

demonstrate how this interaction altered Hg in Walleye between lake invasion status, I estimated slopes and intercepts for the Walleye fork length-[Hg] relationship for large (~10000 Ha: maximum lake size), medium (~5000 Ha: median lake size) and small (~160 Ha: minimum lake size) sized invaded lakes in our dataset (Figure 2.2). Across lake size classes Walleye were compared between invasion class at multiple (fork length) size intervals (Table 2.7). These size intervals were selected because 200mm is a rough size distribution of age 0 Walleye by the end of their first growing season (Neumann and Allen 2007) and fishing restrictions allow the harvest of only one Walleye greater than 460mm in many Northwestern Ontario Fisheries Management Zones (Including Quetico) and not more than one greater than 530mm in other Zones (Ontario Fishing Regulations Summary 2023). Across comparable fish size classes (200-400 mm), Walleye [MeHg] was greater in invaded lakes than noninvaded lakes, with the percent difference between invaded and uninvaded lakes generally increasing with lake size. Walleye [MeHg] was lower in uninvaded than invaded lakes for comparable fish size classes up to approximately 500 mm FL where the percent difference was more similar across lake size classes before reversing for larger-sized fish (Table 2.7).

#### 2.4.2 Temporal Analysis of QPP Lakes

Optimization of random effects revealed that a model with random intercepts and slopes explained significantly more variance in Walleye [MeHg] than a model with only random intercepts (LLR test, *LLR* =65.875, *df* =12,10, p < 0.0001). Optimization of fixed effects revealed that the interaction between Time-Status (before/after *Bythotrephes* Invasion, or historical and contemporary periods for uninvaded lakes) and Walleye fork length was not significant (LLR test, *LLR* =0.861, *df*=12,9, p = 0.417). However, Time-

Status was significant as an additive fixed effect and explained more variance in [MeHg] than a model with only fork length (LLR test, LLR = 13.078, df = 9,6, p = 0.0022; Figure 2.3). Results of the additive (common slope) model indicated that Walleye [MeHg]-at-length did not differ significantly between historical uninvaded lakes and contemporary uninvaded lakes (p = 0.9494). Both uninvaded lake categories (historical and contemporary) differed significantly from lakes invaded by *Bythotrephes*, both prior to invasion (p = 0.0224) and post-invasion (p = 0.0036), with higher Walleye Hg concentrations in invaded lakes compared to uninvaded lakes across all time periods. Though visual inspection suggested that post-invasion mercury concentrations of Walleye were slightly elevated (increased vertical offset) relative to pre-invasion (Figure 2.3), a comparison of pre-and post invasion groups for invaded lakes showed that the difference was not statistically significant (p = 0.2503).

#### 2.4.3 Temporal Analysis of Rainy Lake

The optimization of random effects found that inclusion of "LakeYear" as random intercepts and slopes was no better than a random intercepts model (LLR test, LLR = 4.295, df = 8,6, p = 0.0584). The model with random intercepts explained more variance than a model with just fixed effects therefore I selected the model where "LakeYear" was treated as a random intercept: (LLR test, LLR = 473.841, df = 6,5, p < 0.0001). The optimization of fixed effects revealed that inclusion of "Time-Status" as an interaction with fork length explained significantly more variance than as an additive fixed effect (LLR test, LLR = 3.768, df = 6,5, p = 0.0025), such that the slope of Hg accumulation with fish size was shallower after the invasion of *Bythotrephes* compared to before

invasion, but accompanied by an increase in intercept (Figure 2.4). Very similar to the spatial comparison among QPP lakes, [MeHg] among smaller Walleye was greater after *Bythotrephes* invaded but was roughly equivalent [Hg] in larger Walleye (500-650mm; Table 2.8). Above a size threshold of 650mm, pre-invasion Walleye appear to have greater [MeHg]-at-length than those sampled after the invasion of *Bythotrephes*.

#### 2.5 Discussion

Through two analytical approaches and 3 data sets, I have demonstrated what appears to be a general pattern of elevated Walleye Hg associated with *Bythotrephes* invasions. Comparisons of invaded and uninvaded lakes in QPP using contemporary data (arguably my most robust dataset, representing the greatest number of lakes and the greatest number of observations of Walleye Hg) revealed that [MeHg] in small Walleye from large *Bythotrephes* invaded lakes was significantly higher than [MeHg] of similar sized fish in comparable uninvaded lakes. I also observed that as lake size decreases, so does the difference in Walleye [MeHg] between invaded and uninvaded lakes. Temporal analysis of Rainy Lake Walleye revealed a very similar pattern; [MeHg] in smaller sized Walleye was elevated following Bythotrephes invasion compared to before invasion. Though my temporal analysis of QPP did not show a clear or significant effect of Bythotrephes invasion, Walleye [MeHg] appeared visually to be greater in after Bythotrephes invasion than reported previously, though the pattern was not significant. The sum of evidence among the trends observed in the study suggests that the presence of Bythotrephes may cause increases in [MeHg]-at-length for smaller Walleye, and critically, in Walleye of typical harvested size by anglers for consumption. Creel surveys show that Walleye caught and retained by anglers are generally in the 3- to 6-year age range, with fork lengths between 250-350mm (Patterson and Sullivan 1998, VanDeValk

et al. 2002), this is the size range where the greatest differences between invaded and uninvaded lakes were observed in this study, with Walleye Hg concentrations 17-170% greater in invaded lakes than in non-invaded medium and large-sized lakes and a magnitude of 8-26% in Rainy Lake.

Differences in Walleye [Hg] between invaded and uninvaded systems suggest (1) that fish with greater Hg-at-length may be exposed to higher Hg concentrations, which in fish is primarily through prey consumption (and therefore consuming prey with higher [MeHg]) and/or that (2) that Walleye from invaded lakes may have lower growth efficiency (conversion efficiency) than Walleye from uninvaded systems, due to either higher foraging costs or reduced prey energy densities (Trudel et al. 2006, Rowan et al. 1998, Pazzia et al. 2002). Changes in growth efficiency and activity are known to occur as fish shift foraging strategies from one trophic position to another (Sherwood et al. 2002). Similarly, as Walleye mature, they switch trophic positions from a planktivorous diet when young, to a predatory piscivorous diet later in life (Uphoff et al. 2019). The presence of *Bythotrephes* may impact Walleye before and after their diet switch, by increasing competition for planktivorous YOY and larval Walleye causing reductions in growth efficiency and total length (Gretchen et al. 2020; Gartshore and Rennie 2023). These impacts may alter Walleye growth trajectories and potentially reduce winter survival (Gartshore and Rennie 2023). The surviving Walleye could be subject to potential disadvantages, as early life stressors have been shown to affect fish physiology and biometrics later in life, including juvenile growth rates and adult size (Jonsson and Jonsson 2014). Another mechanism that could be driving the elevation in Walleye [MeHg] is increased [MeHg] of prey fish. While the trophic elevation theory would

suggest this, [MeHg] is significantly greater only in smaller Walleye suggesting [MeHg] may be higher only for juvenile Walleye prey. One reason for elevated Hg in small Walleye and not large Walleye could be increased ecosystem biomagnification of invaded pelagic communities with higher densities of active predatory zooplankton, which have higher concentrations of Hg relative to filter feeding species (Long et al. 2018, Poste et al. 2019).

I believe that a combination of early life competition, altered growth trajectories, as well as access to growth efficient Cisco can account for the results observed in this spatial analysis as well as the results of the Rainy Lake temporal analysis, a very large lake with both Cisco and Rainbow smelt. In this study, invaded large lakes were characterized by greater [MeHg] in small Walleye, but a lower rate of Hg accumulation. This contrasted with uninvaded lakes which were characterized by with lower [MeHg] at small sizes but a greater rate of accumulation. To explain these results, I believe that Bythotrephes have different effects on Walleye at different stages of life when they occupy different trophic positions. Within invaded lakes, evidence suggests that zooplanktivorous YOY Walleye compete with *Bythotrephes*, causing a decline in growth rate and body size (Hansen at al. 2020, Gartshore and Rennie 2023). Once Walleye grow and their diet begins to shift from zooplankton to larger prey (Benthic invertebrates and later fish) they are no longer in direct competition with Bythotrephes but may be on a shallower growth trajectory later in life as result of early life stressors (Sherwood et al. 2002). While this altered growth trajectory would suggest increased Hg in larger fish, the opposite is observed. I believe this is partially a result of the consumption of a prominent prey fish, Cisco (Coregonus artedii). Cisco have been found to favour consuming

*Bythotrephes* when present (Gatch et al. 2021, Martin et al. 2023). Additionally, Cisco seem to benefit from the presence of *Bythotrephes*, where Cisco growth efficiency improved relative to refence conditions resulting in lower Hg burdens (Rennie et al. 2023 in review). If Walleye are feeding on faster growing Cisco with lower Hg burdens (than Cisco not feeding on *Bythotrephes*), then they will also have reduced Hg-at-length, but critically, only when they are large enough to consume them. The presence of Cisco is positively correlated with lake surface area, and they are more likely to be absent or in low abundance in smaller shallower lakes (Kennedy et al. 2018), which may explain why I observe a more drastic relationship of Hg accumulation within larger lakes.

The temporal analyses conducted in this study faced limitations regarding historical data availability but also yielded results to provide some support for the results of the spatial analysis. The temporal analysis was conducted separately on the 3 main bays of Rainy Lake and across 10 QPP lakes. The results of the Rainy Lake temporal analysis showed an effect of *Bythotrephes* invasions in Hg accumulation between preand post-invasion years that was remarkably consistent with the results of the spatial comparison, where Walleye accumulated Hg at a greater rate with size in pre-invasion years but had lower [MeHg] at smaller sizes. I observed a significant increase in Walleye [MeHg] at smaller sizes (<50cm) in post-invasion Walleye compared to pre-invasion. By contrast, the temporal analysis of QPP lakes found no differences within invaded lakes, or within uninvaded lakes over time. However, Walleye [MeHg] in uninvaded lakes was significantly lower than in invaded lakes at any point in time. The lack of change in Hg accumulation within the temporal reference (uninvaded) lakes was surprising given that deposition has been decreasing globally since the 1970s (Engstrom et al. 1997) and

declines in deposition should be detectable over even shorter time scales than examined in this study (Blanchfield et al. 2022). I expected to see a temporal decline in Walleye Hg accumulation in uninvaded lakes however, this study is not the first to report stagnant levels in fish [MeHg] over time despite declines in atmospheric deposition (Tang et al 2013, Gandhi et al. 2014). The sample sizes of lakes for the QPP temporal analysis were a limitation in this study; compared to the spatial analysis which utilized 31 lakes, the temporal dataset had only 10 lakes with data both before and after invasion, providing less statistical power with which to detect effects of *Bythotrephes* on Walleye [MeHg]. In addition to a smaller dataset of waterbodies, the number of Walleye collected per time period was also limited; sample sizes of Walleye for both invaded (Namakan, Lac la Croix, Sandpoint, Little Vermillion) and uninvaded lakes (Knife, Agnes, Birch) were either not balanced across time periods, or were just very small (Table 2.2). As my historical sample sizes were often very small for individual lakes, the slope relationships were more vulnerable to outliers and likely a less robust representation of the Walleye population at that time.

Uncertainty in estimated invasion dates could also have contributed to my inability to detect temporal changes in Walleye Hg. The QPP surveys for *Bythotrephes* are not conducted yearly, and many lakes have not been surveyed since 2009. *Bythotrephes* invasions are recorded as the year they were first detected by humans, which may underestimate their true invasion date. Even with continuous zooplankton surveys, *Bythotrephes* presence may not be identified immediately. For example, continuous estimates of Cladocera zooplankton richness in Harp Lake were observed to decline in 1990 but *Bythotrephes* were not detected there until 1993, suggesting that
*Bythotrephes* may impact the ecosystem at very low densities before they are identified by researchers (Yan et al. 2002). This combination of infrequent sampling and lag time in identification of invasions would impact comparisons of Hg accumulation, specifically regarding fish that are old enough to have lived in a lake both before and after the arrival of *Bythotrephes*. In this study, invasion was simply defined as the year *Bythotrephes* were first detected, which as previously outlined may not be entirely accurate. Thus, the muted effect of *Bythotrephes* in my QPP temporal analysis may be further hindered by the frequency of sampling on either side of invasion, in that *Bythotrephes* may have been present in some years identified as "pre-invasion" samples, or that old Walleye (15 years +) were spawned in "pre" years but were not collected until "post" years. By contrast, I don't believe this to be an issue in my Rainy Lake comparison given that a greater frequency of sampling of Walleye [Hg] has been conducted on either side of estimated invasion dates as well as larger sample sizes in the Rainy Lake complex generally (Table 2.3).

Common to all three datasets was the strong relationship of length and [Hg] regardless of any effect of *Bythotrephes*, which is consistent with the literature (Storelli et al. 2007, Lescord et al. 2018) and should still be considered the most important factor when selecting fish for human consumption. However, my results suggest that resource managers (and anglers themselves) may want to carefully consider the potential of *Bythotrephes* infestation in lakes, and the potential consequences on Hg levels in fish harvested from those lakes. I suggest increased monitoring of fish [Hg] for lakes following *Bythotrephes* establishment to determine if consumption advisories should be adjusted in the best interests of human health. I also suggest that further testing for fish

[MeHg] be done on a wider scale looking at additional species (Cisco, Yellow perch) that are commonly consumed by anglers.

I considered two offshore species as covariates for the spatial model but could not test for their effects given their distribution among my lakes included in the analysis. The first species, Cisco, have the potential to impact both Walleye growth efficiency and Bythotrephes populations (Kaufman et al. 2006, Martin et al. 2023). However, Cisco had a cosmopolitan distribution among the lakes included in this survey, in almost every lake in my dataset (Table 2.1). One issue with this variable is that prey species data in my dataset was recorded as presence or absence and not catch per unit effort (CPUE). However, it is not just the presence of Cisco, but their abundance that might be expected to alter growth efficiency and foraging behaviour of Walleye (Kaufman et al. 2006). Additionally, I would expect Cisco CPUE to increase with lake size (Kennedy et al. 2018) but unfortunately did not have the data to test this effect. While the presence of Rainbow smelt can alter Walleye diet composition (Sheppard et al. 2015), they were only present in a few lakes and reconstructions of QPP Walleye diets revealed only minor differences in energy density when smelt were available (Chapter 3). Additionally, attempts to investigate the role of rainbow smelt on Hg accumulation have found that they do not strongly influence [MeHg] in piscivorous fishes (Johnston et al. 2003).

Other variables that were considered as covariates but could not be tested were effects associated with climate including Growing Degree Day (GDD), annual precipitation, and changes in annual water level/flooding. GDD (species specific value of thermal energy available for growth) influences immature Walleye growth rates (Venturelli et al. 2010), increased precipitation is linked to increased Hg deposition

(Kamman et al. 2004a), and water level/flooding can influence sediment deposition, which contains Hg (Kamman et al. 2004b). While I couldn't test for these as fixed effects explicitly (given gaps in historical data), they were included as random effects within "LakeYear" which accounted for lake-specific annual variation.

In summary, the sum of evidence I collected points toward a potential pattern where *Bythotrephes* invasions increased Hg levels in harvestable size Walleye, suggesting invasion should be of concern to both Walleye anglers who consume their catch and managers of lakes either recently invaded or at risk of invasion where Walleye consumption is common. My findings suggest smaller Walleye are impacted more heavily by invasive *Bythotrephes* than larger Walleye but that the magnitude of this impact is directly related to lake surface area. For future studies, I highlight the importance of more frequent Hg monitoring of Walleye from recently invaded lakes to better understand post invasion conditions and to capture potential shifts in small Walleye Hg dynamics year-to-year. I also suggest targeting smaller Walleye for Hg analysis (as Walleye <50 cm appear to be more significantly impacted than those >50 cm) to provide better baseline of Walleye Hg data from across all QPP lakes to help better understand the potential for increased Hg exposure following *Bythotrephes* invasions.

## 2.6 Tables

**Table 2.1**. Locations used to evaluate Walleye Hg bioaccumulation between *Bythotrephes*-invaded and uninvaded lakes. Abbreviations are QPP: Quetico Provincial Park, RRWS: Rainy River watershed, BWCA: Boundary waters Canoe Area, C = Cisco, RS = Rainbow smelt. N = Number of Walleye included in the analysis. Asterisk\* indicates the presence of *Bythotrephes*.

Waterbody	Location	Surface	Mean Depth	Max Donth (m)	Invasion	Survey Years	Offshore	N
Name		Area (Ha)	(m)	Max Deptil (III)	Year	(Spatial)	Prey	18
French*	QPP	284	12.5	26	2009	2021, 2022	RS+C	55
Iron*	QPP/BWCA	190	6.26	18	2018	2018	С	8
Kawnipi*	QPP	4488	12	40	2003	2021	-	38
Lac La Croix*	RRWS/BWCA	13774	34	51.2	2010	2017	RS+C	10
Little Vermillion*	RRWS/BWCA	-	-	-	1995	1996, 2001, 2010, 2016	С	21
Namakan*	RRWS	10100	13.6	45.7	1995	1996, 2015,	С	12
Pickerel*	QPP	5754	17.7	74.7	2008	2009, 2015, 2021, 2022	RS+C	70
Poohbah*	QPP	1530	16.1	70	2010	2010, 2016	С	22
Saganagons*	QPP	2470	6.9	31.3	2003	2021, 2022	RS+C	53
Sandpoint*	RRWS	3440	-	56	1995	1996, 2013	С	13
Agnes	QPP	2982	19.6	79.3	-	2010, 2017, 2022	С	39
Batchewaung	QPP	1274	19.4	55	-	2021, 2022	RS+C	60
Beaverhouse	QPP	1958	22.3	64.7	-	2010, 2016, 2022	С	32
Birch	QPP/BWCA	174	3.9	10	-	2005, 2015	-	14
Burt	QPP	736	22.4	75.1	-	2010, 2016	С	30
Camel	QPP	233	9.6	33	-	2017	С	10
Cirrus	QPP	2115	21.8	88.6	-	2010, 2017	С	18

Waterbody	Location	Surface Area	Mean Depth	Max Depth	Invasion	Survey Years	Offshore	N
Name		(Ha)	(m)	(m)	Year	(Spatial)	Prey	19
Conmee	QPP	531	6	21	-	2017	С	10
Jean	QPP	1335	17	77.6	-	2010, 2016	С	30
Kashahpiwi	QPP	549	18.2	85.7	-	2010, 2017	С	31
Knife	QPP/BWCA	608	15.43	54.56	-	1993, 2017	С	5
Mack	QPP	726	7	31	-	2017	С	10
McAree	QPP	879	12.7	37.2	-	2016	С	9
Minn	QPP	479	5.9	39.6	-	2016, 2022	С	38
Olifaunt	QPP	561	12.9	39.4	-	2011	RS+C	20
Other Man	QPP	176	10.9	32.6	-	2010	-	7
Robinson	QPP	421	12.7	35.1	-	2021, 2022	С	55
Sarah	QPP	956	16.8	50.9	-	2010	С	8
This Man	QPP	318	11.7	39.3	-	2010, 2016	-	15
Wolseley	QPP	1307	12.6	40	-	2016	С	10
Your	QPP	164	5.1	20.8	-	2010, 2016	С	30

Table 2.1. Continued.

**Table 2.2.** Lakes used to evaluate temporal changes in Walleye [MeHg] in *Bythotrephes*-invaded (\*) and non-invaded lakes. All uninvaded lakes use 2009 as a common "invasion year" for comparisons, which is the median of all detection dates of *Bythotrephes* across the dataset of invaded lakes. N = sample size of Walleye collected per time period. Note: All lakes also appear in Table 2.1, but with a truncated time period to facilitate spatial comparisons (see text).

Waterbody Name	ame Invasion Pre years Year		N (Pre)	Post years	N (Post)
Pickerel*	2008	1977, 1991, 1999	72	2009, 2015, 2021, 2022	55
Namakan*	1995	1990, 1995,	50	1996, 2010, 2015	18
Poohbah*	2010	2010	15	2016	7
Sandpoint*	1995	1977, 1982, 1990, 1995	36	1996, 2013	13
Lac La Croix*	2010	1984, 1994, 1999, 2000, 2003, 2004	48	2017	10
Little Vermillion*	1995	1991	9	1996, 2001, 2016	16
Agnes	-	1985	15	2010, 2017, 2022	39
Robinson	-	1985	27	2021, 2022	55
Birch	-	1976, 1984, 1990, 1991, 2005	28	2015	8
Knife	-	1993	3	2017	5

**Table 2.3**. Dataset used to analyze temporal trends in Walleye Hg accumulation in the Rainy Lake complex. Physical characteristics, *Bythotrephes* invasion dates, samples sizes and years for Rainy Lake bays included within the temporal analysis. N = sample size of Walleye collected per time period.

Waterbody name	Surface Area (Ha)	Mean Depth (m)	Max Depth (m)	Invasion Year	Pre years	N (Pre)	Post years	N (Post)
Rainy-Red Gut Bay	8,320	6.89	31	2006	1983, 2003	50	2008, 2016, 2018	76
Rainy-North Arm	34,570	7.96	41	2006	1986, 1990, 1993, 1999, 2000	100	2007, 2015, 2018	74
Rainy-South Arm	27,260	11.5	49	2006	1978, 1983, 1990, 1999	117	2009, 2017, 2018	64

**Table 2.4.** Models used to evaluate and optimize individual fixed effects for the spatial comparison of size-Hg relationship in Walleye of Quetico provincial Park. Models are compared using log-likelihood ratio tests to determine the significance of the effect excluded from the comparison.

Effect being	Model Comparison	
tested		
Interaction of	Log <sub>10</sub> [Hg] ~ Lake size*Invasion* fork length + Lake size*	2.4
Lake size,	Invasion + fork length * Invasion + Lake size* fork length +	
Invesion	Invasion + fork length + Lake size, random = fork length	
mvasion,	Lake	
Fork length		
	$Log_{10}$ [Hg] ~ Lake size * Invasion + fork length * Invasion +	2.5
	Lake size * fork length + Invasion + fork length + Lake size,	
	random = fork length   Lake	

**Table 2.5.** Models that were used to evaluate and optimize individual fixed effects for the temporal comparisons of size-Hg relationship in Quetico provincial Park. Models are compared using log-likelihood ratio test to determine the significance of the fixed effect excluded from the comparison.

Effect being	Model Comparison	Eq
tested		
Fork	$Log_{10}$ [Hg] ~ 1 + fork length * Time, random = ~1 + fork length	2.8
length*	LakeYear	
Time		
	$Log_{10}$ [Hg] ~ 1 + fork length + Time, random = ~1 + fork	2.9
	length   LakeYear	
Time	$Log_{10}$ [Hg] ~ 1 + fork length + Time, random = ~1 + fork	2.9
	length   LakeYear	
	$Log_{10}$ [Hg] ~ 1 + fork length, random = ~1 + fork length	2.10
	LakeYear	

**Table 2.6**. Models used to evaluate and optimize individual fixed effects for the temporal comparisons of size-Hg relationship for Rainy Lake. Models are compared using log-likelihood ratio test to determine the significance of the fixed effect excluded from the comparison.

Effect being	Model Comparison	Eq
tested		
Fork length* Time	Log <sub>10</sub> [Hg] ~ 1 + fork length * Time, random = ~1   LakeYear	2.7
	Log <sub>10</sub> [Hg] ~ 1 + fork length + Time, random = ~1   LakeYear	2.11
Time	$Log_{10}$ [Hg] ~ 1 + fork length + Time, random = ~1   LakeYear	2.11
	$Log_{10}$ [Hg] ~ 1 + fork length, random = ~1   LakeYear	2.12

**Table 2.7.** Differences in Walleye [MeHg] ppm organized by lake category, invasion class, and Walleye length from lakes in the QPP spatial analysis. **Percent difference** (Bolded rows) indicate the difference in [MeHg] ppm between invaded lake Walleye and uninvaded lake Walleye of the same size and lake category. Bolded values calculated as the percent difference between the two not bolded values in the rows directly above said bolded value (Eq 2.13). Large lakes (~10000 Ha), Medium lakes (~5000 Ha), Small lakes (~160 Ha).

Lake Category			Length (mm)		
	200	300	400	500	600
Large Uninvaded	0.01	0.036	0.145	0.589	2.39
Large Invaded	0.128	0.226	0.398	0.7	1.23
Large Lake (% Difference)	171.0	145.0	93.2	17.2	-64.1
Medium Uninvaded	0.044	0.11	0.276	0.695	1.75
Medium Invaded	0.173	0.292	0.492	0.831	1.41
Medium Lake (% Difference)	119.6	90.5	56.3	17.8	-21.5
Small Uninvaded	0.214	0.335	0.524	0.82	1.28
Small Invaded	0.233	0.376	0.608	0.982	1.588
Small Lake (% Difference)	8.5	11.5	14.8	18.0	21.5

**Table 2.8**. Differences in Walleye [MeHg] ppm organized by invasion category, and Walleye length from the Rainy Lake Temporal analysis. **Percent difference** (Bolded rows) indicates the difference in [MeHg] ppm between pre-invasion Walleye and post-invasion Walleye of the same size. Bolded values calculated as the percent difference between the two not bolded values in the rows directly above said bolded value (Eq 2.13).

Invasion Category			Length (mm)			
	200	300	400	500	600	700
Pre-Invasion	0.129	0.204	0.324	0.513	0.813	1.288
Post invasion	0.168	0.251	0.374	0.558	0.834	1.245
Percent Difference	26.3	20.4	14.5	8.5	2.5	-3.5

## 2.7 Figures



**Figure 2.1.** Map of thesis study area in northwestern Ontario and northern Minnesota for both the spatial and temporal comparisons (QPP and Rainy Lake). Invaded lakes are defined by the established presence of *Bythotrephes*.



**Figure 2.2.** 3-way interaction between Walleye fork length (mm, z-score standardized), Lake Surface Area (Ha, z-score standardized) and *Bythotrephes* invasion status with Log<sub>10</sub> [Hg] as the response variable. Gradient color emphasizes predicted level of response variable (Log<sub>10</sub> [Hg]). Panels represent spatial comparison between the bioaccumulation relationship of invaded lake Walleye (left) and uninvaded lake Walleye (right).



**Figure 2.3.** Temporal slopes of Hg accumulation with body size for Walleye in northwestern Ontario common slope model. Invaded lakes pre- and post- designations selected as years on either side of when *Bythotrephes* were first detected. Reference (uninvaded) lake time periods designated with 2009 as the cut-off year. Each point represents an individual Walleye, color and shape indicate the Time-Status category of when it was sampled.



**Figure 2.4.** Temporal slopes of Hg accumulation (fork length-Hg) of Rainy Lake Walleye (Redgut Bay, North and South Arm) random slopes model. All sections of the lake were assumed invaded by *Bythotrephes* in 2006 which divides the years into pre and post invasion. Each point represents an individual Walleye, color and shape indicate the Time-Status category where it was sampled.

## Chapter 3: Impacts of Invasive Spiny Water Flea on Whole Ecosystem Biomagnification and Walleye Bioenergetics *3.1 Abstract*

Food chain elongation and increased contaminant concentrations are predicted to result from the invasion of mid-trophic species. However, several tests of this prediction have failed to find any evidence of this phenomenon. One hypothesis for the lack of increased contaminant concentration following mid-trophic invasion is that conversion efficiency increases and "dilutes" the effect of elongated food chain biomagnification. In this study, I test this hypothesis first through a whole-ecosystem analysis of biomagnification slopes in lakes with and without the spiny water flea (Bythotrephes cederströmii), and by examining bioenergetic-derived conversion efficiency of Walleye from these same lakes. Concentrations of methylmercury [MeHg] were regressed against stable isotopes of nitrogen as an indicator of trophic position to generate slopes of bioaccumulation, while a bioenergetics model paired with a mercury mass balance model was used to describe the daily activity budget and conversion efficiency of Walleye. Though  $\delta^{15}N$  of invaded lake Walleye was elevated, no increase in [MeHg] was observed, and food chains in invaded lakes were not elongated relative to uninvaded lakes. Bioenergetic analysis showed differences among individual lakes which did not appear to be associated with Bythotrephes invasion. Contrary to investigations on juvenile Walleye, I found little evidence to support trophic or bioenergetic impacts of *Bythotrephes* invasion on Walleye. Future analyses should attempt to refine this analysis with a larger suite of lakes to improve comparisons of invasion status.

Key Words: Bioenergetics, Biomagnification, Invasive Species, Methylmercury, Zooplankton

## 3.2 Introduction

Mercury (Hg) is both a naturally occurring and anthropogenically produced toxic heavy metal (Pirrone et al. 2010) that has severe negative effects on the health of humans and wildlife of many taxa (Yang et al. 2020). Sources of Hg include geological activity (Gustin 2003), fossil fuel combustion, and chemical manufacturing (Ajsuvakova et al. 2020, Pirrone et al. 2010, Kudo et al. 1998). Mercury from combustible sources is often emitted into the atmosphere where it can be transported and deposited in remote and previously pristine locations (Lodenius 1991, Pirrone et al. 2010). However, Hg containing emissions in North America have been reduced significantly over recent decades (Engstrom and Swain 1997, Diaz-Somoano et al. 2007).

Regardless of its initial source, when inorganic Hg enters an aquatic ecosystem the process of methylation occurs, by which inorganic Hg is processed by mostly sediment-dwelling bacteria which chemically alter inorganic Hg into methylmercury (Lavoie et al. 2013, Bringham et al. 2021) but it can also be methylated in the water column (Eckley and Hintelmann 2006). Organic methylmercury (MeHg) is both more bioavailable and more toxic than inorganic forms of Hg (Gilmour et al. 2013). Methylmercury is introduced to ecosystems at the lowest trophic level (autotrophs) and is transported through the food web through consumption of prey (Mathers and Johansen 1985, Hill et al. 1996, Kidd et al. 2011). As MeHg is transported up the food chain, biomagnification occurs, demonstrated by a general increase in consumer [MeHg] relative to its prey (Kidd et al. 2011, Gobas et al. 1999). Biomagnification is a consequence long lasting bioaccumulation (faster rates of dietary accumulation of MeHg relative to elimination rates in organisms) across multiple trophic levels with increasing [Hg] per trophic level (Mathers and Johansen 1985, (Kidd et al. 2011, Ajsuvakova et al.

2020). Therefore, organisms can have 2-4 times greater Hg concentrations than their prey (Watras et al. 1998), though a meta-analysis has indicated biomagnification of MeHg by up 15 times between adjacent trophic levels (Lavoie et al. 2013). Across an entire food chain, top level consumers can have [MeHg] many thousands of times greater than ambient environmental concentrations.

Biomagnification and bioaccumulation can be impacted by various environmental variables including water chemistry, waterbody type, and atmospheric Hg deposition (McMurtry et al. 1989, Kamman, et al. 2005, Harris et al. 2007, Sumner et al. 2020). Biological factors affecting [MeHg] include organism size and species (Sotrelli et al. 2006, Kamman et al. 2005), as well as variation in food chain length (Vander Zanden and Rasmussen 1996, Cabana et al. 1994). In a predatory fish (the Lake Trout, Salvelinus namaycush) higher concentrations of mercury were detected in naturally occurring lakes with longer food chains (Cabana et al. 1994). In these communities, the trophic position of Lake trout increased with food chain length due to the presence of mid-trophic organisms such as freshwater shrimp (Mysis diluviana) or pelagic forage fish (Cabana et al. 1994). If greater contaminant accumulation rates are associated with longer food chains, as suggested by these comparative studies, then the potential elongation of food chains by the addition of a mid-trophic invasive species might also be expected to increase contaminant concentrations of mercury (Swanson et al. 2003, Rennie et al. 2011). Attempts to test this theory directly have generally found no effect (Rennie et al. 2010, Hogan et al. 2007, Johnston et al. 2003) or have reported a decline in contaminant concentrations (Rennie et al. 2023 in review). One explanation for a failure to observe increased contaminant accumulation with elevated trophic position is an increase in

consumer growth efficiency associated with mid-trophic invasions (Rennie et al. 2011, Johnston et al. 2003, Swanson et al. 2003); if mid-trophic invasions permit more efficient foraging by consumers (e.g. reduced search times, increased prey size or energy densities), the energy obtained (and therefore somatic growth) per unit food item consumed increases, resulting in a reduction in Hg accumulation per unit food consumed (Trudel et al. 2006, Rowan et al. 1998, Pazzia et al. 2002). However, if the presence of an invasive species improves growth efficiency (negative impact on consumer Hg) while simultaneously elevating trophic position (positive impact on consumer Hg), these processes may counteract one another to yield either no net effect or unpredictable results on consumer Hg concentrations (Simoneau et al. 2005, Essington et al. 2011).

A mid-trophic invasive species that has been studied for its potential impacts on fish contaminant accumulation and growth rates is the Eurasian Spiny Water flea (*Bythotrephes cederströmii*). Spiny water flea (hereafter *Bythotrephes*) is a large predatory zooplankton with a voracious appetite. Following establishment, *Bythotrephes* can severely decrease the richness and diversity of native zooplankton species (Kerfoot et al. 2016, Yan et al. 2002). *Bythotrephes* effects on ecosystems extend past zooplankton, and it has been demonstrated that they have potential impacts on fish growth efficiency. In planktivorous Cisco, the presence of *Bythotrephes* was found to be associated with a decline in slope of biomagnification compared to uninvaded systems and these differences were attributed to increased rates of growth efficiency in lakes with *Bythotrephes* has been shown to negatively impact the growth rates of YOY Walleye and vellow perch (Hansen et al. 2020, Gartshore and Rennie 2023). While *Bythotrephes*  appear to have impacts on the growth and energetics of planktivorous juvenile fishes, their impacts on mature fish are less clear.

Walleye are an economically significant source of food and recreation in Canada, but especially within Ontario. In 2021 Ontario was the province with the greatest commercial landing (by mass) of Walleye. (Department of Fisheries and Oceans 2023), while recreational fishing contributed billions of dollars in revenue (Department of Fisheries and Oceans 2019). In Ontario, Indigenous communities' food security is heavily linked to availability of healthy fish populations (Marshuka et al. 2021). This study aims to directly test the hypothesis that a mid-trophic invasive species (*Bythotrephes*) cause food chain elongation through a whole ecosystem approach, and then attempt to understand if a systematic shift in conversion efficiency is occurring and interfering with biomagnification and accumulation of Hg in Walleye.

# Objectives

**Objective 1**: The first objective of this study was to measure the potential effect of invasive *Bythotrephes* on ecosystem biomagnification and food chain length in Quetico provincial park. In order to estimate ecosystem biomagnification slopes across QPP lakes I applied stable nitrogen isotope analysis and mixed effects modelling and compare slopes of ecosystem biomagnification between invaded and uninvaded lakes.

**Hypothesis 1**: I hypothesize that invasion of *Bythotrephes* will be associated with elongation of foods chain and increase trophic position of Walleye. Invaded lakes will have steeper slopes of ecosystem biomagnification compared to uninvaded lakes.

**Objective 2**: The second objective of this study was to measure the effects of invasive *Bythotrephes* on Walleye bioenergetics (growth efficiency). In order to measure these effects I parameterized a Bioenergetic + mercury mass Balance model for QPP Walleye populations integrating water temperature, Walleye age cohorts, and prey Hg.

**Hypothesis 2**: I hypothesize that lakes fish in lakes invaded by *Bythotrephes* will have lower growth efficiency compared to those in lakes without *Bythotrephes*, and therefore greater rates of Hg bioaccumulation.

#### 3.3 Methods

## 3.3.1: Study Area and Dataset.

The study was conducted in the lakes of Quetico Provincial Park (QPP). Quetico lakes are not subject to the same fishing or anthropogenic disturbances as other waterbodies in Ontario given their provincial park status. Lakes were selected for ecosystem biomagnification and bioenergetics analysis based on availability of Walleye age, Hg, and size data, accessed through the Ontario Ministry of Environment, Conservation and Parks (MECP) fish contaminants database, and the Broad-scale Monitoring (BsM) database maintained by the Ontario Ministry of Natural Resources and Forestry (MNRF) (Tables 3.1, 3.2). The MECP Fish Contaminant Monitoring Program (FCMP) analyzes contaminants (including Hg) in sportfish and has catalogued these data for over 40 years (Gewurtz et al. 2011). The MNRF maintains a database of fish lengths and ages from fish sampled in BsM surveys (Sandstrom et al. 2013). Data provided through these databases were used to supplement data collected in 2021 and 2022 to estimate bioenergetics model parameters (Table 3.1).

Fish collection during 2021 and 2022 adapted the methods outlined in the BsM procedures and protocols (Sandstrom et al. 2013). Modifications were made to better target a range of sizes of Walleye with the goal of increasing the probability of returning fish to the lake that were not needed and avoid large numbers of bycatch. Sampling consisted of setting 4 single-gang North American/ Large mesh gill nets for a duration of approximately 3 hours repeated a maximum of 4 times daily (12 sets). These nets target fish  $\geq$  20 cm in length with panels of differing mesh widths increasing by 13 mm per panel, with panels randomly placed along the gang (Sandstorm et al. 2013). When travelling to new lakes, gill nets and all other sampling gear were cleaned with chlorinated tap water and hung up to be air dried for up to a week to prevent the spread of invasive species. Sampling was targeted to obtain at least 20 individual Walleye per lake, with as wide a size range of fish as possible. Walleye tissue samples were collected following the Protocol for the Collection of Sport Fish for Contaminant Analyses section of the Broadscale Monitoring Procedures and Protocols (Sandstrom et al. 2013). Epaxial muscle tissue was taken in the form of skinless boneless fillets. After collection all samples were placed on ice and stored at Lakehead University where they were frozen at -20°C for later processing of Hg and stable isotopes.

Collection of Walleye stomach contents for isotope and bioenergetic analysis was conducted by netting during crepuscular and overnight hours when Walleye were more actively foraging (Swenson 1977). Stomach contents were obtained in the field from euthanized fish. Stomachs were incised from the lower esophagus to the pyloric sphincter and removed from the carcass. Contents were removed from the stomachs immediately after dissection to halt digestion by enzymes (Sandstrom et al. 2013). Stomach contents

were placed on ice in the field for a maximum of 4 days and frozen at -20°C for storage prior to further analysis. Stomachs consisting primarily of bile or chyme were not retained and reported as empty (Sandstrom et al. 2013). Fish collections were made under the authorization of the Lakehead University Animal Care Committee (Lakehead AUP # 1468680), the Ontario MNRF (collection permit # 1101743) and under the authorization of the Ontario MECP.

A travelling benthic kick and sweep method (Wesolek et al. 2010) was used to collect benthic invertebrates from each lake sampled in 2022. To characterize pelagic invertebrates, zooplankton were collected with a 2m long, 50cm diameter, 150um mesh net. The net was lowered to one metre off the bottom at the deepest part of the lake and hauled quickly to the surface. Water was strained out and zooplankton were concentrated into a 50ml centrifuge tube. This process was repeated 2-3 times per lake until a 50 ml centrifuge tube was full of a concentrated zooplankton mixture. Samples were kept on ice in the field for a maximum of 4 days until they could be returned to the lab, where they were frozen prior to analysis.

#### 3.3.2. Hg determination

Estimates of [Hg] for Walleye tissue and stomach contents were obtained using a DMA-80, under the assumption that the total Hg measured in fish is ≥95% MeHg (Bloom 1992, Porcella 1994, Becker and Bingham 1995). Walleye stomach contents were almost entirely fish and analyzed similarly. All samples were run following EPA method 7473 SW-846 (Environmental Protection Agency, 2003). All stomach contents from each fish were desiccated individually in a drying oven at 60°C overnight and ground into a dry homogenate with a mortar and pestle. Stomach contents were analyzed for [Hg] as a dry

homogenate, while Walleye muscle was run as wet tissue. Duplicate samples of Walleye and stomach contents were run every fifth sample to determine the repeatability of results. If duplicate samples within a run exceeded an average percent difference of 10%, then the run was re-analyzed. The certified reference material (SRM) TORT-3 was analyzed approximately 6 times on each run to indicate the ongoing accuracy of the DMA. The SRM has a certified range for [Hg] of  $0.292 \pm 0.022$  ppm (~7.5%). If the SRM within a run showed evidence of drift (SRM [Hg] deviating consistently in one direction across a run of a magnitude greater than 5%), then all samples on the run were reanalyzed. Across all runs, duplicate samples of Walleye tissue had a mean percent difference of 5.9%. A paired t-test revealed no significant differences between samples and their duplicates ( $t_{59} = -0.717$ , p = 0.476). The average SRM concentration for Walleye runs was  $0.2773 \pm 0.001$  ppm and within the acceptable range. Stomach content duplicates had a mean percent difference of 8.7% and duplicates did not significantly differ between duplicate pairs ( $t_{20} = -1.0182$ , p = 0.3). Average SRM concentration for stomach content runs was  $0.2765 \pm 0.0002$  ppm, within the acceptable range. Invertebrate samples (benthic and limnetic) were sent to the ISO-17025 certified Lakehead University Environmental Laboratory for [MeHg] analysis and processed by a Brooks Rand MERX-M analyzer following EPA Method 1630 using the SRM "DORM-4"  $[0.412 \pm 0.0036]$ ppm]. Both Walleye stomach contents and invertebrates were analyzed for mercury concentrations as dry tissue. I measured wet to dry mass ratios of benthic invertebrates, zooplankton, and Walleye prey which were used to estimate Hg in ppm (wet mass) for use in ecosystem biomagnification analysis and bioenergetic models.

#### 3.3.3: Whole Ecosystem Biomagnification Slopes: stable isotope analysis

To estimate potential impacts of *Bythotrephes* on ecosystem biomagnification slopes, a subset of tissues analyzed for [Hg] were also analyzed for stable nitrogen isotopes (Kiriluk et al. 1995). To characterize the whole ecosystem biomagnification slope, organisms were collected to represent three trophic positions, characterized by benthic macroinvertebrates and zooplankton (Position 1, primary consumers), Walleye stomach contents (mainly prey fish- Position 2) and the top position was characterized by Walleye muscle tissue (Position 3). Walleye tissue samples for which Hg data were already available (see Chapter 2) were selected for stable isotope analyses. I chose Walleye from the minimum, first quartile, median, third quartile and maximum fork length values for each Walleye population for which tissues were available in order to represent size variation in fish Hg and  $\delta^{15}$ N.

Samples were prepared for stable isotope analysis by drying at 60°C overnight or until fully desiccated. Once dry, samples were crushed into a fine powder using a mortar and pestle. Between 0.2-0.4 mg from each sample was weighed and loaded into tin cups. Fifteen samples were selected from every lake to estimate ecosystem biomagnification slopes; 5 Walleye from across the size range collected (see above), 5 samples from Walleye diets, and the lowest trophic level using 3 benthic and 2 pelagic samples. A duplicate was run every tenth sample for quality assurance. A paired *t*-test demonstrated good agreement between duplicate samples for  $\delta^{15}$ N ( $t_{11}$ = 1.2029, p = 0.3) with an average difference between duplicates of 0.2‰. All stable isotope analysis was conducted by Isotope Tracer Technologies INC and were analyzed with a DeltaPlus Isotope Ration Mass Spectrometry (IRMS) accompanied by Elemental Analyzer EA 1110 CHN. The

instruments used in this stable nitrogen analysis have a standard deviation:  $\pm 0.3\%$ . The standards used in this analysis were IT2-601, IAEA-N1, IAEA-N2, and Acetanilide (B2000).

#### 3.3.4. Food chain length

Assuming Walleye were the top predators in my lakes, food chain length was estimated according to (Vander Zanden and Rasmussen 1999) as:

$$TP_{Walleye} = ((\delta^{15}N_{Walleye} - \delta^{15}N_{baseline})/3.4) + 2 \qquad Eq 3.1$$

where  $\delta^{15}N_{Walleye}$  was the mean  $\delta^{15}N$  from Walleye samples for a lake,  $\delta^{15}N_{baseline}$  was the mean of all zooplankton and benthos  $\delta^{15}N$  for a lake and 3.4 is the factor by which adjacent trophic levels in a food chain differ in  $\delta^{15}N$  enrichment. Walleye trophic position was calculated for each lake.

#### 3.3.5: Bioenergetics Modelling

To understand how *Bythotrephes* invasions may impact the bioenergetics of QPP Walleye, I used a bioenergetics model paired with a mercury mass balance model (MMBM) to estimate daily consumption, activity budgets and growth efficiency of Walleye across lakes. The MMBM is linked to the bioenergetics model through consumption, and simultaneously balances the equations between observed initial and final mass and Hg concentrations based on parameters provided (Table 3.3) by adjusting both the p-value (proportion of maximum consumption) and activity multiplier (Trudel et al. 2000; Rennie et al. 2005). Only female Walleye were included in bioenergetic models as they were better represented in the data and avoided confounding results based on physiological differences between the sexes (Rennie et al. 2008, Rennie and Venturelli 2015; Madenjian et al. 2014, 2016). The bioenergetics model was run by estimating single-year age cohorts (the change in Walleye [Hg] and mass (g) modelled over a single year for a particular age) under the assumption that age-at-length curves and Hg-at-length slopes were representative of Walleye populations within each lake. All data included in the bioenergetics model were compiled from surveys conducted after the arrival of *Bythotrephes* in QPP (Table 3.1). Population estimates of mass and Hg-at-length were approximated for each lake (Table A.1) and I only modelled age cohorts which had been observed in the field (Table A.2), which included those that were invaded with *Bythotrephes* and those that were uninvaded. Further details on model input parameterization and model structure are provided below.

#### 3.3.6: Bioenergetic model structure

A bioenergetics model combined with a mercury mass balance model was applied to estimate Walleye consumption, activity, and growth efficiency (Trudel et al. 2000; Rennie et al. 2005). Bioenergetic model parameters and appropriate sub-equations for Walleye consumption, metabolism, and other loss functions (Kitchell et al. 1977, Deslauriers et al. 2017) are summarized in Table 3.

The general bioenergetics equation applied daily is:

$$Growth = C - (A*SMR+F+U+SDA)$$
 Eq 3.2

Where C is consumption (J), A is Activity (Unitless), SMR is Standard metabolic rate (J), F is egestion (J), U is excretion (J), and SDA is the specific dynamic action (J).

Following Deslauriers et al. (2017), Walleye consumption was estimated using Consumption Model 2 (temperature dependence) and respiration was estimated using Respiration Model 2 (activity dependence) equations (Kitchell et al. 1977, Deslauriers et al. 2017). Walleye egestion and excretion were both estimated using Model 2 temperature and ration dependency equations (Elliott 1976, Deslauriers et al. 2017).

Consumption from the bioenergetics model was replaced with consumption from the MMBM and estimated as:

$$C = \frac{Hgt - Hg0 \cdot e^{-(E+G+K)t}}{\alpha - Cd \cdot (1 - e^{-(E+G+K)t})} \cdot (E+G+K)$$
 Eq 3.3

 $Hg_{0}$  and  $Hg_{t}$  are the concentration of mercury at time 0 (initial) and at time t (estimated using VBGM, Section 3.3.12),  $\alpha$  is the assimilation efficiency of food, Cd is the [Hg] of prey (Section 3.3.8), G is the growth rate (day<sup>-1</sup>), K is Hg loss to the gonads (day<sup>-1</sup>) and Eis the elimination rate (day<sup>-1</sup>). Daily elimination rate (E) of Hg was estimated using the chronic exposure equation:

$$E = EA^* \cdot \text{mass}^{EB*} \cdot e^{EQ^* \text{temp}}$$
 Eq 3.4

Where mass is the weight of the fish (g), *EA*, *EB*, and *EQ* are coefficients (Table 3), and temp is the temperature of the water (°C, Section 3.3.7). A modified version of the chronic Hg elimination equation was used in this study. A recent study by Madenjian et al. (2021) suggested that the standard chronic mercury elimination equation overestimates elimination in percids. To account for this discrepancy, Madenjian et al. (2021) proposed a modification of this standard equation for Walleye of; 0.5E, which halves the modeled elimination rate. I applied 0.5E to all cohorts to be consistent across all ages.

The model was solved by expressing variables as mass-specific quantities (Consumption; converted from j/day to g/day, Activity; j/day), which along with Walleye mass (g) were averaged over the modelling period (365 days) and reported for statistical analysis (Table A.3; as per Trudel et al. 2000; Rennie et al. 2005). Growth efficiency was estimated as the relationship between consumption ( $g_{food}/day$ ) and growth ( $g_{growth}/day$ ). Walleye biomagnification factor (BMF) is also estimated through consumption, as calculated through the bioenergetics model. After converting consumption from joules to grams, Walleye BMF can be expressed as:

BMF = 
$$(\alpha \cdot C(day^{-1}))/(E+G+K)(day^{-1})$$
 Eq 3.5

Walleye BMF is a unitless quantity, where the numerator represents daily Hg assimilated through consumption, relative to the total daily losses of Hg to elimination, growth, and gonads. This quantity was averaged over the entire modelling period (365 days) for reporting (Table A.2).

#### 3.3.7: Water Temperature Data

Surface lake water temperatures for the open water season were measured using HOBO loggers in 3 QPP lakes (Table 2). These three lake sizes are representative of small, medium, and large lakes in QPP, and were used to represent water temperatures for other QPP lakes included in bioenergetics analyses which did not have water temperature data available. Loggers were deployed in the littoral zone of the lakes in the spring. Buoys were anchored in at least 4 m depth with 4.5kg weights, with a float holding the line taught. One buoy was deployed per lake with 2 loggers attached, located at 1m and 2m below the surface. Each logger recorded the surrounding water temperature every four hours allowing the calculation of daily average epilimnetic water temperatures for most of the ice off months (Table 3.2).

For days or years where no temperature data were available, water temperatures were estimated from air temperatures using a statistical model (Matuszek and Shuter 1996). Based on the data available, single-year models were produced for each of the lakes with measured water temperature data and air temperature data accessed through The Government of Canada: Environment and Natural Resources (Government of Canada 2023). Mean daily water and air temperature were calculated as the average of maximum and minimum temperature for a given day.

All mean daily surface water temperatures (WTEMP) were estimated with the formula:

$$WTEMP = C_0 + C_1(ATEMP) + C_2(YDAY)$$
 Eq 3.6

Where *ATEMP* is daily mean air-temperature and *YDAY* is the day of the year and  $C_{0,1,2}$  are lake specific regression coefficients. For bioenergetic analysis, observed water temperature values were used where available, but were predicted using this model for days/years when direct measurements were not available. Given similarities in thermal characteristics among waterbodies, lakes that did not have loggers deployed were modeled using the data from the nearest lake where loggers were deployed.

As Walleye behaviorally thermoregulate, the assumption was made that the maximum temperature Walleye would experience was 22.1°C (Kitchell et al. 1977, Hasnain et al. 2010). Therefore, any daily mean water temperatures that exceed 22.1 degrees were capped at that threshold to simulate the behavior of Walleye seeking ideal

habitat and temperature. Temperatures under the ice are relatively stable and range from 1-3°C in temperate lakes a few meters below the surface (Ellis et al. 1991). Therefore, I applied a constant 2°C temperature when estimated water temperatures reached 2°C, assuming this represented the period of ice cover on these lakes.

#### 3.3.8: Walleye Prey Hg and Stomach Contents

Prey mercury (*Cd*) data were estimated from Walleye stomach contents collected during 2022. In this study, *Cd* was estimated for each lake as the average of all collected and measured prey item concentrations, and a single lake specific value was applied to all age cohorts. Species identification of stomach contents was difficult due to various levels of digestion. However nearly all stomach contents could be identified as prey fish with the occasional invertebrate also present. Lake-specific *Cd* was calculated from the stomach contents collected from each lake. Initially, a *Cd*-fork length relationship was attempted to provide a unique *Cd*-at-length for each age cohort as diet length relationships have been established in previous bioenergetics studies (Rennie et al. 2012). However, attempts to produce a linear relationship yielded weak correlations ( $R^2 \le 0.1$ ), and fitting a general linear model showed that the fork length-*Cd* relationship was not significant (p = 0.2546) for the Walleye in this study. This application of lake-specific mean *Cd* is consistent with previous studies using similar applications which have used constant *Cd* estimates in Walleye of age ranges 2 – 11 years (Trudel et al. 2001).

### 3.3.9: Prey Energy Density

Prey energy densities and prey composition for QPP Walleye was estimated from published dietary proportions and energy densities derived by season long observations of Walleye in Lake Winnipeg (Sheppard et al. 2015) and bomb calorimetry. Walleye diets were calculated as the sum of the products of the proportion of a prey item and its mean energy density (Table 3.4) based on estimates from Sheppard et al. (2015). In this study, two separate diet types for QPP Walleye were estimated based on published values. The first diet type was for lakes with rainbow smelt, and the second type for lakes where smelt were rare or absent. Sheppard et al. (2015) reported that white bass and freshwater drum were prey items of Lake Winnipeg Walleye. Given that these fish do not occur within the lakes in this study, their dietary proportion was replaced with yellow perch across both diet types, given they are all spiny-rayed fish with relatively similar energy densities. It was assumed that QPP Walleye were almost entirely piscivores based on stomach contents collected during 2022 sampling.

#### 3.3.10: Walleye Age Determination and Age-at-length Assignment

Walleye collected during 2021-22 were aged following protocols outlined elsewhere (Pritchard et al. 2019). Briefly, Walleye fin rays were emersed proximal side first in an epoxy-resin mixture and dried for 24 hours to reduce damage to the structure when cutting. Once dry, dorsal spines were cut with a low speed isomet saw (Buehler: 11-1280-170) with a single 10.2 cm diamond edge wafering blade. Cuts were made at a speed of ~200 rpm. Dorsal spines were cut perpendicular to the axis of the fin to obtain multiple cross sections. The initial cut was made as close to the proximal end to capture the first annulus. Dorsal spines were sectioned 2-3 times, in widths of ~0.85-0.95 mm. Sections were mounted to microscope slides with clear mounting fluid and left to dry 48 hours. Age was interpreted by counting annuli; seasonal increments are visible as rings in calcified structures, including dorsal spines (Pritchard et al. 2019). Annuli were counted manually after taking high resolution images through a digital SeBaCam5C camera mounted to a Laxco LMC4-BF280 microscope at a magnification of 4x. For quality assurance, a blind read was conducted on 20% of the fish ages in 2022 selected at random. Results of blind read showed 87.5% accuracy rate on estimating ages. Those that were not successfully re-read during the blind read were found to be off by only a single year and the initial read was retained.

### 3.3.11: Fish Mass at age

In my dataset distributions of fish mass and size were not equally represented across all ages classes of the population, making it necessary to estimate age-at-length was estimated with a standard von Bertalanffy growth model for all ages across each Walleye population (VBGM; Ogle and Iserman 2017, Schnute and Fournier 1980). The standard VBGM function is expressed as:

$$Lt = (L_{\infty})^{*} [1 - e^{-k(t-t_{0})}]$$
 Eq 3.7

Where  $L_{\infty}$  is the asymptotic length (mm),  $L_t$  is the expected length at time (Age), K is the exponential rate at which Lt approaches  $L_{\infty}$ , and  $t_0$  is the hypothetical age-atlength 0. In this model,  $t_0$  was estimated as 0 to force the VBGM through the origin, as relatively few juvenile fish were sampled. Early growth rates ( $\Omega$ ) were estimated as the product of  $L_{\infty}$ , and K (Gallucci and Quinn 1979).

To estimate mass-at-age, length-weight relationships were established for each lake using log-transformed ( $log_{10}$ ) variables in a linear regression, producing a slope (*B*)

and intecept (A) for each lake (Table A.1). After generating a linear relationship for each lake the following equation was applied:

Mass-at-age = 
$$(age-at-length^B) \cdot 10^A$$
 Eq 3.8

#### 3.3.12: Fish Hg at size

A relationship between Walleye age and [Hg] was established through a linear regeression of Walleye [Hg] and length. A linear regression was performed on the fork length-Log<sub>10</sub>(Hg) relationship for each lake, generating a lake specific equation (Table A.1). To estimate Hg at age, I substituted age-at-length calculated from the VBGMs in the equation for all cohorts represented in the data. The estimated log10[Hg]-at-age values were raised to the power of 10 to transform back from the logarithimc relationship.

## 3.3.13: Statistical Analysis

Ecosystem biomagnification slopes were estimated as the relationship between  $\delta^{15}N$  and [MeHg]. To analyze variation in slopes among lakes, I used a test for heterogeneity of slopes followed by ANCOVA where appropriate. Further statistical analysis sought to determine if mean [MeHg] or  $\delta^{15}N$  varied between invaded and uninvaded lakes at each trophic level. Differences in means were evaluated with t-tests for both variables ( $\delta^{15}N$ , MeHg) and at each trophic level (benthos, zooplankton, Walleye stomach contents/prey, and Walleye) for a total of 8 comparisons. Walleye trophic positions for each lake were assigned to their respective invasion status, and invasion class means were compared with a *t*-test.

Both the bioenergetic model inputs (VBGM and Hg-at-length) and outputs (growth efficiency, consumption, activity, and Walleye biomagnification factors) were evaluated. VBGM inputs were evaluated by comparing mean asymptotic length ( $L_{\infty}$ ), and *K* across lakes using t-tests. While Hg-length, and all output variables were analyzed across lakes with tests for heterogeneity of slopes. Bioenergetic estimates of consumption (g/day), activity (g/day), and biomagnification factor (unitless) were plotted against fish mass (g) while growth efficiency was calculated as the slope of consumption (g/day) regressed against growth (g/day). All bioenergetics estimates and fish mass were log<sub>10</sub> transformed to perform linear analysis. Conversion/growth efficiency (Growth (g/day) ~ Consumption (g/day)) did not respond to data transformations and a linear fit was not appropriate to describe the relationship. Visual inspection showed changes in the slope of conversion efficiency (positive to negative slope) with maturation status. The decision was thus made to split between mature and immature fish for each lake, and then conduct tests for heterogeneity of slopes separately for mature and immature fish.

Finally, I evaluated the effect of waterbody surface area on Walleye bioenergetics. To do so, consumption, activity, and Walleye BMF were converted to from mass relative estimates (g/day or J/day) to mass-specific quantities (g/g/day or J/g/day) to allow for accurate mean estimates to be calculated for each lake (Hewett and Kraft 1993, Rennie et al. 2012). Mass-specific quantities for consumption were calculated as:

$$C_{(g/g/ay)} = C_{(g/ay)} / (W_{avg}^{B})$$
 Eq 3.9

Where  $W_{avg}$  was the average mass of the cohort of the period being modelled (g), *C* (g/day) was the mean daily consumption of the cohort and *B* was the lake-specific mass scaling exponent (e.g. slope of the log10-log10 relationship between consumption(g/day)
and  $W_{avg}$  from Figure 3.7; Table A.2). Activity was converted from units of J/day to J/g/day, and Walleye BMF was converted from a unitless parameter to units of g<sup>-1</sup> using the same approach. Mass-specific quantities of consumption, activity, and Walleye biomagnification factor were then evaluated against log<sub>10</sub>-transformed lake surface area with a correlation test. Correlation tests were also evaluated between lake surface area and von Bertallanfy parameter estimates (*K* and  $L_{\infty}$ ) of Walleye populations.

#### 3.4 Results

### 3.4.1: Ecosystem Biomagnification Slopes

There were no significant differences in ecosystem biomagnification slopes across lakes (test for heterogeneity of slopes,  $F_{15,111} = 19.35$ , p = 0.63). Further testing was conducted on differences in intercepts using ANCOVA which indicated significant differences in intercepts among lakes ( $F_{8,118} = 18.64$ , p < 0.0001). However, differences in intercepts could not be attributed to invasion status, as invaded lakes (French, Saganagons and Pickerel) did not group together apart from uninvaded lakes (Figure 3.3).

Mean  $\delta^{15}$ N and [MeHg] (Table A.4) were compared by invasion class at each trophic level using *t*-tests. I found only marginal differences in zooplankton  $\delta^{15}$ N between invaded and uninvaded lakes ( $t_6 = 2.12$ , p = 0.08), however, Walleye mean  $\delta^{15}$ N was significantly greater in invaded lakes than in uninvaded lakes ( $t_6 = 4.4$ , p = 0.0046, Figure 3.4 Panel B). I followed up with a Levene's test and found that variances in Walleye  $\delta^{15}$ N between invaded and uninvaded lakes were equal ( $F_{1,6}=0.0134$ , p = 0.912). Despite the increase of mean invaded Walleye  $\delta^{15}$ N no difference in mean Walleye [MeHg] was detected between invasion classes ( $t_6 = 1.44$ , p = 0.20). Further, despite differences between Walleye  $\delta^{15}$ N and no difference in zooplankton  $\delta^{15}$ N, Walleye trophic positions were not significantly different between lakes invaded with *Bythotrephes* and those without ( $t_6 = 0.031$ , p = 0.976).

### 3.4.2: Walleye Population Parameters

A VBGM was fitted to the age-at-length data for each lake (Figure A.1). Analysis of VBGM parameters revealed that while growth rates varied among lakes, differences in Walleye asymptotic length were not associated with *Bythotrephes* invasions (Table 3.5, Figure 3.5A). While lakes with *Bythotrephes* present were more tightly grouped relative to one another, they were within the range of variation observed for uninvaded lakes. A *t*-test revealed no significant difference across estimates of mean asymptotic length ( $t_6 = 0.3110, p = 0.7663$ ) or *k* between invasion classes ( $t_6 = 0.8587, p = 0.4235$ ). Analysis of Hg-at-length (Figure 3.5C) among lakes indicated differences in the slope of the relationship between fish Hg and body size among lakes (test for heterogeneity of slopes,  $F_{15, 386} = 76.24, p < 0.0001$ ), however, lakes with similar slopes did not group clearly on the basis of *Bythotrephes* invasion (group 1, Agnes, Pickerel, Saganagons; group 2, French, Robinson, and group 3: Beaverhouse, Batchewaung and Minn).

#### 3.4.3: Bioenergetics Model Outputs

Significant differences in slopes were detected across lakes describing the allometric relationship of consumption (g) with body size (Figure 3.6. Panel A: test for heterogeneity of slopes,  $F_{15,96} = 4681$ , p < 0.0001); however, these differences did not appear to be associated with *Bythotrephes* invasion status. One group of similar slopes consisted of: Agnes, French, and Minn while another group of similar slopes consisted of Saganagons, Batchewaung and Pickerel (both groups consisted of invaded and uninvaded

lakes). Similarly, the scaling of daily activity budgets (J) with body mass were found to be heterogenous (Figure 3.6. Panel B:  $F_{15,96}$  = 2694, p < 0.0001), but did not group based on *Bythotrephes* invasion status; Agnes, Beaverhouse, and Saganagons grouped together while Robinson and French and Minn grouped together. A test for homogeneity of slopes for Walleye Biomagnification factors with body size across lakes revealed that slopes differed among lakes (Figure 3.6. Panel C:  $F_{15,96}$  =1130, p < 0.0001). As with other bioenergetic estimates, the differences did not seem to be associated with invasion as one group was Batchewaung, Beaverhouse, and Pickerel and another group consisted of French, Minn, and Robinson.

For immature Walleye, conversion efficiency (estimated as the slope of growth (g/day) with consumption(g/day) on a log10-log10 scale) varied among lakes (heterogeneity of slopes test,  $F_{15,6} = 7.793$ , p < 0.0001, Figure 3.7; Panel A). Both positive and negative slopes were observed, however, lakes did not group with invasion status. Agnes, Batchewaung, Pickerel, and French had similar slopes while Saganagons, Beaverhouse and Min had similar slopes. For mature Walleye, slopes of G(g/day) vs C(g/day) (as an estimate of conversion efficiency) were all negative; however, log10 transformation failed to sufficiently linearize relationships between growth and consumption. While slopes differed significantly among lakes ( $F_{15,74} = 171.3$ , p < 0.0001, Figure 3.7; Panel B), groupings among lakes were not associated with invasion status: Pickerel, Minn, and Saganagons formed one group, while another group consisted of: Agnes, Batchewaung, Robinson, and French.

#### 3.4.4: Effect of Lake Surface Area

Pearson Correlation tests revealed that Walleye activity had a strong negative correlation with lake surface area ( $r_6 = -0.82$ , p = 0.012, Figure 3.8; Panel A) as did Walleye BMF ( $r_6 = -0.75$ , p = 0.03 Figure 3.8; Panel B) and Consumption ( $r_6 = -0.89$ , p = 0.003 Figure 3.8; Panel C). Correlation tests between slopes of growth efficiency and lake size for both immature ( $r_6 = 0.104$ , p = 0.807, Figure 3.8; Panel D) and mature Walleye ( $r_6 = 0.137$ , p = 0.546, Figure 3.8; Panel E) were not significant, and neither was the correlation of intercepts (vertical offset) of ecosystem biomagnification calculated from isotope data ( $r_6 = -0.21$ , p = 0.62, Figure 3.8; Panel F). Removal of Agnes (outlier) from the test for mature growth efficiency yielded a stronger albeit still insignificant result ( $r_5 = 0.5685$ , p = 0.183). Correlation tests between lake surface area on parameters associated with the input variables of the Bioenergetics model revealed a significant correlation between the slope of Hg-length and lake size ( $r_6 = 0.825$ , p = 0.012, Figure 3.8; Panel I). No correlation was detected between lake size and  $L_{\infty}$  ( $r_6 = 0.116$ , p =0.7855, Figure 3.8; Panel G) or  $K (r_6 = 0.001, p = 0.99)$ , Figure 3.8; Panel H). However, the removal of Agnes Lake (outlier) results in a slightly stronger negative correlation for K with lake size ( $r_5 = -0.625$ , p = 0.133). No correlation between  $\Omega$  and lake area was detected ( $r_5 = -0.0487$ , p = 0.91, not pictured).

### 3.5 Discussion

Using stable isotope and [Hg] data collected from 8 QPP lakes in 2022, I found no supporting evidence for an effect of the presence of invasive *Bythotrephes* on ecosystem biomagnification or food chain length with respect to Walleye and their prey. Slopes of ecosystem biomagnification were not different among lakes, and while some differences in intercepts (elevation) among ecosystem biomagnification relationships were detected, they did not group by invasion status. Further, food chain length was found to be similar

between lakes with and without *Bythotrephes* present, despite Walleye  $\delta^{15}$ N being elevated in invaded lakes relative to uninvaded lakes and no difference in zooplankton  $\delta^{15}$ N with invasion status. A potential reason behind this could be differences in the mean baseline  $\delta^{15}$ N between invaded and uninvaded lakes; differences in mean zooplankton  $\delta^{15}$ N were only marginally non-significant between invaded lakes and uninvaded lakes with zooplankton  $\delta^{15}$ N tending to be higher in the former. As such, relative 'trophic distance' between baselines and Walleye were similar between invaded and uninvaded lakes. This lack of systematic difference in ecosystem biomagnification was also reflected by the results of the bioenergetics model, which indicated that differences in Walleye body size-BMF slopes did not group based on invasion and differences in slopes were more attributed to differences among individual lakes. Similarly, other mass-relative Walleye bioenergetic estimates yielded similar results, with no clear effect of *Bythotrephes* on activity, consumption, or growth efficiency.

Interestingly, analysis of mass-specific bioenergetic estimates showed that several bioenergetics variables were associated with lake size. Strong negative correlations were detected for Activity, Consumption, and Walleye BMF with lake surface area. Associations between Walleye Hg and lake size were also reported in my first data chapter, interacting with fish body size. This study is not the first to find an effect of lake size on ecosystem biomagnification or bioenergetics. In another predatory fish (Lake trout), asymptotic length was found to be positively correlated to lake surface area (McDermid et al. 2010) likely due to increased availability of preferred prey (Pazzia et al. 2002). In addition to asymptotic length ( $L_{\infty}$ ), Lake trout grew slower before maturity in larger lakes (McDermid et al. 2010). While my study did not find a correlation between

lake size and  $L_{\infty}$ , or  $\Omega$  an examination of K with the removal of one outlier (Agnes Lake) showed weak evidence that the rate (K) that Walleye approach  $L_{\infty}$  was negatively correlated to lake surface area. Other variables related to lake size include ecosystem biomagnification, where ecosystem biomagnification intercepts (derived from  $\delta^{15}N$ ) negatively correlate with lake surface area (Kidd et al. 2012). In my study, though the relationship between ecosystem biomagnification intercept and lake size was nonsignificant, the direction of the association was indeed negative. However, I did find a strong negative correlation between mass-specific Walleye BMF and lake surface area. Kidd et al. (2012) also reported a positive correlation of lake surface area with the slope of biomagnification, and while my differences in the slopes of ecosystem biomagnification among lakes were not statistically significant, my slopes of Walleye-Hg with body size were positively correlated to lake surface area. My observations regarding slopes of Walleye Hg-at-length with lake size are supported by my first data chapter where I also found an increase in the Walleye [Hg] vs body size slope with increasing lake surface area. Combined with strong negative correlations of both mass specific activity and consumption with lake surface area, this suggests that Walleye foraging effort may decline as lake size increases. Offshore prey (Cisco) presence and abundance is correlated to lake surface area (Kennedy et al. 2018) and the presence of Cisco is shown to reduce activity costs in Walleye (Kaufman et al. 2006). Additionally, Cisco have greater energy densities than comparable inshore prey like yellow perch (Pazzia et al. 2002, Sheppard et al. 2015). Suggesting that Walleye may not need to expend much effort into foraging for an energetically favorable prey species.

The results of my stable isotope ecosystem biomagnification analysis are not the first to suggest that mid-trophic invasions are not associated with food chain elongation and do not impact ecosystem biomagnification or accumulation among fishes (Rennie et al. 2010, Hogan et al. 2007, Johnston et al. 2003). Similar studies (Rennie et al. 2011, Johnston et al. 2003) have suggested that changes in growth efficiency could be responsible for "masking" effects of mid-trophic invasions, and some studies have shown that the presence of *Bythotrephes* likely does improve the growth efficiency of some fishes (Rennie et al. 2023 in review). However, examination of both immature and mature Walleye growth efficiencies from bioenergetics models applied to these populations did not support this hypothesis. Differences in slopes of growth efficiency (Figure 3.7) for both mature and immature Walleye were attributed to individual lake differences other than invasion status and do not suggest differences in growth trajectories. Bioenergetic input variables (age- and [Hg]-at-length) for the 8 QPP lakes also did not display any clear or significant patterns with respect to invasion status. The only trend to emerge from analysis of VBGM plots was that invaded lakes were more tightly clustered for both  $L_{\infty}$  and K and there was also no clear separation with lake invasion status for Hg-at-length relationships.

Despite these results that seem to suggest that increasing lake size provides Walleye with a greater abundance of easier to capture and energy dense offshore prey, thereby reducing their activity costs and improved energy yields of their prey, no increase in growth (conversion) efficiency with lake size was observed. However, the removal of Agnes Lake provides some evidence of a weak trend suggesting the growth efficiency may increase in mature Walleye with increasing lake size. This study is not the first to

report differences in mass specific consumption and activity but no significant change in growth efficiency. Similar to my study, increased rates of mass specific consumption and activity suggest a less preferable prey community resulting in increased foraging efforts (Rennie at al. 2012) suggesting that changes in growth efficiency may be less sensitive to environmental fluctuations than fish activity and consumption.

The results presented in this data chapter were potentially impeded by three limitations; 1) the number of lakes included in this analysis, 2) sample sizes of Walleye and ecosystem components within lakes, and 3) seasonal variation in prey [MeHg] and  $\delta^{15}$ N isotopes that I was unable to account for. In my first data chapter, I demonstrated that invasion does impact Hg-at-length, when interacting with lake surface area, but it required more data than 8 lakes to reveal these patterns and overcome among-lake variation. My sample sizes were limited primarily due to logistic constraints (number of each lake type that could reasonably be sampled in a single summer). Sample size likely impacted my examination of correlations with lake size, where several comparisons (mature growth efficiency, ecosystem biomagnification intercepts, and K) that show similar trends to published studies were not statistically significant. In studies where lake size was associated with biomagnification (Kidd et al. 2012) and fish life history (McDermid et al. 2010) sample sizes were larger than in my study (14 and 114 lakes, respectively) and used a larger range of lake sizes (Kidd et al. 2012: 10,000-1,000,000 Ha), (McDermid et al. 2010: 30-380,000 Ha) compared to my study (8 lakes, 280-5,750 Ha). I argue that a larger sample size of lakes as well as a wider size range of lakes would strengthen the correlations observed in my study.

The second limitation that I have identified with this analysis is the sample size of Walleye and other food web components within lakes, specifically a lack of relevant historical data. Data collections done in QPP before the start of this thesis did not focus on biomagnification or bioenergetics and as such did not target Walleye stomach contents, prey fish or primary consumers (e.g. zooplankton or benthic invertebrates) for consistent Hg analysis Stable isotopes were also not collected for any organisms prior to this study. While VBGMs and Hg-at-length (which in future studies should be estimated from mass-at-age, Essington et al. 2001), were aided by data obtained from previous collections, all stable isotopes, prey [Hg], and water temperature data were collected within a single summer (2022). This limited my ability to compare bioenergetic estimates and ecosystem biomagnification before and after the invasion of *Bythotrephes* as well as limited the statistical power and accuracy of lake specific variables. Bioenergetic modelling has previously been applied temporally to estimate the impact of invasive species. In the South Bay of Lake Huron, the introduction of invasive dreissenid mussels impacted lake whitefish (*Coregonus clupeaformis*) populations resulting in greater rates of activity and consumption and declines in growth efficiency (Rennie et al. 2012). Additionally temporal analysis of biomagnification has reported strong trends of increasing [MeHg] linked to anthropogenic emissions using decades worth of MeHg and stable isotope data (Vo et al. 2011). I believe that the approaches highlighted in the two studies (Rennie et al. 2012 and Vo et al. 2011) demonstrate that temporal analyses can be used to evaluate trends in bioenergetics associated with invasive species and biomagnification. The data collected within this thesis could be used in future studies as the "historical data" to perform temporal analyses.

The third limitation that I have identified is the lack of seasonal sampling of the waterbodies in this analysis. Each lake was only visited once during the summer of 2022, meaning that aside from water temperature, all data were collected at a single point in time. While this has implications for the seasonal accuracy of Walleye diets (Rennie 2003, Sheppard et al. 2015) my greatest concern is in regard to the shorter-lived organisms collected in this study. Zooplankton have very prominent changes in their population density and biomass throughout the open-water season (Coguiec et al. 2021, Bellier et al. 2022). Zooplankton isotopic composition appears to be highly variable throughout the season with individual lakes having highly enriched  $\delta^{15}$ N peaks at different times of the year with seasonal patterns differing across years (Matthews and Mazumder 2005, Rennie et al. 2013). Additionally, zooplankton populations have seasonal fluctuations in mercury concentrations, generally lowest in early spring and increasing steadily to a peak in autumn (Marziali et al. 2022). I would advise caution when interpreting the lowest trophic positions in this study (zooplankton, benthos) and suggest that isotopes and [Hg] should be collected at several points throughout the season where possible (spring, summer, fall) to improve estimation of baseline  $\delta^{15}$ N in QPP lakes.

I also want to highlight an issue with the modelling presented in this thesis used to estimate size classes and age cohorts of Walleye populations. The consistent trend of negative slopes in mature Walleye growth efficiency (Figure 3.7, Panel B) can likely be attributed to using an asymptotic growth model to estimate age-at-length and then estimate mass-at-age from the asymptotic population estimate. Future studies should favor a mass-at-age model (Essington et al. 2001) to make initial population estimates for

bioenergetic analysis over the harsh asymptotic relationship demonstrated in estimates of growth efficiency and Hg-at-age (Figure 3.5, Panel B) presented in this thesis. Switching to a mass-at-age model for initial estimates will reduce the severity of the asymptote in Walleye Hg-at-age presented in this study and provide a more realistic estimate of large Walleye (>50cm) bioaccumulation.

In summary, lake surface area, rather than *Bythotrephes* invasion appears to have a strong relationship with Walleye biomagnification and bioenergetics. Increases in lake surface area were significantly correlated to declines in mass specific consumption, activity and Walleye BMF while the slope of Walleye Hg-length was positively correlated to lake surface area. The effects of lake surface area on Walleye observed in this chapter are supported by the results of the spatial analysis presented in my first data chapter. For the weaker correlations (growth efficiency, *K*, and ecosystem biomagnification intercepts) I believe a larger sample size (number of lakes) with a greater range in surface area could yield significant results similar to those observed in the literature. Extending this analysis to more lakes will allow for a more robust spatial comparison like the one presented in my first data chapter enabling the investigation of all the physical variables associated with lake size that may be contributing to these observations.

## 3.6 Tables

**Table 3.1.** Waterbodies included in whole ecosystem Biomagnification and Bioenergetics analysis. *Bythotrephes*-invaded lakes denoted with an asterisk\*. Lakes that did not have temperature loggers deployed were modeled using the data collected from the closest lake with loggers. Batchewaung = Model 1, French = Model 2, Pickerel = Model 3. Prey mercury (Cd) was collected for all Lakes in 2022.

				Invasion			
Waterbody	Surface	Mean Depth	Max Depth	Year	Survey Years	Cd	Temperature Model
Name	Area (Ha)	(m)	(m)				
Pickerel*	5754	17.7	74.7	2008	2009, 2015, 2021, 2022	2022	Model 3
Saganagons*	2470	6.9	31.3	2003	2021, 2022	2022	Model 3
French*	284	12.5	26	2009	2021, 2022	2022	Model 2
D . 1	1074	10.4			2021 2022	2022	
Batchewaung	1274	19.4	55	-	2021, 2022	2022	Model I
Minn	479	5.9	39.6	-	2016, 2022	2022	Model 1
Agnes	2982	19.6	79.3	-	2010, 2017, 2022	2022	Model 2
Robinson	421	12.7	35.1	-	2021, 2022	2022	Model 1
Beaverhouse	1958	22.3	64.7	-	2010, 2016, 2022	2022	Model 1

Location	Model Type	Logger depth (m)	Logger Latitude	Logger Longitude	Km from weather station	Start Date	End Date	Days Active
	HOBO UA-001-							
Pickerel	64	1	48.6548	-91.2087	32.43	2022-05-25	2022-10-15	143
	HOBO UA-001-							
Pickerel	64	2	48.6548	-91.2087	32.43	2022-05-25	2022-10-15	143
	HOBO UA-001-							
French	64	1	48.6729	-91.1503	35.68	2022-05-25	2022-10-15	143
	HOBO UA-001-							
French	64	2	48.6729	-91.1503	35.68	2022-05-25	2022-10-15	143
	HOBO UTBI-001							
Batchewaung		1	48.6751	-91.5266	11.76	2022-06-03	2022-10-22	141
-	HOBO UTBI-001							
Batchewaung		2	48.6751	-91.5266	11.76	2022-06-03	2022-10-22	141

**Table 3.2.** Water temperature data-logger summary used to estimate season long ice-off epilimnion temperatures for Bioenergetics modelling. Data loggers were programmed to record water temperature on 4-hour intervals.

#	Symbol	Parameter description	Value	Source
1	α	Assimilation Efficiency	0.8	1
2	Cd	Prey Mercury Concentration	Lake Specific	This Study
3	Ep	Prey Energy Density (J)	Lake Specific	2, This Study
4	CA	Intercept of mass dependence function (Cmax for a 1-g fish) at CTO	0.25	3
5	CB	Coefficient of Mass Dependent Function	0.73	3
6	CTM	Maximum Consumption Temperature (°C)	28	3
7	СТО	Optimal Consumption Temperature (°C)	22	3
8	CQ	Rate of function increase over low water temperature	2.3	3
9	RA	$(O^2/g/d)$ consumed by a 1-gram fish at RTO	146.406528	3
10	RB	Slope of allometric mass function of SMR	0.8	3
11	RTM	Maximum Respiration temperature (°C))	32	3
12	RTO	Optimal Respiration Temperature (°C)	27	3
13	RQ	Rate of function increase over low water temperatures	2.1	3
14	FA	Intercept of proportion consumed energy egested ~water temperature and ration	0.158	3,
15	FB	Egestion water temperature dependence Coefficient	0.778	3, 4
16	FG	Egestion feeding level dependence Coefficient	0.631	3, 4
17	UA	Intercept of proportion consumed energy excreted ~water temperature and ration	0.0292	3, 5
18	UB	Excretion water temperature dependence	0.58	3, 5
19	UG	Excretion feeding level dependence	-0.299	3, 5
20	EA	Coefficient of Hg elimination	0.0029	6
21	EB	Allometric exponent of Hg elimination	-0.2	6
22	EQ	Temperature coefficient of Hg elimination	0.066	6

**Table 3.3.** Bioenergetics and MMBM parameters used to estimate daily activity budget for Walleye. Definitions of #4-19 obtained
from fb4 manual (Deslauriers et al. 2017).

**3** 1, Norstrom et al. 1976; 2, Sheppard et al. 2015; 3, Kitchell et al. 1977; 4, Elliot 1976; 5, Hanson et al. 1997; 6, Trudel and Rasmussen 1997.

Diet					
Туре	Prey Species	Proportion	ED (J)	ED x P	Total ED (J)
	Rainbow Smelt	0.86	5840	5005	
	Cisco (Lake herring)	0.12	5140	622	
1	Yellow perch	0.0007	5040	4	5756
	Troutperch	0	5700	0	
	Shiner	0.016	6820	109	
	Walleye/Sauger	0.003	5100	16	
	Cisco (Lake herring)	0.27	5140	1362	
	Yellow perch	0.16	5040	818	
2	Troutperch	0.095	5700	542	5592
	Shiner	0.40	6820	2728	
	Walleye/Sauger	0.028	5100	143	

**Table 3.4.** Energy densities and proportions of two estimated Walleye diets (Type 1 = smelt present, Type 2 = smelt absent). Proportions and energy density values obtained from (Sheppard et al. 2015) with slight modifications amended for QPP species distributions (see text).

**Table 3.5.** von Bertalanffy growth model parameter estimates for Walleye populations of lakes included in Bioenergetics analysis.  $L_{\infty}$  = the Asymptotic length. K = exponential rate that  $L_T$  approaches  $L_{\infty}$ .  $\Omega$  is the product of  $L_{\infty}$  and K and is an estimate of early growth rates. *Bythotrephes*-invaded lakes denoted with an asterisk\*.

Waterbody Name	$L_\infty$	K	arOmega
Pickerel*	594.69	0.23	136.78
French*	568.10	0.31	176.11
Saganagons*	609.44	0.25	152.36
Minn	517.80	0.24	124.27
Beaverhouse	639.47	0.28	179.05
Agnes	518.55	0.39	202.23
Batchewaung	592.90	0.27	160.08
Robinson	628.94	0.29	182.39

# 3.7 Figures



**Figure 3.1.** Study Area of thesis research. Highlighted lakes used in Data Chapter 2 (Thesis Chapter 3) for the analysis of whole ecosystem Hg biomagnification slopes and Walleye Bioenergetics. Quetico provincial Park boundaries highlighted in Green.



**Figure 3.2.** Single year water temperature models were generated by multiple linear regression of daily mean air and water temperatures (Eq 3.6). Estimated surface temperature (blue) plotted against observed water temperature data (pink). Walleye thermal optima = 22.1°C and temperature during Ice-on period was assumed 2°C. Panel A= Batchewaung (Model 1), Panel B = French lake (Model 2). Panel C = Pickerel Lake (Model 3). Model numbers reflected which lakes they are applied to in table 3.1.



**Figure 3.3.** Ecosystem biomagnification slopes of  $Log_{10}$  [MeHg] vs  $\delta^{15}$ N‰ across invasion classes of QPP lakes. Biomagnification slopes estimated from 3 trophic levels collected during 2022 and fitted with a common slope. Invaded lakes (Saganagons, Pickerel, French) are denoted by warm colors: light red to Maroon. While uninvaded lakes (Agnes, Batchewaung, Beaverhouse, Minn, Robinson,) are denoted by cool colors: Light blue to Navy. Each point represents an individual organism and shape indicates trophic position.



**Figure 3.4**. Boxplots of Log10 Hg concentrations (ppm) (Panel A) and Nitrogen stable isotope ratios ( $\delta^{15}$ N‰) (Panel B) for *Bythotrephes*-invaded lakes\* (red) and uninvaded lakes (blue) at each of four trophic levels.



**Figure 3.5.** von Bertalanffy growth functions representing age-at-length (mm) for Walleye from 8 QPP lakes (Panel A). Single phase von Bertalanffy functions were used in combination with length-weight relationships (Table A.1) to estimate mass-at-age inputs for the bioenergetics model. For individual model fits to age-at-length data, see Figure A.1. Inputs of Hg-at-age per lake (Panel B) calculated from the asymptotic age-at-length relationship. Relationship of Walleye log10[Hg] (ug/kg)-length for the 8 QPP lakes (Panel C). *Bythotrephes*-invaded lakes are denoted by warm colors (red, orange, brown) while uninvaded lakes are denoted by cool colors (light to dark blue).



**Figure 3.6.**  $Log_{10}$  transformed average Walleye mass (g) plotted against  $Log_{10}$  transformed daily prey consumption in grams (Panel A),  $Log_{10}$  daily energy allocated for activity in joules (Panel B), and  $Log_{10}$  Walleye biomagnification factor (Panel C). *Bythotrephes*-invaded lakes are denoted by warm colors while uninvaded lakes are denoted by cool colors. Each point represents the mean estimate from a single-year cohort.



**Figure 3.7.** Log<sub>10</sub> transformed Growth (g/day) ~ Log<sub>10</sub> Consumption (g/day) of immature Walleye (Age 1-4; Panel A) and mature Walleye (Age  $\geq$ 5; Panel B) from QPP lakes. Note: Differences in both x and y axis scales between Panel A and B. Invaded lakes are denoted by warm colors while uninvaded lakes are denoted by cool colors. Each point represents an estimate for single-year age cohort of Walleye.



**Figure 3.8.** Scatter plots of mean mass-specific (allometrically corrected) Walleye Activity (A), Walleye Biomagnification Factor (B) and Consumption (C), Slopes of Immature (D) and Mature (E) Walleye growth efficiency, Ecosystem Biomagnification intercepts (F), Asymptotic length (G), K (H), and slopes of Walleye length-[Hg] (I) all plotted against Log<sub>10</sub> lake surface area. Red points representative of invaded lakes and blue points are uninvaded lakes. Note: Differences in y-axis scales and increased size of Agnes Lake datapoint to highlight outlier. Significant correlations indicated with an asterisk\*.

## **Chapter 4: Synthesis**

Throughout this thesis, I have examined the potential impacts of invasive *Bythotrephes* on the mercury dynamics of Walleye in northwestern Ontario ecosystems. Examining a broad set of contemporary data, I found that Walleye Hg concentrations varied with lake surface area, fish length, and *Bythotrephes* invasions between lakes. This is not the first study to find an impact of Bythotrephes on percids (Hansen et al. 2020, Gartshore and Rennie 2023; both of which examined juvenile percids only), nor is it the first to find an effect of lake size on Hg accumulation and biomagnification (Kamman et al. 2005, Kidd et al. 2012). The relationship between fish body size and mercury concentration is also well documented (Scott and Armstrong 1972, Storelli et al. 2007, Dang and Wang 2012). However, this study differs from previously published research in that it is among the first to examine the effects of *Bythotrephes* invasions on large piscivorous Walleye. Extending this analysis to temporal scales, comparing lakes before and after invasion, I uncovered a significant effect of *Bythotrephes* on [MeHg] bioaccumulation in Walleye of the Rainy Lake complex. In both the temporal and spatial analyses, smaller Walleye had greater [Hg] in the presence of *Bythotrephes* than without. In my spatial analysis, differences between invaded and uninvaded lakes increased with lake size; for small lakes, small (20cm) Walleye predicted [Hg] was 8.5% higher in invaded lakes than uninvaded lakes while for large lakes predicted [Hg] was 171% greater in invaded lakes. This difference associated with lake size decreased as fish size increased; for large (50 cm) Walleye, the difference between invaded and uninvaded lakes was ~17% regardless of lake size. At smaller sizes (20cm) Rainy Lake Walleye after Bythotrephes invasions had a 26% more Hg than a similar sized Walleye collected before invasion. Elevation of Walleye Hg in Rainy Lake following invasion were apparent up

until large sizes ( $\geq$ 60cm) where differences were moderate (~2%). Smaller Walleye are likely more susceptible to increases in [Hg] as a result of *Bythotrephes* invasions given declines in growth and prey availability within invaded lakes (Hansen et al. 2020, Gartshore and Rennie 2023). If small Walleye have reduced prey availability in an invaded lake and must expend extra foraging effort to obtain prey, I hypothesized that their growth efficiency likely suffers resulting in increased contaminant concentrations.

To attempt to explain these results associated with *Bythotrephes* invasions, I tested my hypothesis that changes in growth efficiency or ecosystem biomagnification (food chain length) would be impacted by *Bythotrephes*. Trophic elevation and food chain elongation have long been predicted to result from mid-trophic invasions in aquatic ecosystems (Swanson et al. 2003, Rennie et al. 2011). In my second data chapter (Chapter 3), I tested these hypotheses through examination of ecosystem biomagnification of Hg derived from stable isotope data and bioenergetics modelling of a set of 8 Walleye populations. The analysis of ecosystem biomagnification of Hg did not support this hypothesis: I found that food chain length derived from stable isotopes was not longer for invaded lakes than uninvaded lakes, and that no significant differences in ecosystem biomagnification slopes were associated with *Bythotrephes*. Using a bioenergetics model, I tested another mechanism for mid-trophic invasion elevating predatory fish Hg, which is that a systematic shift in decreased growth efficiency will results in greater accumulation of mercury per prey item consumed. The bioenergetics model generated mass-relative slopes of Consumption, Activity, and Walleye BMF as well as slopes of Walleye (immature and mature) growth efficiency that differed among lakes but did not seem to group based on whether lakes were invaded with *Bythotrephes* 

or not. Similar results were reported for the inputs of the model ( $L_{\infty}$ , K, and slopes of Hglength) where differences were not associated with invasion status. These results offer no support for either mechanism I proposed to explain my Chapter 2 results.

Somewhat surprisingly though, further testing yielded significant correlations between lake surface area and mass-specific bioenergetics outputs. The relationship between lake size (surface area) and Walleye Hg in the set of lakes I examined appeared to be a an easier to detect regarding Walleye bioenergetics and Hg bioaccumulation patterns than those associated with Bythotrephes invasions. I observed significant declines in mass-specific Walleye activity, consumption and BMF with increasing lake size, and a positive correlation with Hg-length slope with lake size. This is supported by my first data chapter where lake size was found to significantly interact with Walleye-Hglength and *Bythotrephes* invasions. The positive correlation of lake surface area on Walleye [Hg] is the exact trend observed in Chapter 2, where the slope of Walleye Hglength increased with lake size regardless of invasion status. The correlation test of growth efficiency was not significant, but a slightly stronger relationship with the removal of a potential outlier value (Agnes Lake) provides weak evidence that growth efficiency may increase with lake size. Walleye in Agnes Lake reach asymptotic size very quicky compared to other lakes and appear to stop growing in length entirely after reaching maturity. This means that mature Walleye in Agnes Lake have some limiting factor resulting in a relatively small asymptotic size for the population. Seeing as they grow very quickly before maturity this limiting factor likely has a disproportionate effect on mature Walleye and could be the result of limited access to a favourable prey type, or increased competition. Regardless given the small sample size (number of lakes) included

in this analysis, extreme values like Agnes Lake have a significant effect on my ability to interpret that data and assess the overall pattern.

The observation of a potential correlation between lake size and growth efficiency, combined with the significant decease in mass-specific activity in larger lakes provides some evidence for the mechanism of systematic changes to bioenergetics and growth efficiency proposed in my first data chapter. In both data chapters I found significant effects of lake size, but only found an effect of *Bythotrephes* in the first chapter which had a much larger sample size. In Chapter 3, I compared my sample size and lake surface area range to other studies which found similar trends but report stronger correlations in biomagnification and life history with lake size than observed in my study (Kidd et al. 2012, McDermid et al. 2010). Here, I make the same comparison between my two data chapters where the sample size of the spatial analysis in my data first chapter is large enough for detection of the weaker effect (Bythotrephes) within the larger effect (surface area), but the sample size of the second data chapter is not. Comparison of the sample sizes between data chapters and surface area ranges are such that analysis in Chapter 2 were able to utilize 23 more lakes than Chapter 3, as well has having an additional range of 4000 hectares to test the relationship of surface area with Bythotrephes invasions. A larger sample size for bioenergetics and ecosystem biomagnification analyses would be beneficial to further testing, as a better ratio of predictor variables to lakes would allow for environmental variables like lake surface area to be tested as fixed effects along with invasion status.

A recurrent issue of this study was sample size. Due to relatively weak historical sampling, I could not discern a significant effect on Walleye [MeHg] over time in QPP

for either invaded lakes or uninvaded reference lakes in Chapter 2. Similarly in Chapter 3, my analysis was potentially limited by a relatively small sample of lakes. The primary reason that Chapter 3 could only utilize data from 8 lakes comes down to logistic challenges, specifically those posed by the landscape of the study area. QPP is a pristine wilderness but is traversable almost exclusively by canoe, which would not suit my sampling needs. Coupled with the size of the park and the time it would take to travel by portage to each lake I had to prioritise those with the most data and fly into the ones not accessible by land. In hindsight a more effective way to amass a larger sample size (specifically for ecosystem biomagnification and bioenergetics) would be to perform this analysis in a location better equipped to support extensive sampling, for example the Experimental Lakes Area. An alternative would be to conduct this analysis across a regional/provincial scale.

After completing this study, I have several suggestions regarding contaminant sampling, fisheries management, and future studies investigating the effects of invasive *Bythotrephes*. I suggest during future sampling efforts in QPP, [Hg] collection and analysis should be extended to prominent forage fish species (Yellow perch, Cisco, Rainbow smelt). These are important Walleye prey species (Sheppard et al. 2015) and studies have already shown that *Bythotrephes* can impact contaminant accumulation patterns in prey fish (Rennie et al. 2023 in review). In addition to being important for Walleye, Cisco, smelt, and yellow perch are often consumed by humans; in 2021 those species combined accounted for over 4000 tonnes in commercial landings (Department of fisheries and Oceans 2021), with significant quantities of these fish harvested by recreational anglers (Department of fisheries and Oceans 2019). The results of this study

also suggest the potential need for revisions to consumption recommendations for Walleye in lakes where guidelines are not based on recent sampling data (i.e., data collected before invasions). I have demonstrated the [Hg] in small Walleye has increased with the presence of invasive Bythotrephes both in QPP and in Rainy Lake. This is concerning as the greatest differences (8-170%) were observed in Walleye of harvestable size, which are most likely to be consumed by anglers. Specifically, within Quetico PP there is elevated concern as anglers on extended backwoods canoe trips often supplement their diet with Walleye and are likely to consume more fish than the average angler during these trips. My final suggestion is to highlight areas where future studies could improve upon the approach taken in the thesis. Specifically, (1) organizing sampling efforts that would allow a larger sample size for bioenergetics and ecosystem biomagnification investigation (including a larger surface area range for comparison to other studies) and (2) seasonally repeated sampling of zooplankton. As demonstrated in both my data chapters, lake size is related to Walleye bioaccumulation and bioenergetics, but only in my first data chapter did I find a significant effect of *Bythotrephes*. A likely reason behind this discrepancy is the difference in the number of lakes in both analyses as well as the range that lake surface area is examined over, to improve the analysis of Walleye bioenergetics impacted by Bythotrephes. As such I suggest sampling more lakes across a larger range of surface areas. The second recommendation for future studies would be the sampling of zooplankton MeHg and isotopes at more than one point per year given fluctuations in zooplankton population abundance (Coguiec et al. 2021, Bellier et al. 2022) and isotopic composition (Matthews and Mazumder 2005; Rennie et al. 2013); additional sampling of this component of the food web would likely provide a

better estimation of baseline trophic positions integrated over similar time scales as the tissue measurements reflected in the Walleye collected as part of this study.

Through the research presented I have filled gaps in knowledge regarding *Bythotrephes* invasion status on fish mercury accumulation, ecosystem biomagnification, and bioenergetics. Specifically, I found that the presence of *Bythotrephes* increased [Hg] of small (but harvestable) Walleye both across lakes, and over time. I found that lake size significantly correlates to mass specific Walleye bioenergetics and is a greater determinant of Hg-at-length and bioenergetics than *Bythotrephes* invasion status. While uncertainties exist regarding the mechanisms responsible, I am very confident in both the spatial (QPP) and temporal (Rainy Lake) analysis and the results of my Bioenergetics-Lake size analysis. In my first data chapter I suggested early life stressors may alter growth trajectories later in life. However, when I applied a bioenergetics model to many of the same populations included in that analysis, I found no evidence to support this hypothesis, as there was no clear trend between fish growth efficiency, asymptotic size, *K*, or any other bioenergetics variable associated with *Bythotrephes* invasions.

Overall, the findings of both data chapters suggest that lake surface area is a stronger effect on Walleye bioenergetics and mercury accumulation than *Bythotrephes* invasions, but that *Bythotrephes* do appear to have a significant effect over time and across space. Applying a larger sample size (~3 times larger than what is presented here) for either temporal analysis or spatial approach to bioenergetic or ecosystem biomagnification investigations of *Bythotrephes* impacts may be required to be able to address mechanistic hypotheses for patterns of elevated Walleye Hg in lakes invaded by *Bythotrephes*. I believe these findings provide a justification for both (a) better

monitoring of uninvaded lakes that are at high risk of invasion (e.g. close to invaded lakes on common canoe routes in the park, or those frequently visited by fly-in outfitters, etc), (b) more comprehensive sampling of lakes at high risk of invasion, specifically sampling dedicated to documenting lower food web biomagnification characteristics (Hg and  $\delta^{15}$ N) for future investigations and/or modelling efforts in evaluating species invasion impacts, and (c) investigation of physical environmental parameters related to lake surface area that may be driving this relationship.

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Figure A.1. Individual von Bertalanffy growth function (VBGM) inputs for lakes included within the bioenergetics analysis. Invaded lakes denoted with an asterisk\*.

	Log <sub>10</sub> (Mass)-	Log <sub>10</sub> (length)	h) $Log_{10}(Hg)$ -length			
Waterbody Name	Intercept	Slope	Intercept	Slope		
Pickerel*	-5.21	3.09	1.53	0.0029		
Saganagons*	-5.09	3.04	1.36	0.0026		
French*	-4.59	2.86	2.11	0.002		
Batchewaung	-3.49	2.44	1.74	0.0024		
Minn	-5.10	3.04	1.94	0.0022		
Agnes	-5.15	3.06	1.15	0.0034		
Robinson	-5.04	3.02	1.93	0.0019		
Beaverhouse	-5.95	3.37	1.63	0.0023		

Table A.1. Regression coefficients used to calculate mass at age and Hg at age. Invaded lakes denoted with an asterisk\*.

		Mass Scaling Exponent	
Waterbody Name	Activity	Consumption	BMF
Agnes	1.48	1.11	0.71
Batchewaung	1.32	1.21	0.59
Beaverhouse	1.45	1.12	0.59
French*	1.12	1.04	0.52
Minn	1.22	1.07	0.51
Pickerel*	1.32	1.21	0.59
Robinson	1.13	1.05	0.56
Saganagons*	1.47	1.20	0.63

Table A.2. Mass scaling exponents for 3 Walleye bioenergetics variables. Exponents are calculated as the slope coefficient of each variable  $(log_{10})$  plotted against  $log_{10}$  Walleye mass (g). Invaded lakes denoted with an asterisk\*.

Table A.3: Bioenergetics + MMBM inputs and outputs for QPP Walleye populations. Weight (W) in grams, Hg and *Cd* in ppm. Sex not listed as only female Walleye bioenergetics was conducted. Maturity: immature = 1, mature = 2. *Act* = activity multiplier. *C* = consumption, *G* = growth, *SMR* = standard metabolic rate, *A* = Activity *F*= Egestion, U= Excretion, *SDA*= Specific dynamic action,  $W_{avg}$  = Average mass over modelled time, *BMF* = Biomagnification factor. All outputs in Joules except  $W_{avg}$  (g), *C*, and *G* (g/g/day). Activity multiplier and *BMF* (unitless). Lakes invaded with *Bythotrephes* denoted with an asterisk\*. Lake Names abbreviated as French; FR, Pickerel; PC, Saganagons= SA, Agnes = AG, Minn = MN, Beaverhouse = BH, Batchewaung = BT, Robinson = RB.

			Input									Output				
Lake	Age	Mat	Wo	$W_t$	$Hg_{\theta}$	$Hg_t$	Cd	С	G	SMR	A	$\boldsymbol{F}$	U	<b>SDA</b>	Wavg	BMF
FR*	3	1	446	707	0.62	0.81	0.086	16.9	0.7	10134	51911	15469	2378	13991	878	7.2
FR*	4	1	707	953	0.81	1	0.086	23.4	0.66	13368	74081	21512	3299	19416	1235	9.3
FR*	5	2	953	1166	1	1.16	0.086	33.8	0.75	16460	110715	31206	4765	28060	1612	9.1
FR*	6	2	1166	1341	1.16	1.29	0.086	39.4	0.69	18687	130379	36433	5554	32716	1885	10.2
FR*	7	2	1341	1480	1.29	1.4	0.086	43.6	0.63	20383	144969	40312	6139	36164	2098	11
FR*	8	2	1480	1589	1.4	1.49	0.086	46.6	0.57	21651	155392	43088	6558	38631	2260	11.6
FR*	9	2	1589	1672	1.49	1.55	0.086	48.6	0.52	22589	162694	45038	6852	40363	2381	12
FR*	10	2	1672	1735	1.55	1.6	0.086	50.1	0.49	23280	167769	46399	7057	41571	2470	12.4
FR*	11	2	1735	1783	1.6	1.64	0.086	51.1	0.46	23787	171297	47349	7200	42414	2536	12.6
FR*	12	2	1783	1818	1.64	1.67	0.086	51.8	0.44	24159	173762	48015	7300	43006	2585	12.8
FR*	13	2	1818	1845	1.67	1.69	0.086	52.3	0.42	24432	175497	48485	7371	43423	2621	12.9
FR*	15	2	1864	1879	1.71	1.72	0.086	52.9	0.4	24780	177606	49059	7458	43933	2666	13
PC*	3	1	274	488	0.26	0.38	0.063	8.5	0.58	6781	23496	7761	1198	7039	529	5.1
PC*	4	1	488	721	0.38	0.53	0.063	15.1	0.63	10062	45304	13799	2123	12484	865	7.2
PC*	5	2	721	952	0.53	0.68	0.063	25.4	0.77	13470	81298	23410	3584	21096	1255	7.8
PC*	6	2	952	1167	0.68	0.83	0.063	34.8	0.76	16616	114547	32170	4910	28920	1633	9.3

Table A.3 Continued.

			Input									Output				
Lake	Age	Mat	$W_{\theta}$	$W_t$	$Hg_{\theta}$	$Hg_t$	Cd	С	G	SMR	$\boldsymbol{A}$	$oldsymbol{F}$	$oldsymbol{U}$	<b>SDA</b>	Wavg	BMF
PC*	7	2	1167	1359	0.83	0.98	0.063	44	0.72	19417	147044	40672	6193	36486	1985	10.5
PC*	8	2	1359	1525	0.98	1.11	0.063	52.3	0.67	21824	176734	48412	7357	43354	2299	11.6
PC*	9	2	1525	1666	1.11	1.23	0.063	59.4	0.62	23841	202592	55143	8367	49310	2569	12.5
PC*	10	2	1666	1784	1.23	1.33	0.063	65.5	0.57	25500	224361	60806	9214	54311	2795	13.2
PC*	11	2	1784	1882	1.33	1.42	0.063	70.4	0.53	26847	242261	65463	9910	58417	2981	13.8
PC*	12	2	1882	1961	1.42	1.49	0.063	74.4	0.49	27931	256745	69232	10472	61735	3133	14.3
PC*	13	2	1961	2026	1.49	1.55	0.063	77.6	0.46	28799	268341	72250	10922	64390	3255	14.7
PC*	14	2	2026	2078	1.55	1.6	0.063	80.2	0.43	29490	277563	74652	11280	66501	3353	15
PC*	15	2	2078	2120	1.6	1.64	0.063	82.2	0.41	30040	284867	76555	11563	68172	3432	15.2
PC*	16	2	2120	2154	1.64	1.68	0.063	83.8	0.4	30476	290638	78059	11787	69493	3494	15.4
PC*	19	2	2202	2219	1.72	1.74	0.063	86.8	0.36	31312	301628	80925	12214	72008	3614	15.8
PC*	21	2	2233	2244	1.76	1.76	0.063	87.2	0.35	31524	302697	81227	12259	72275	3643	15.8
SA*	2	1	133	325	0.1	0.16	0.063	2.7	0.53	3953	4096	2423	376	2205	269	2.3
SA*	3	1	325	565	0.16	0.23	0.063	5.8	0.66	6919	12621	5280	817	4795	536	3.6
SA*	4	1	565	820	0.23	0.31	0.063	9.7	0.69	9984	24520	8878	1371	8050	845	5
SA*	5	1	820	1066	0.31	0.39	0.063	13.8	0.66	12856	37540	12641	1949	11447	1157	6.4
SA*	6	2	1066	1290	0.39	0.46	0.063	20.9	0.9	15438	61146	19160	2942	17298	1464	6.4
SA*	7	2	1290	1486	0.46	0.54	0.063	25.4	0.87	17694	76670	23400	3589	21102	1735	7.3
SA*	8	2	1486	1652	0.54	0.6	0.063	29.4	0.84	19576	90132	27051	4144	24373	1968	8
SA*	9	2	1652	1791	0.6	0.65	0.063	32.6	0.8	21113	101301	30069	4603	27074	2163	8.5
SA*	10	2	1791	1904	0.65	0.7	0.063	35.2	0.76	22352	110294	32496	4971	29245	2322	9
SA*	11	2	1904	1997	0.7	0.74	0.063	37.3	0.73	23340	117396	34413	5262	30958	2450	9.3

Table A.3 Continued.

			Input									Output				
Lake	Age	Mat	W <sub>0</sub>	$W_t$	$Hg_{\theta}$	$Hg_t$	Cd	С	G	SMR	A	F	$oldsymbol{U}$	<b>SDA</b>	Wavg	BMF
SA*	12	2	1997	2071	0.74	0.77	0.063	38.9	0.7	24123	122940	35910	5489	32295	2553	9.6
SA*	13	2	2071	2130	0.77	0.79	0.063	40.2	0.68	24741	127237	37072	5665	33333	2634	9.8
SA*	14	2	2130	2177	0.79	0.81	0.063	41.1	0.66	25227	130559	37971	5801	34136	2699	10
SA*	15	2	2177	2215	0.81	0.83	0.063	41.9	0.64	25608	133123	38666	5907	34757	2750	10.1
SA*	16	2	2215	2244	0.83	0.84	0.063	42.5	0.63	25908	135104	39204	5988	35237	2790	10.2
SA*	17	2	2244	2268	0.84	0.85	0.063	42.9	0.62	26142	136636	39620	6051	35608	2821	10.3
SA*	18	2	2268	2286	0.85	0.86	0.063	43.2	0.62	26326	137822	39943	6100	35896	2845	10.3
SA*	19	2	2286	2301	0.86	0.87	0.063	43.5	0.61	26470	138742	40193	6138	36120	2865	10.4
SA*	20	2	2301	2312	0.87	0.87	0.063	43.7	0.6	26582	139456	40388	6168	36294	2880	10.4
SA*	21	2	2312	2321	0.87	0.88	0.063	43.9	0.6	26671	140012	40539	6191	36429	2892	10.4
AG	2	1	226	469	0.14	0.24	0.097	3.7	0.67	5476	5644	3290	510	2992	401	2.5
AG	3	1	469	707	0.24	0.36	0.097	6.9	0.65	8501	14597	6118	946	5553	690	4.1
AG	4	1	707	905	0.36	0.47	0.097	9.5	0.54	10945	22375	8444	1305	7655	942	5.4
AG	5	2	905	1057	0.47	0.57	0.097	13.3	0.7	12646	34178	11838	1824	10707	1134	5.5
AG	6	2	1057	1169	0.57	0.64	0.097	14.9	0.63	13938	39041	13244	2039	11973	1279	6.1
AG	7	2	1169	1248	0.64	0.69	0.097	15.8	0.58	14822	41829	14069	2166	12716	1380	6.4
AG	8	2	1248	1304	0.69	0.73	0.097	16.3	0.54	15420	43330	14531	2237	13132	1449	6.7
AG	9	2	1304	1342	0.73	0.76	0.097	16.6	0.51	15821	44111	14786	2276	13361	1495	6.8
AG	10	2	1342	1368	0.76	0.78	0.097	16.7	0.49	16090	44510	14926	2297	13487	1526	6.9
AG	11	2	1368	1386	0.78	0.79	0.097	16.8	0.48	16270	44715	15004	2309	13558	1548	6.9
AG	12	2	1386	1398	0.79	0.8	0.097	16.9	0.47	16391	44822	15049	2316	13599	1562	6.9
AG	13	2	1398	1406	0.8	0.81	0.097	16.9	0.46	16472	44879	15076	2321	13623	1571	7
AG	16	2	1415	1418	0.82	0.82	0.097	16.9	0.45	16588	44939	15109	2326	13653	1585	7

Table A.3 Continued.

			Input									Output				
Lake	Age	Mat	W <sub>0</sub>	$W_t$	$Hg_{\theta}$	$Hg_t$	Cd	С	G	SMR	A	F	U	<b>SDA</b>	Wavg	BMF
AG	19	2	1420	1421	0.82	0.82	0.097	17	0.45	16623	44952	15118	2327	13661	1589	7
AG	20	2	1421	1422	0.82	0.82	0.097	17	0.45	16628	44954	15119	2327	13662	1589	7
MN	2	1	74	182	0.24	0.34	0.079	2.6	0.3	2723	5464	2229	345	2027	171	3.3
MN	3	1	182	319	0.34	0.44	0.079	5	0.37	4682	12192	4369	675	3966	332	4.7
MN	4	1	319	465	0.44	0.55	0.079	7.7	0.4	6685	20306	6803	1050	6167	515	6
MN	5	2	465	608	0.55	0.65	0.079	11.9	0.51	8605	33733	10584	1628	9569	710	6.3
MN	6	2	608	739	0.65	0.74	0.079	15.1	0.51	10321	43947	13441	2064	12138	889	7.2
MN	7	2	739	854	0.74	0.82	0.079	17.9	0.49	11794	53123	15982	2452	14419	1050	8
MN	8	2	854	953	0.82	0.89	0.079	20.3	0.47	13027	60961	18141	2781	16355	1187	8.6
MN	9	2	953	1037	0.89	0.95	0.079	22.3	0.45	14040	67432	19918	3051	17948	1303	9.1
MN	10	2	1037	1105	0.95	1	0.079	23.9	0.43	14862	72656	21351	3269	19231	1398	9.5
MN	11	2	1105	1162	1	1.04	0.079	25.1	0.41	15523	76812	22492	3443	20252	1476	9.8
MN	12	2	1162	1207	1.04	1.07	0.079	26.1	0.4	16050	80088	23391	3579	21057	1538	10
MN	13	2	1207	1244	1.07	1.1	0.079	26.9	0.39	16471	82659	24097	3687	21689	1588	10.2
MN	14	2	1244	1273	1.1	1.12	0.079	27.5	0.37	16804	84670	24651	3771	22184	1628	10.4
MN	15	2	1273	1297	1.12	1.14	0.079	28	0.37	17068	86242	25083	3836	22571	1660	10.5
MN	16	2	1297	1316	1.14	1.15	0.079	28.4	0.36	17276	87470	25422	3888	22874	1685	10.6
BH	2	1	193	496	0.19	0.3	0.131	3.7	0.83	5414	5433	3384	525	3080	398	2.1
BH	3	1	496	877	0.3	0.43	0.131	7.9	1.04	9614	16507	7220	1117	6556	808	3.3
BH	4	1	877	1273	0.43	0.56	0.131	12.7	1.07	13838	30478	11590	1790	10508	1267	4.5
BH	5	2	1273	1644	0.56	0.69	0.131	19.8	1.41	17495	53085	18209	2802	16463	1706	4.9
BH	6	2	1644	1969	0.69	0.8	0.131	24.9	1.39	20770	69264	22846	3511	20634	2111	5.6

Table A.3 Continued.

			Input									Output				
Lake	Age	Mat	$W_{\theta}$	$W_t$	$Hg_{\theta}$	$Hg_t$	Cd	С	G	SMR	$\boldsymbol{A}$	F	$oldsymbol{U}$	<b>SDA</b>	$W_{avg}$	BMF
BH	7	2	1969	2243	0.8	0.9	0.131	28.9	1.34	23441	82520	26614	4087	24018	2453	6.2
BH	8	2	2243	2465	0.9	0.98	0.131	32.1	1.28	25560	92704	29506	4528	26614	2731	6.7
BH	9	2	2465	2643	0.98	1.04	0.131	34.4	1.22	27210	100218	31648	4855	28536	2951	7
BH	11	2	2782	2891	1.1	1.14	0.131	37.3	1.13	29450	109492	34319	5263	30933	3254	7.5
BT	3	1	453	694	0.34	0.48	0.062	13.7	0.65	9491	39158	12219	1881	11059	803	6.7
BT	4	1	694	922	0.48	0.62	0.062	20.3	0.61	12592	60837	18110	2780	16355	1141	8.9
BT	5	2	922	1122	0.62	0.76	0.062	30.8	0.73	15573	96546	27564	4211	24794	1500	9
BT	6	2	1122	1292	0.76	0.89	0.062	37.7	0.67	17941	120580	33890	5166	30429	1790	10.2
BT	7	2	1292	1431	0.89	1	0.062	43.6	0.62	19852	140778	39189	5965	35138	2031	11.2
BT	8	2	1431	1544	1	1.09	0.062	48.3	0.56	21361	156985	43435	6603	38905	2226	12
BT	9	2	1544	1633	1.09	1.17	0.062	51.9	0.52	22535	169605	46742	7100	41835	2379	12.6
BT	10	2	1633	1704	1.17	1.24	0.062	54.7	0.48	23440	179256	49272	7480	44074	2499	13.1
BT	11	2	1704	1760	1.24	1.29	0.062	56.8	0.45	24134	186559	51187	7767	45768	2591	13.5
BT	12	2	1760	1803	1.29	1.33	0.062	58.4	0.43	24666	192060	52632	7983	47045	2662	13.7
BT	13	2	1803	1836	1.33	1.36	0.062	59.6	0.41	25072	196198	53719	8146	48006	2717	13.9
BT	14	2	1836	1862	1.36	1.39	0.062	60.5	0.39	25381	199312	54539	8269	48730	2758	14.1
BT	15	2	1862	1882	1.39	1.41	0.062	61.1	0.38	25618	201661	55157	8362	49276	2790	14.2
RB	2	1	220	509	0.29	0.43	0.057	12.1	0.78	7241	34569	10700	1649	9706	585	4.9
RB	3	1	509	843	0.43	0.57	0.057	21.7	0.9	11732	65766	19362	2972	17494	1058	7.3
RB	4	1	843	1172	0.57	0.71	0.057	31.6	0.88	15968	98810	28302	4331	25501	1548	9.6
RB	5	2	1172	1469	0.71	0.84	0.057	47.1	1.01	20153	151865	42310	6440	37943	2083	9.6
RB	6	2	1469	1722	0.84	0.94	0.057	56.5	0.94	23305	184161	50850	7723	45510	2494	10.9

Table A.3 Concluded.

			Input									Output				
Lake	Age	Mat	$W_{\theta}$	$W_t$	$H_{0}$	$H_t$	Cd	С	G	SMR	$\boldsymbol{A}$	$\boldsymbol{F}$	$oldsymbol{U}$	<b>SDA</b>	$W_{avg}$	BMF
RB	7	2	1722	1929	0.94	1.03	0.057	63.8	0.85	25781	209380	57513	8721	51400	2825	11.9
RB	8	2	1929	2095	1.03	1.11	0.057	69.2	0.78	27681	228208	62492	9466	55794	3084	12.6
RB	9	2	2095	2225	1.11	1.16	0.057	73.2	0.71	29120	241898	66119	10008	58992	3283	13.2
RB	10	2	2225	2326	1.16	1.21	0.057	76.1	0.66	30200	251718	68726	10398	61290	3433	13.6
RB	11	2	2326	2404	1.21	1.24	0.057	78.1	0.61	31008	258729	70592	10677	62934	3546	13.9
RB	12	2	2404	2463	1.24	1.27	0.057	79.6	0.58	31613	263761	71934	10877	64117	3630	14.2
RB	13	2	2463	2508	1.27	1.29	0.057	80.6	0.55	32062	267331	72890	11020	64959	3694	14.4

Lake Name	Sample Type	δ <sup>13</sup> C	$\delta^{15}N$	Hg (ppm)
French*	W	-27.6	8.7	0.596
French*	W	-27.1	9.6	1.007
French*	W	-27.3	10.2	1.22
French*	W	-28.4	11.2	1.431
French*	W	-28.3	11.4	2.114
French*	W	-28.2	11.3	2.114
French*	S	-29.2	8.4	0.102
French*	S	-29.4	9.1	0.131
French*	S	-29.3	8.3	0.104
French*	S	-29.9	8.9	0.102
French*	S	-30.5	7.5	0.046
French*	В	-27.3	1	0.01
French*	В	-26.7	0.6	0.006
French*	В	-31.6	4.5	0.006
French*	Ζ	-31.8	4.4	0.021
French*	Ζ	-26.5	0.8	0.007
Batchewaung	W	-25.4	7.7	0.405
Batchewaung	W	-24	7.1	0.435
Batchewaung	W	-26.2	7.7	0.471
Batchewaung	W	-25.4	7.6	0.904
Batchewaung	W	-23.2	10.1	2.09
Batchewaung	W	-29.2	6.9	0.047
Batchewaung	S	-28.5	5.6	0.083
Batchewaung	S	-29.5	7.2	0.093
Batchewaung	S	-22.6	4.5	0.025
Batchewaung	S	-30.2	7.7	0.045
Batchewaung	S	-29.1	7.8	0.045
Batchewaung	В	-28	-1.2	0.012
Batchewaung	В	-28	-0.4	0.005
Batchewaung	В	-31	6.3	0.008
Batchewaung	Ζ	-30.7	7.3	0.002
Batchewaung	Z	-26.8	0.6	0.002

Table A.4. Raw stable isotope data. Invaded lakes denoted with an asterisk\*. Sample type abbreviations are W = Walleye, S= Walleye Stomach Contents, B = Benthic Invertebrates, Z = Zooplankton.

Table A.4. Continued

Lake Name	Sample Type	$\delta^{13}C$	$\delta^{15}N$	Hg (ppm)
Pickerel*	W	-27.5	8.8	0.444
Pickerel*	W	-26.8	8.2	0.399
Pickerel*	W	-26.8	9.4	0.718
Pickerel*	W	-25.7	7.8	0.969
Pickerel*	W	-27.7	11.1	1.58
Pickerel*	W	-27.2	10.3	1.58
Pickerel*	S	-28.6	6.7	0.059
Pickerel*	S	-31.4	2.5	0.302
Pickerel*	S	-25.9	2	0.047
Pickerel*	S	-31	4.4	0.143
Pickerel*	S	-30.1	3.6	0.066
Pickerel*	В	-27.5	0.2	0.004
Pickerel*	В	-26.8	0.1	0.007
Pickerel*	В	-32.7	4.4	0.007
Pickerel*	Ζ	-33	4.5	0.002
Pickerel*	Z	-26.5	0.1	0.002
Beaverhouse	W	-24.1	7.5	0.295
Beaverhouse	W	-24	7.9	0.235
Beaverhouse	W	-24.1	8	0.394
Beaverhouse	W	-20.6	7.7	0.453
Beaverhouse	W	-24.1	9.3	1.759
Beaverhouse	W	-26.3	5.8	0.05
Beaverhouse	S	-26.1	6.7	0.044
Beaverhouse	S	-20.5	1.7	0.12
Beaverhouse	S	-26.7	3.8	0.214
Beaverhouse	S	-28.4	7.5	0.227
Beaverhouse	S	-28.5	7.2	0.227
Beaverhouse	В	-27.7	0.1	0.001
Beaverhouse	В	-18.6	2.8	0.006
Beaverhouse	В	-36	1	0.009
Beaverhouse	Ζ	-35.7	0.9	0.002
Beaverhouse	Z	-26.8	-0.2	0.002

Table A.4. Continued

Lake Name	Sample Type	δ <sup>13</sup> C	$\delta^{15}N$	Hg (ppm)
Robinson	W	-26.2	8.3	0.656
Robinson	W	-24.6	6.2	0.203
Robinson	W	-26.2	8.3	0.561
Robinson	W	-27.7	9.2	1.112
Robinson	W	-27.9	10.4	1.041
Robinson	S	-18.2	5.8	0.024
Robinson	S	-26.8	3.8	0.046
Robinson	S	-28.2	5.5	0.04
Robinson	S	-27.2	3.8	0.087
Robinson	S	-26.1	4.3	0.052
Robinson	В	-23.9	2.4	0.006
Robinson	В	-28.8	-3	0.012
Robinson	В	-33.8	1.8	0.011
Robinson	Ζ	-33.4	1.9	0.002
Robinson	Ζ	-28.3	-2.4	0.002
Minn	W	-25.5	8	0.262
Minn	W	-27.1	8.6	0.502
Minn	W	-27.7	8.5	0.721
Minn	W	-27.2	9.1	1.037
Minn	W	-27	9.1	0.849
Minn	W	-29.5	5.8	0.228
Minn	S	-28.2	6.8	0.062
Minn	S	-28.6	6.2	0.041
Minn	S	-28.4	6.1	0.068
Minn	S	-27.3	5.7	0.051
Minn	S	-27.4	5.7	0.051
Minn	В	-27.3	1.9	0.009
Minn	В	-26.4	1.5	0.009
Minn	В	-34.4	2.2	0.025
Minn	Z	-35	-0.1	0.008
Minn	Z	-28.7	-2.8	0.007

Table A.4. Concluded

Lake Name	Sample Type	$\delta^{13}C$	$\delta^{15}N$	Hg (ppm)
Agnes	W	-24.1	7.2	0.323
Agnes	W	-24.1	6.7	0.243
Agnes	W	-24.6	7.6	0.94
Agnes	W	-24.4	7.4	1.043
Agnes	W	-24.9	7.7	0.949
Agnes	W	-24.4	7.4	0.949
Agnes	S	-25.1	3.5	0.049
Agnes	S	-25.7	4.4	0.071
Agnes	S	-28.6	2.4	0.021
Agnes	S	-18.2	5.6	0.122
Agnes	S	-28.1	2.4	0.032
Agnes	В	-28.5	-3.5	0.006
Agnes	В	-29.5	-2.6	0.007
Agnes	В	-28.2	-2.1	0.015
Agnes	Ζ	-28.5	-0.9	0.002
Agnes	Z	-29.2	-1.8	0.002
Saganagons*	W	-26	8.6	0.216
Saganagons*	W	-26.2	9.9	0.5
Saganagons*	W	-25	9.8	0.595
Saganagons*	W	-27	10.4	0.685
Saganagons*	W	-26.9	10.2	0.973
Saganagons*	W	-28.6	6.9	0.057
Saganagons*	S	-31.8	6.6	0.036
Saganagons*	S	-30.5	7.3	0.054
Saganagons*	S	-30.9	7.7	0.188
Saganagons*	S	-30.3	7.1	0.029
Saganagons*	S	-30.2	7.1	0.029
Saganagons*	В	-27.4	-1.3	0.002
Saganagons*	В	-27.1	1.1	0.001
Saganagons*	В	-30	1.5	0.014
Saganagons*	Z	-30.1	1.3	0.003
Saganagons*	Z	-27.6	8.7	0.006