

The Role of Invasive *Bythotrephes longimanus* in Lake Food Webs

by

Elizabeth Courtney Hatton

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Abstract

Bythotrephes longimanus, a predatory exotic cladoceran, has spread rapidly to numerous lakes through the Laurentian Great Lakes region of Ontario and North America. Post-invaded lakes are known to have reduced zooplankton species richness, biomass and altered community structure. *Bythotrephes* may also affect the diet and trophic position of macroinvertebrate predators and prey species for fish (e.g., *Mysis relicta*). However, the effects of this species in altering higher trophic levels remain largely unexamined. Using a combined approach of stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and THg analysis, the trophic position of *Bythotrephes* in two invaded lakes was investigated. Based on $\delta^{15}\text{N}$ values, *Bythotrephes* shared a similar trophic position to native macroinvertebrate predators (9 and 7‰ for Peninsula and Harp lakes, respectively). Using a mixing model and stomach content analysis we show that, despite low and patchy lake abundance, *Bythotrephes* may be a key prey item to fish and has approximate dietary contributions similar to native prey items, such as zooplankton, *Chaoborus* and *Mysis*. In both lakes, Hg conformed to predicted biomagnification trends as indicated by $\delta^{15}\text{N}$. When *Bythotrephes* invades lakes with native macroinvertebrate predators, it inserts itself into the same trophic position and does not have major effects on food web length.

Co-Authorship

This thesis conforms to the manuscript format as outlined by the School of Graduate Studies and Research. The manuscript that is a direct result of this thesis and its co-authors is:

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List of Abbreviations

| | |
|-----------------------|---|
| $\delta^{13}\text{C}$ | stable isotope ratio of carbon |
| $\delta^{15}\text{N}$ | stable isotope ratio of nitrogen |
| %MeHg | percent methylmercury of total mercury |
| B | <i>Bythotrephes longimanus</i> |
| C | carbon |
| Ch | <i>Chaoborus spp.</i> |
| DOC | dissolved organic carbon |
| dw | dry weight |
| Hg | mercury |
| LH | lake herring (<i>Coregonus artedii</i>) |
| LT | lake trout (<i>Salvelinus namaycush</i>) |
| M | <i>Mysis relicta</i> |
| MeHg | methylmercury |
| N | nitrogen |
| OME | Ontario Ministry of the Environment |
| PS | pumpkinseed (<i>Lepomis gibbosus</i>) |
| RB | rock bass (<i>Ambloplites rupestris</i>) |
| SM | rainbow smelt (<i>Osmerus mordax</i>) |
| SMB | smallmouth bass (<i>Micropterus dolomieu</i>) |
| THg | total mercury |
| TP | total phosphorus |
| ww | wet weight |
| YP | yellow perch (<i>Perca flavescens</i>) |
| Z | zooplankton |
| Z _{max} | maximum depth |
| Z _{mean} | mean depth |

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Chapter 1:
General Introduction

1.1 Invasive species

Invasive species, also referred to as exotic, non-native or non-indigenous, are defined as successfully reproducing organisms that have been transported to ecosystems in which they previously did not exist (Mills et al. 1994; Mack et al. 2000). Well known examples of invasive species in Ontario aquatic systems include: zebra mussel (*Dreissena polymorpha*), round goby (*Apollonia melanostoma*), fishhook water flea (*Cercopagis pengoi*), Eurasian watermilfoil (*Myriophyllum spicatum*) and purple loosestrife (*Lythrum salicaria*). Due to their impact on the environment and the economy (Pimentel et al. 2000; Simon and Townsend, 2003), species introductions are the subject of increasing ecological interest. Many scientists believe that the introduction of invasive species into lakes will rank among the greatest threats to aquatic biodiversity over the next several decades (Sala et al. 2000).

Historically, geographic barriers to dispersal have restricted the movement of species making species introductions at both local and global scales difficult (Rahel 2007, Maguire 1963). With increased globalization and trans-oceanic shipping routes, humans have not only reduced many of these geographic barriers, but have aided in large-scale dispersal of species. For example, since the opening of the St. Lawrence Seaway in 1959, over seventy new species introductions in the Laurentian Great Lakes have been documented (Ricciardi 2006). The global aquarium and ornamental trade is another significant contributor to exotic species introductions (Padilla and Williams 2004)

It is estimated that of all the exotic species released, only 10% will establish self-sustaining populations in an invaded ecosystem (Kolar and Lodge 2001). Of those that are successful, many are “r” strategists that can promote rapid population growth over

short periods of time (Lodge 1993) and often cause significant ecological change and economic damage (Kolar and Lodge 2001). For example, the invasion of zebra mussels into North American lakes has shifted the importance from pelagic to benthic processes and zebra mussel fouling of industrial and recreational structures (Mills et al. 2003, MacIsaac 1996).

An invasive zooplankton species in the Great Lakes is *Bythotrephes longimanus* Leydig (spiny water flea), hereafter referred to as *Bythotrephes*. *Bythotrephes* is a cladoceran and a zooplankton predator originally from Eurasia (Figure 1-1). Like many other aquatic invasive species, it is hypothesized that *Bythotrephes* was introduced into North America through the unregulated dumping of ballast water by transoceanic ships (Sprules et al. 1990). Since *Bythotrephes* invasion of the Great Lakes in the mid-1980s, it has spread rapidly through the region and into inland lakes (MacIsaac et al. 2004). *Bythotrephes* is very successful at dispersal and invasion in three ways: 1) it can reproduce swiftly through parthenogenesis; 2) its spines are readily caught on rope, fishing line, trailers and boats; and 3) diapausing eggs can survive desiccation and gut passage (Jarnagin et al. 2004). To date, *Bythotrephes* has been identified in over 100 Ontario lakes (ND Yan *personal communication*) with more invaded lakes being discovered each year.

There is growing evidence that *Bythotrephes* invasion can alter the structure of aquatic pelagic food webs, as well as affect fundamental ecosystem processes. Predation by *Bythotrephes* has led to shifts in crustacean zooplankton composition, size structure and total biomass in several of the Great Lakes (Barbiero and Tuchman 2004) and several Ontario inland lakes (e.g., Strecker et al. 2006; Boudreau and Yan 2003, Dumitru et al. 2001). Zooplankton is a key component of freshwater food webs, transferring energy from primary producers (i.e., algae at the base of the food web) to higher trophic levels

(e.g., forage and juvenile fish) (Figure 1-2). A change at this trophic level of the food web could have serious ecosystem consequences.

In the Muskoka region of Ontario, *Bythotrephes* has invaded many lakes already containing communities of native macroinvertebrate predators, including *Mysis relicta* and *Chaoborus spp.* *M. relicta*, hereafter referred to as *Mysis*, is a glacial relict found in lakes formed after the last ice age (Dadswell 1974) and *Chaoborus spp.*, the aquatic larval stage of the phantom midge, is found in most lakes. Both *Mysis* and *Chaoborus* species play an important role in lake food webs, consuming large portions of zooplankton and acting as a key energy link between small zooplankton and fish (Dumitru et al. 2001).

Because it also preys upon zooplankton, it is thought that *Bythotrephes* will enter the food web at about the same trophic position as its analogous native macroinvertebrate predators, including *Mysis* and/or *Chaoborus* (Figure 1-2). However, *Bythotrephes* may not act in a similarly effective trophic pathway to transfer energy from zooplankton to fish; *Bythotrephes* has a caudal spine for protection against predation by small gape-limited fish (Branstrator and Lehman 1996). *Bythotrephes* could also out-compete other planktivores in an invaded lake. *Bythotrephes* can have dramatic effects on native zooplankton communities (Boudreau and Yan 2003, Strecker et al. 2006) and may reduce the food supply to forage fish and native macroinvertebrate predators. For example, a four-fold decrease in abundance of cladoceran zooplankton was reported in 10 Muskoka Ontario lakes with *Bythotrephes* compared to 4 lakes without *Bythotrephes* (Strecker et al. 2006). These effects could directly and indirectly alter the trophic transfer of energy and translate to changes in contaminant concentrations at higher trophic

levels. The role of many invasive species on energy pathways and contaminant transfer is still poorly understood (Shuter and Mason 2001).

1.2 Mercury in aquatic ecosystems

By altering food web interactions and trophic transfer of energy, invasive species may affect contaminant transfer (Vander Zanden and Rasmussen 1996, Cabana et al. 1994). One of the major contaminants of concern in aquatic ecosystems is mercury (Hg), a ubiquitous contaminant distributed through natural and anthropogenic processes. Since industrialization, the majority of non-point Hg emissions to the atmosphere and subsequent addition to aquatic and terrestrial ecosystems are anthropogenic: metallurgy and combustion of fossil fuel and waste are main contributors (Mohapatra et al. 2007). Due to elemental mercury's high volatility, it can travel long distances in the atmosphere, up to tens of thousands of kilometers and thus pollute remote ecosystems by atmospheric deposition (Schroeder and Munthe 1998).

Mercury is of concern in aquatic systems primarily due to the contamination of food webs with an organic form of Hg, methylmercury (MeHg), a neurotoxic chemical. MeHg forms from the methylation of elemental mercury. Elevated levels of MeHg in freshwater and marine fish have led to consumption guidelines or advisories throughout North America. For example, over 80% of all Ontario consumption restrictions for sport fish are based on Hg concentrations (OME, 2007). Thus, factors that increase the Hg burdens of fish are of important ecological concern.

Atmospheric inputs of Hg, along with the chemical, physical and ecological properties of a lake will affect Hg concentrations in biota. Even lakes with similar Hg inputs and

physiochemical properties can vary substantially in Hg concentrations in biota; water concentrations of Hg typically do not explain all lake-to-lake variation in Hg found in biota (Gorski et al. 2003). For example, there are strong correlations between Hg in fish and water pH (Rask et al. 2007) and between Hg in fish and dissolved organic carbon (DOC) in water (Ravichandran 2004). Understanding key chemical, physical and biological factors is crucial to assessing the complexities involved with the aquatic Hg cycle (Figure 1-3).

Mercury enters the aquatic food web through direct deposition and watershed loading. The uptake of MeHg is greater than that of inorganic mercury and for zooplankton (Watras et al. 1998) and fish (Hall et al. 1997) the primary pathway of Hg uptake is through the diet. As trophic level increases so does the ratio of MeHg to inorganic Hg (May et al. 1987). For example, of the total Hg (inorganic and organic Hg) burden in phytoplankton, MeHg ranges from 13 to 30% (Kirkwood et al. 1999) compared to 95% in most fish (Bloom 1992). MeHg biomagnifies through aquatic food webs and results in concentrations several orders of magnitude higher in predators than in water.

The rate at which MeHg is eliminated is much slower than the rate of uptake in most organisms (Trudel and Rasmussen 1997) so that with chronic exposure, the concentration of MeHg in an organism increases with time. Bioaccumulation is well correlated to age, weight and length of most fish species. Although a rare phenomenon, MeHg does not biomagnify in all organisms. For example, through physiological mechanisms, including growth effects and energy use, *Chaoborus* contain lower concentrations of MeHg than its prey (Back and Watras 1995).

Due to the bioaccumulative and biomagnification properties of MeHg, any change in food web structure and energy flow may affect MeHg burdens at any level of the food web. The introduction of an invasive species may lengthen the trophic pathway to top predator fish and result in an increase in mercury burdens (Cabana et al. 1994); however, in some cases, the trophic pathway to top predator fish is not lengthened by an invasive species. Instead, the invasive species assumes a similar trophic position to a native species and predicted post-introduction Hg burdens do not increase (Swanson et al. 2006). The objective of this study is to determine the trophic position of *Bythotrephes* in order to examine whether top-down trophic effects of *Bythotrephes* introductions observed in the native zooplankton community will translate to changes in the food web trophic structure as measured by Hg concentrations and stable isotope ratios.

1.3 Stable isotope analysis of food web structure

To assess trophic structure through food webs, stable isotopes are increasingly used in aquatic ecology. Stable isotope techniques are based on the principle that the change in isotopic ratios between predator and prey tissues reflect the fractionation through feeding, food processing and excretion processes (Hobson and Clark 1992).

The trophic position of an organism can be estimated using stable nitrogen isotope ratios ($\delta^{15}\text{N}$) because ^{14}N is preferentially excreted over the heavier ^{15}N isotope. This trophic fractionation of $\delta^{15}\text{N}$ leads to a predator that is commonly 2-4‰ enriched compared to its prey (Peterson and Fry 1987; Vander Zanden and Rasmussen 2001; Post 2002). However, there may be larger variability in $\delta^{15}\text{N}$ enrichment due to many factors. For example, carnivores, vertebrates and ureotelic organisms are all shown to have higher $\delta^{15}\text{N}$ enrichment than herbivores, invertebrates and ammonotelic organisms (Vander

Zanden and Rasmussen 2001, Post 2002, Vanderklift and Ponsard 2003; Goedkoop et al. 2006). When combined with traditional gut content analysis, $\delta^{15}\text{N}$ can provide a comprehensive assessment of an organism's diet (Johannsson et al. 2001).

Unlike $\delta^{15}\text{N}$, stable carbon isotope ratios ($\delta^{13}\text{C}$) are generally conserved from predator to prey. According to Post (2002), the average trophic fractionation is only 0.4‰ (± 1.3 , $n=107$) from prey to predator in both aquatic and terrestrial organisms. Differences in $\delta^{13}\text{C}$ values occur between food webs with energy sources in different food webs (i.e., pelagic versus benthic). For example, due to decreased availability of CO_2 and therefore less ^{12}C , energy created from the primary producers in the benthic food web is enriched in ^{13}C (less negative $\delta^{13}\text{C}$) relative to the pelagic food web (Vander Zanden and Rasmussen 2001, France 1995).

Stable isotope ratios of carbon and nitrogen have become a widespread tool in ecology and can provide a measure of trophic position of an organism, integrating energy assimilation through different trophic pathways. Stable isotopes have also been successfully used to quantify food web consequences associated with invasive species. Vander Zanden and Rasmussen (1999b) measured the impact that invading smallmouth and rock bass had on Ontario inland lake trout populations. Due to bass-mediated reductions in the abundance of littoral prey fish species, lake trout were forced to shift to a more planktivorous diet, as indicated by more negative $\delta^{13}\text{C}$ and reduced $\delta^{15}\text{N}$ values (Vander Zanden and Rasmussen 1999a). Gorokhova et al. (2005) used stable isotopes to show that the invasive zooplankton *Cercopagis pengoi* formed a new trophic level in the Baltic Sea, elevating the trophic position of forage fish.

1.4 Study objectives

The goal of this thesis is to determine the trophic position of invasive *Bythotrephes* in Muskoka lake food webs using two approaches: stable isotope and Hg analyses. Investigating where *Bythotrephes* is inserting itself into lake food webs will help predict the effects of invasion.



Figure 1-1: (a) *Bythotrephes longimanus* (body length, including tail spine, is approximately 2 cm). This third instar female has several embryos in her brood pouch that are in the black-eyed stage and nearing the end of their development. Picture used with permission from AL Strecker. (b) *Mysis relicta* (top) and *Bythotrephes longimanus* (bottom) collected from a lake trout stomach.

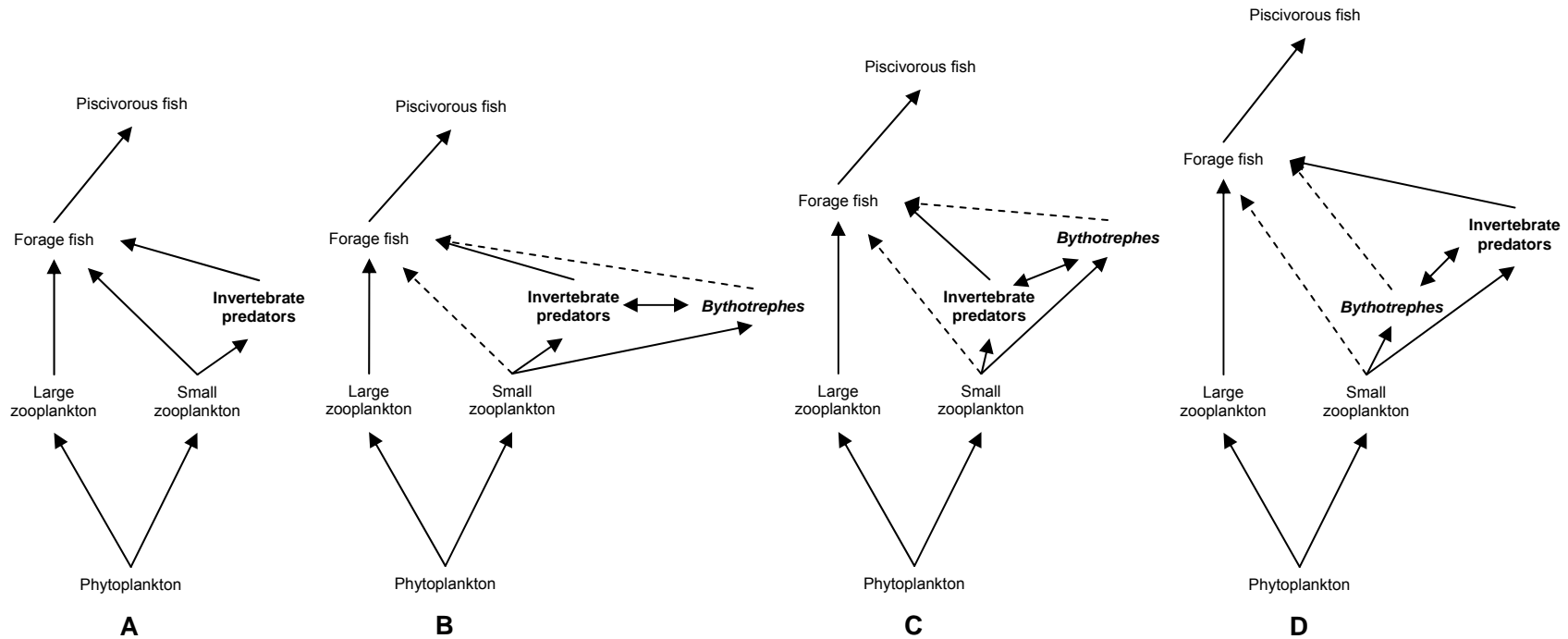


Figure 1-2: Simplified lake food web: A) with macroinvertebrate predators, B) with *Bythotrephes* not increasing the trophic length of the food web, C) and D) with *Bythotrephes* increasing the length of the food web. Trophic position ($\delta^{15}\text{N}$) and mercury concentrations increase from phytoplankton to piscivorous fish. Dashed arrows indicate a possible decrease in predation.

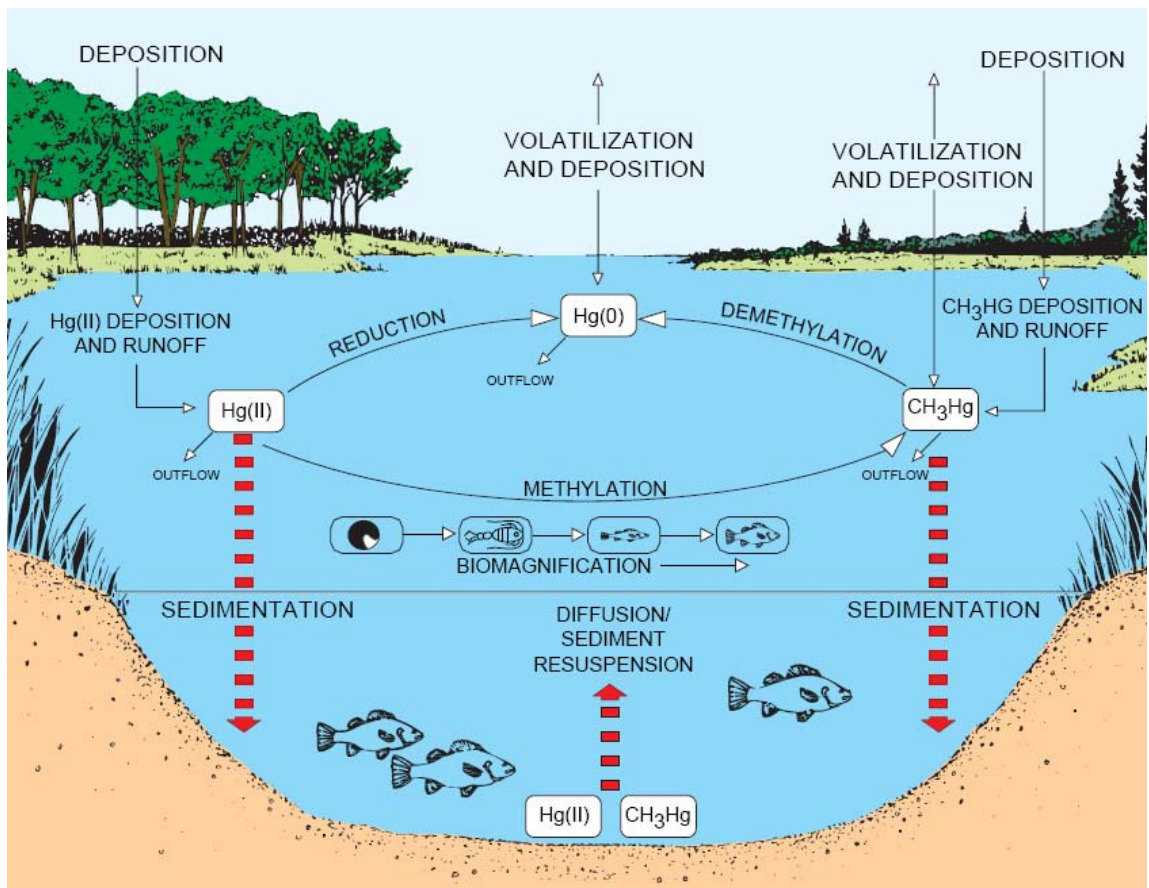


Figure 1-3: Mercury cycling pathways in aquatic environments (USGS, 2000).

Chapter 2:

**The trophic position of *Bythotrephes* in
Harp and Peninsula Lakes using $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ and THg**

2.1 Introduction

The invasion of species to North American lakes is of growing ecological concern and is one of the major threats to aquatic biodiversity (Sala et al. 2000). Invasive species have greatly altered community and ecosystem structure in Ontario aquatic ecosystems through alterations in water clarity, nutrient ratios and habitat (Vanderploeg et al. 2002). *Bythotrephes longimanus* (spiny water flea), is a predatory cladoceran originally from Eurasia that has been transported to North America in the ballast water of transoceanic ships (Sprules et al. 1990). Since its invasion of the Laurentian Great Lakes in the mid-1980s, *Bythotrephes* has spread rapidly through the region into many inland lakes (MacIsaac et al. 2004). *Bythotrephes* preys upon small crustacean zooplankton, a key component of freshwater food webs that transfers energy from primary producers, such as algae at the base of the food web, to higher trophic levels, planktivorous macroinvertebrates and fish. There is growing evidence that *Bythotrephes* invasion alters aquatic food web structure. Predation by *Bythotrephes* has led to shifts in crustacean zooplankton composition, size structure and total biomass in several studied lakes (Strecker et al. 2006; Boudreau and Yan 2003; Yan et al. 2002; Yan and Pawson 1997). While not yet fully understood, the potential disruption of food webs by invasive species is of great importance due to impacts on higher trophic level communities (Shuter and Mason 2001). The zooplankton shifts seen in *Bythotrephes* invaded lakes may have both indirect and direct effects on food resources of planktivorous macroinvertebrates and fish.

It is hypothesized that *Bythotrephes* will enter the food web at a similar trophic position to that of native macroinvertebrate predators that also prey on zooplankton. Common macroinvertebrate predators in Ontario lakes include the glacial relict, *Mysis relicta*

(opossum shrimp), and the dipteran larvae, *Chaoborus* spp. (phantom midge). Both of these species play important roles in lake food webs. By traveling between the benthic and pelagic zones of a lake and transferring energy between the two, *Mysis* and *Chaoborus* connect these two environments and act as a key energy link between the zooplankton and the forage and juvenile fish. *Bythotrephes* may not assume a similar significant role in trophic transfer to fish as these other native invertebrate predators. The spine of *Bythotrephes* protects it from predation by small fish (i.e., 3-5 cm) (Branstrator and Lehman 1996) and *Bythotrephes* does not have a similar fatty acid composition to *Mysis* (Nordin et al. *in press*) therefore it may not be as effective a link to transfer energy from zooplankton to fish. *Bythotrephes* also significantly reduces the zooplankton abundance in a lake, reducing the food supply to planktivorous predators, including macroinvertebrates and fish. By avoiding predation and/or out-competing native planktivores, *Bythotrephes* may be altering energy flow in the food web.

If *Bythotrephes* does not assume a similar role to native macroinvertebrate predators, it may create a new trophic level, thereby increasing the length of the entire food web. Longer food webs are associated with increased contaminant burdens at high trophic levels. One contaminant of concern in aquatic ecosystems is mercury (Hg). Mercury, and more specifically a neurotoxic organic form of Hg, methylmercury (MeHg), can biomagnify in the food web (Cabana et al. 1994, Vander Zanden and Rasmussen 1996). By creating a novel trophic position, *Bythotrephes* may be increasing Hg concentrations in higher trophic levels, including fish and other top predators.

Stable isotope analysis is useful in assessing trophic relationships in food webs. Unlike traditional investigations that use stomach contents to investigate predator-prey relationships, stable isotopes provide estimates based on diet assimilation over a longer

time period (Post 2002). Naturally occurring stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) have been previously used to understand the trophic interactions between an invasive and native species in an invaded food web (Gorokhova et al. 2007, 2005, Vander Zanden and Rasmussen 1999b). Stable isotopes have not been previously used to assess *Bythotrephes* invasion into North American lakes. Mercury concentrations and nitrogen and carbon stable isotopes of zooplankton, macroinvertebrates and fish were compared in two lakes invaded by *Bythotrephes*. These two approaches will provide complimentary information about trophic relationships in lakes invaded by *Bythotrephes* and determine the role of *Bythotrephes* in invaded lakes.

2.2. Methods

2.2.1 Site description

Five lakes with and five lakes without *Bythotrephes* in the Muskoka and Haliburton regions of south-central Ontario, Canada were surveyed May through August 2006. However, data from only Peninsula and Harp lakes in the Muskoka region (Chaffey Township, Figure 2-1) were examined due to dissimilar pelagic food web structure between the lakes surveyed (i.e., no forage fish or lake trout), (see Appendix I for a list of species caught). Like many boreal shield lakes, both are oligotrophic and have a Precambrian bedrock catchment overlaid with a thin covering of soil (Devito et al. 1999) (Table 2-1). Both lakes have dense shoreline development in the form of cottages and/or resorts. Harp is a headwater lake, with Peninsula Lake directly downstream; both are remote from anthropogenic point sources of Hg. The study lakes both have similar benthic and pelagic invertebrate and fish species, with documented *Bythotrephes* invasions taking place in the early 1990s (Yan et al. 1992). Harp Lake has been

intensively monitored by the Ontario Ministry of the Environment (OME) since 1980 and this monitoring program has provided clear evidence that *Bythotrephes* has decreased zooplankton community species abundance and richness (Yan et al. 2001, 2002).

2.2.2 Sample collection

Crustacean zooplankton samples for laboratory analyses and composition were collected the second and third weeks of June, before *Bythotrephes* was sufficiently abundant to impact the zooplankton community. Using clean techniques, crustacean zooplankton were collected by daytime vertical hauls 2 m from the bottom to the surface of each lake at the deep station using an 80 μm mesh net. One sample was preserved in 4% sugared and buffered formalin for later enumeration. Four other samples were placed in plastic bags and transported back to the laboratory where they were rinsed in reverse osmosis (RO) water and passed through a series of mesh screens (153, 243 and 500 μm) to split the sample into 3 different size fractions (153-243, 243-500 and >500 μm). Initially, separation based on family was attempted, but due to time constraints, separation based on size was chosen. Macroinvertebrate predators and any foreign items were removed. Before drying, a subsample of each zooplankton size fraction was preserved in 4% sugared and buffered formalin for later qualitative identification to family.

Because many invertebrate predators migrate down in the water column during the day to avoid fish predation, *Bythotrephes*, *Mysis* and *Chaoborus* were collected from each lake at night using vertical tows with a 35 cm diameter zooplankton net with 400 μm mesh taken from 2 m above the bottom to the surface. Five vertical tows with a minimum depth of 10 m were taken along a transect from the center of the lake (the

“deep” station) to the shore. Visual assessment of the presence/absence of *Bythotrephes* was recorded after each sample. At each station, one sample was preserved in 4% sugared and buffered formalin. Additional samples at the deep station from 2 m above the bottom to the surface were obtained using clean techniques to collect approximately 10-15 animals each of *Bythotrephes*, *Mysis* and *Chaoborus*. These were rinsed in RO water, enumerated and sorted. *Mysis* were grouped into 3 size categories: small/juvenile (<1 cm), intermediate (1-1.5 cm) and large/adult (>1.5 cm) as they undergo an ontogenic shift towards increased carnivory (Branstrator et al. 2000).

Fish were collected with NORDIC and FWIN gill nets set perpendicular to shore (as described in Morgan and Snucins 2005, Morgan 2002) using 12-hour sets. To allow for the uptake and incorporation of prey items over the season for stable isotope analyses, fish were collected in late August 2006. All fish caught were sacrificed, enumerated, and identified to species. Captured species included lake trout (*Salvelinus namaycush*), rainbow smelt (*Osmerus mordax*), lake herring (*Coregonus artedii*), yellow perch (*Perca flavescens*), rock bass (*Ambloplites rupestris*), smallmouth bass (*Micropterus dolomieu*) and pumpkinseed (*Lepomis gibbosus*). Littoral fish species, including smallmouth bass, are present in both Peninsula and Harp lakes; however, for this study, littoral species were only captured in Peninsula Lake. Length, weight and sex were recorded for each individual. Lake trout scales (n=8 for each individual) were collected and stored in paper envelopes for aging by plastic impression by Aquatech Services, Perth, Ontario. Skinless and boneless dorsal muscle tissue was frozen at -20°C for future stable isotope and THg determination. In the case where a fish was too small to obtain enough dorsal muscle tissue for analysis, the whole fish or a composite sample of multiple whole fish was analysed. Whole fish have approximately half the THg concentration of muscle tissue (Goldstein et al. 1996). Fish stomachs were preserved in ethanol for later

taxonomic identification of contents and stable isotope analysis. Only stomachs estimated to be greater than 25% full were included in analyses in order to get enough sample mass that wasn't too digested. Due to the duration of the gill net sets, only 41 of the 110 (37%) fish stomachs were greater than 25% full. Prey items not digested in the stomach were identified to order and the mass (wet weight) of each group was recorded. Stomach contents that were too digested to identify were recorded as "unknown".

Samples for water chemistry were obtained in August 2006 from the deep station of each lake using an integrated tube sampler with a 5 cm diameter from 5 m to the water surface. Water samples were analysed at the OME Dorset Environmental Science Centre (Table 2-1) following OME protocols for conductivity (DOCOND-E3024), dissolved organic carbon (DOC; DOT-E3422), pH (DOCSI-E3042) and total phosphorus (TP; DOP-E3036).

2.2.3 Sample preparation and analyses

All invertebrate, fish and known stomach content samples collected for stable isotope analyses were dried at 60°C for 24-48 hours and stored in aluminum foil or glass vials. Samples were homogenized to a fine powder with a ball mill or a mortar and pestle and analysed for stable isotopes or THg. Fish samples collected for THg analysis were shipped frozen to the OME.

The formalin-preserved zooplankton samples were enumerated and identified to family or genus (Table 2-1). The protocol used included counting a series of subsamples of known volumes such that a minimum of 250 individuals were identified. A subsample was enumerated completely, yet once a maximum of 50 copepodids per order, 30 nauplii per order and 50 individuals from each taxa was reached, they would not be counted

towards the total 250 individuals. *Bythotrephes* were enumerated, sexed and length, instar and number of eggs measured. *Mysis* and *Chaoborus* were also enumerated and length recorded.

Carbon and nitrogen stable isotope analyses were performed using the continuous-flow isotope ratio mass spectrometer (CF-IRMS) at Queen's Facility for Isotope Research (Geological Sciences and Geological Engineering, Queen's University, Kingston, Canada). Data are expressed in the standard δ unit notation:

$$\delta X = [(R_{\text{sample}}/R_{\text{reference}})-1] \times 1000 \quad [1]$$

where X = is the heavier isotope, R_{sample} is the ratio of the heavy to light isotope (i.e., $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) and $R_{\text{reference}}$ is the ratio of the heavy to light isotope of a reference standard. Isotope ratios were expressed in parts-per-thousands (‰). Random duplicates were performed on approximately 10% of samples (n=22) with a mean difference and standard deviation between duplicate samples of $0.09 \pm 0.06\text{‰}$ and $0.08 \pm 0.07\text{‰}$ for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, respectively. The mean $\delta^{15}\text{N}$ value for the N reference standard, ammonium sulphate (Certified Reference Material (CRM) 8548, International Atomic Energy Agency, Vienna, Austria) was $20.25 \pm 0.24\text{‰}$. For the C reference standard, graphite (NBS21, VPDB), the mean $\delta^{13}\text{C}$ was $28.00 \pm 0.12\text{‰}$. Internal reference standards Atlantic salmon ($\delta^{15}\text{N}$: 8.61 ± 0.28 , $\delta^{13}\text{C}$: -18.62 ± 0.11 , n=7) and red tilapia ($\delta^{15}\text{N}$: 5.87 ± 0.33 , $\delta^{13}\text{C}$: -20.92 ± 0.16 , n=9) showed consistency.

Total Hg concentrations were determined for zooplankton and macroinvertebrate predators using ultra-clean protocols at the Mercury Analytical Laboratory (Dorset

Environmental Science Centre, OME) via atomic fluorescence spectroscopy (AFS) as described in Rasmussen et al. (1991). There were three changes to this method: sample weights were low (4-6 mg) so only 2 mL digestion acid was added to each sample; and a purge and trap procedure was used to remove and concentrate Hg from the digested sample (Greg Mierle, OME *personal communication*). Due to low amounts of THg and low mass of each individual, samples were pooled by genus into composite samples for analysis. Random duplicates were performed on approximately 10% of samples (n=4) and the mean difference and standard deviation between duplicate samples was 13.7 ± 9.7 ng/g dw. Digestion blanks had a mean value of 1.1 ± 0.05 ng/g dw. Certified reference materials (DOLT2 from the National Research Council of Canada, Ottawa, Ontario, CITRUS and OYSTER) were all within range of their expected values. The average measured concentrations for DOLT2, CITRUS and OYSTER were 1913.1 ± 80.1 (n=3), 75.3 ± 8.3 (n=3) and 45.4 ± 0.3 (n=3) ng/g dw, respectively.

Fish tissue samples were analysed for THg by the Sport Fish Contaminant Monitoring Program (Environmental Monitoring and Reporting Branch, OME, Etobicoke, Ontario) via cold vapour atomic absorption spectroscopy as described in OME method HGBIO-E3057 (OME, 2005). Internal reference materials MC1 (0.762 ± 0.0442 $\mu\text{g/g}$ ww, n=372) and MC5 (0.2602 ± 0.0187 $\mu\text{g/g}$ ww, n=317) were consistent. Certified reference material (DORM2 from the National Research Council of Canada) was in the range of expected values (4.13 ± 0.18 $\mu\text{g/g}$ ww, n=20). Fish samples with measurable trace THg levels were assumed to be at half the method detection limit (0.02 $\mu\text{g/g}$ ww or 100 ng/g dw).

2.2.4 Data analyses

Fish and zooplankton samples were analysed for THg concentration by wet weight and dry weight, respectively. Because fish tissue has a relatively standard moisture content compared to the variety of invertebrates analysed, the wet weight of fish samples were converted to dry weights to compare between THg concentrations. This conversion factor of 80% (a multiplication factor of 5) was chosen based on the moisture content of hatchery lake trout (Gunther et al. 2007) and wild yellow perch dorsal muscle tissue from the US Great Lakes (Gonzales et al. 2006).

All statistical analyses were done using JMP 6 (SAS Institute, Cary, NC, USA). Student's t-tests were used to compare the stable isotope ratios and THg concentrations between organisms in the same food web. Multiple regressions were used to compare relationships between THg and fish age, length and weight. Linear regressions were used to compare between fish length and THg concentrations and trophic position ($\delta^{15}\text{N}$ values) and THg concentrations.

2.2.5 Food source modelling

Using stable isotope ratios, the contributions of each prey item to a consumer were investigated with a mixing model, *Isosource*, available through the US Environmental Protection Agency (Phillips and Gregg 2003, Phillips et al. 2005). All possible combinations of each prey item contribution were examined in small increments (i.e., 1%) and with a minimum contribution or tolerance of 0.5% for each prey item (Phillips and Gregg 2003). The mean trophic fractionation from prey to predator was assumed to be consistent; $\delta^{13}\text{C}=0.4\text{‰}$ and $\delta^{15}\text{N}=3.4\text{‰}$ (Post 2002) were subtracted from the consumer's stable isotope values (Riera 2007). Due to the discrepancy in stable isotope ratios between the lake-collected organisms and the preserved stomach content

samples (Appendix II), possibly due to ethanol (Kelly et al. 2006, Feuchtmayr and Grey 2003, Sweeting et al. 2004) or stomach acid effects on stable isotope ratios, only lake-collected samples were applied to the model. However, because benthic invertebrates were not collected from the lake, the mean of the preserved benthic invertebrate stomach content stable isotope ratios was used as an *approximation* of this important prey item. This estimation was based solely on Peninsula Lake benthic invertebrates as only pelagic zooplankton were collected from the stomach contents of Harp Lake fish. Zooplankton, *Mysis*, *Bythotrephes*, *Chaoborus*, lake herring, rainbow smelt, benthic fish (i.e., yellow perch, rock bass, smallmouth bass and pumpkinseed) and/or benthos (estimated from Peninsula benthic fish stomach content stable isotope values) were used as potential prey items for lake trout and rainbow smelt. Only certain predator-prey scenarios could be investigated (e.g., *Bythotrephes* could not be a consumer) because of the requirements and/or constraints of the mixing model. This model serves to assess the probability of multiple prey items' isotopic importance to a single predator.

Lipid synthesis favours ^{12}C over the heavier ^{13}C isotope, therefore tissues high in lipids tend to have more negative $\delta^{13}\text{C}$ values. Kiljunen et al. (2006) showed that normalization of lipid rich $\delta^{13}\text{C}$ values can have a significant impact on proportion estimates of dietary sources; however, this is only the case when lipid concentrations are variable and/or high (Post et al. 2007). Based on C:N values, the fish species sampled had relatively low lipid concentrations, 3.34 ± 0.28 (Appendix III), compared to ratios greater than 5 found in lipid-rich fish (Kiljunen et al. 2006, Post et al. 2007). Lipid normalization of $\delta^{13}\text{C}$ values using Kiljunen et al.'s (2006) revised model resulted in little change between original and normalized values ($0.87 \pm 0.49\text{‰}$, mean $\pm 1\text{SD}$). C:N ratios were higher for invertebrates (5.13 ± 1.17); however, normalizing for lipids is not recommended for whole invertebrate samples as the C:N ratio is dependent upon more than lipid content,

including chitin and glycogen stores (Kiljunen et al. 2006). Due to the low C:N values in fish and the uncertainties involved with zooplankton C:N values, only non-normalized $\delta^{13}\text{C}$ values were used.

2.3 Results

Both Peninsula and Harp lakes had similar pelagic food web structure with lake trout as the top trophic pelagic predator (Figure 2-2). Harp Lake had significantly similar trophic positions ($\delta^{15}\text{N}$ values) for *Mysis* and *Bythotrephes* (t-test, $p=0.358$), while Peninsula did not ($p<0.002$). In both lakes, *Bythotrephes* had a less negative $\delta^{13}\text{C}$ value relative to *Mysis*.

Total Hg followed a predicted bioaccumulation trend of increasing THg concentration with increasing trophic level. The linear regression equations for log THg concentrations versus $\delta^{15}\text{N}$ relationships for Peninsula and Harp lakes were $y = 0.169x + 0.757$ (adjusted $r^2=0.87$, $n=18$, $p<0.002$) and $y = 0.195x + 0.1.165$ (adjusted $r^2=0.78$, $n=31$, $p<0.002$), respectively. Overall, both lakes had similar THg concentrations in the pelagic food webs (Figures 2-3). Mean THg concentrations in zooplankton in Peninsula and Harp lakes were 75.1 ± 16.4 and 108.5 ± 13.2 ng/g dw, respectively. From Peninsula Lake, *Mysis* ranged from 106.2 to 379.91 ng/g dw, with samples following a pattern of increased THg with increased body length (Table 2-2). *Bythotrephes* in Peninsula ($n=2$) and Harp ($n=1$) lakes had a THg concentrations of 117.8 ± 11.8 and 152.8 ng/g dw, respectively. While *Mysis* showed a general trend of increasing THg concentration and trophic position with increasing size, there was no trend observed between THg concentration, trophic position and the three zooplankton size fractions or the different *Bythotrephes* instars.

The average THg concentration of the larger lake trout of Peninsula Lake (t-test, $p < 0.05$) was elevated relative to the smaller lake trout from Harp Lake (2550 ± 1124 versus 1650 ± 951 ng/g dw, respectively) (Table 2-3). Due to their small size, Peninsula Lake rainbow

smelt were analysed as a composite sample of 11 whole fish and had only trace THg concentrations (100 ng/g dw; n=1). The larger bodied lake herring from Harp Lake had higher THg concentrations (1870 ±675 ng/g dw; n=10). Of the benthic fish collected from Peninsula Lake, smallmouth bass had the highest THg concentrations (2385 ±1841; n=10), while pumpkinseed had the lowest (267 ±127, n=9). Lake trout from both Peninsula and Harp Lake fish showed large positive correlations between THg with fish length, mass and age ($p \leq 0.002$) (Table 2-4). THg concentrations in other species in both lakes were also positively correlated to fish length and mass ($p \leq 0.002$).

Stomach contents were variable between and within species (Table 2-5) with zooplankton and *Mysis* occurring most frequently in the stomach of lake herring from Harp Lake. The most frequent prey items for lake trout in Peninsula Lake were fish and fish eggs; however, *Mysis* and *Bythotrephes* occurred most frequently in lake trout from Harp Lake. This dissimilarity in stomach contents between lake trout from the two lakes is due to the significantly smaller size of lake trout of Harp Lake which may have been more planktivorous. When present, *Bythotrephes* and *Bythotrephes* spines made up 2-100% of the total mass of the stomach contents. *Bythotrephes* was also found in the stomachs of yellow perch and rock bass.

Results from the mixing model indicated that *Bythotrephes* made up a significant portion of diet (>15%) for rainbow smelt, lake herring and the smaller lake trout from Harp Lake (Table 2-6, Figure 2-4). In contrast, fish (*i.e.*, rainbow smelt and benthic fish) made up the majority of the lake trout diet in Peninsula Lake. For all the fish diets, there were a large number of possible dietary combinations (*i.e.*, omnivory) as indicated by multiple overlaps for contribution of prey items (Figure 2-4). Due to the large number of prey items for each consumer, the number of trial diets or scenarios was high.

2.4 Discussion

The results of the stable isotope analysis and THg bioaccumulation slopes, as well as stomach content analysis and food source modelling suggest that *Bythotrephes* is assuming a comparable trophic position to native macroinvertebrate predators in Peninsula and Harp lakes. This is similar to observations in its native European range in Lake Geneva (Perga and Gerdeaux 2006) and Loch Ness (Grey et al. 2001) where *Bythotrephes* has higher $\delta^{15}\text{N}$ values than copepods and cladocerans. Because *Bythotrephes* may be inserting itself into a trophic level that is already occupied by other organisms in the lake (e.g., *Mysis*, *Chaoborus*), food web and mercury bioaccumulation shifts at higher trophic levels may not occur in the study lakes.

Based on stomach content analysis from both lakes, *Bythotrephes* is a prey item of lake trout, lake herring, rainbow smelt, yellow perch and rock bass. The proportion of *Bythotrephes* in the stomachs was highly variable, with the highest percent occurrence in the stomachs of lake trout from Harp Lake. The variation in both mean proportion and percent occurrence of *Bythotrephes* in stomachs among fish species could be due to opportunistic feeding or specific fish within a species preferring *Bythotrephes* to other prey. For example, the rock bass that consumed *Bythotrephes* all had relatively high mean proportions of *Bythotrephes* in their stomachs (76.0 ± 38.7). All of the fish species in this study have been previously documented to consume *Bythotrephes* in the Great Lakes or Ontario inland lakes (Stetter et al. 2005, Truemper and Lauer 2005, Coulas et al. 1998, Hudson et al. 1995, Schneeberger 1991).

Native macroinvertebrate predator abundances, including *Mysis* and *Chaoborus*, are low in Peninsula and Harp lakes (S Foster *personal communication*), but are actively

selected for by planktivorous fish (Dryer et al. 1965). As suggested by stomach content analysis and stable isotope ratio food source modelling, zooplanktivorous fish are consuming *Bythotrephes* at a higher frequency than suggested by its low average abundance in the lake (<5 individuals/m³). This may be because *Bythotrephes* is a highly visible prey item in the water column owing to its large size and eye spot. This disparity between the abundance of *Bythotrephes* in the lake and the abundance in the diet suggests that fish are also actively selecting for *Bythotrephes*.

The stable isotope food source modelling results suggest that zooplankton comprised the majority of the diets of rainbow smelt and lake herring; however, *Bythotrephes* also made up a large portion of these forage fish diets. Lake herring and rainbow smelt were primarily zooplanktivores with some reliance on benthic invertebrates. When present, *Bythotrephes*, along with zooplankton, *Mysis* and benthos made up the majority of lake herring and rainbow smelt diets (Stetter et al. 2005, Johnson et al. 2004, Coulas et al. 1998, Vander Zanden and Rasmussen 1996). Like forage fish, young lake trout relied on zooplankton and macroinvertebrates to make up the majority of their diet, while older lake trout preyed primarily on a variety of pelagic and benthic fish. This difference in diets between young and older fish was seen in lake trout's modelled diets for Peninsula and Harp lakes (Appendix IV). *Bythotrephes* was only a major contributor in the smaller and younger Harp lake trout diet. While the food source modelling results were a simplified version of a much more complex food web, the results were comparable to the stomach content analysis.

Considered primarily benthic species, yellow perch and rock bass had *Bythotrephes*, a pelagic species, in their stomach contents. After examining both fish species diets, it may not be as surprising that these two fish species were preying upon *Bythotrephes*;

Vander Zanden and Vadeboncoeur (2002) examined 7377 yellow perch from 90 populations and 1620 rock bass from 20 different north-temperate North American populations and found that their reliance on benthic invertebrates was highly variable among populations. As discussed above, *Bythotrephes* consumption by yellow perch was documented in Lakes Michigan and Erie (Truemper and Lauer 2005, Baker et al. 1992, Bur and Klarer 1991, Schneeberger 1991) and in rock bass in Lake Michigan (Schneeberger 1991). It has been hypothesized that the consumption of *Bythotrephes* in “benthic” species could be due to a shift in available food sources (Bur and Klarer 1991) or perhaps through opportunistically feeding upon this highly visible prey item.

Bythotrephes was found to have inconsistent distribution throughout Peninsula and Harp lakes (Table 2-1), which also has been seen in other lakes (McCarthy et al. 2006) and may be attributed to clustering and/or wind. Wind-induced movement could push *Bythotrephes* into littoral sections downwind in a lake, making them readily accessible to predation by yellow perch and rock bass. The consumption of *Bythotrephes*, rather than smaller zooplankton or benthos, may effect Hg concentrations in both food webs. This evidence of benthic fish consuming pelagic zooplankton suggests that *Bythotrephes* invasion should not be thought of in discrete terms of pelagic and benthic food webs, but rather should be viewed on a whole-lake basis.

In both lakes, *Mysis* had lower $\delta^{13}\text{C}$ values than *Bythotrephes*, which may be due to its diel migration. Unlike *Bythotrephes*, *Mysis* remains in the profundal benthic zone during the day and migrates up through the water column at night to feed on zooplankton when predation pressure by fish is less intense (Johannsson et al. 2001). Vander Zanden and Rasmussen (1999a) divided pelagic and profundal primary consumers in 14 Ontario and Quebec lakes and found that profundal $\delta^{13}\text{C}$ is more negative than pelagic $\delta^{13}\text{C}$ values,

by a difference of -2‰. While there is some evidence that *Bythotrephes* will also perform diel vertical migration (Lehman 1987, Straile and Halbach 2000), it is suggested that the amplitude of migration is relatively small (Straile and Halbach 2000, Mookerji et al. 1998). The vertical habitat separation of *Bythotrephes* and *Mysis* along with the different $\delta^{13}\text{C}$ values suggests that there is some niche separation between these two predators. *Chaoborus* will also perform diel migration in response to fish predation (Dawidowicz et al. 1990); however, the only sample from Peninsula Lake had similar $\delta^{13}\text{C}$ values to *Bythotrephes*.

The potential competition between native macroinvertebrate predators and *Bythotrephes* may be reduced by not only this habitat separation, but also by differences in preferred prey. While Foster (*personal communication* (from University of Toronto Ph.D. thesis)) found that *Bythotrephes*' appetite increases total macroinvertebrate predator demand in a lake by 25%, there were no statistically correlated effects on *Mysis* and *Chaoborus* abundance between invaded (n=4) and non-invaded lakes (n=3). Only *Leptodora* had lower abundance attributed to *Bythotrephes* invasion. *Mysis* and *Chaoborus* have more varied diets and consequently may not be as affected by *Bythotrephes*. *Mysis* is an opportunistic omnivore and as an adult will consume a wide variety of prey, including diatoms, phytoplankton, amphipods, *Mysis*, *Bythotrephes*, *Chaoborus* and copepod and cladoceran zooplankton (Nordin et al. *in press*, Johannsson et al. 2001, Lasenby and Langford 1973). *Chaoborus* prefer rotifers, but will also prey upon small cladocerans and copepods (Moore et al. 1994, Yan et al. 1991). *Bythotrephes* and *Leptodora* share a more comparable diet, consisting primarily of smaller sized cladoceran and copepod zooplankton (Dumitru et al. 2001, Schulz and Yurista 1994; Browman et al. 1994) and therefore may compete for resources.

Zooplankton stable isotope signatures vary both temporally and taxonomically, mirroring the variation of primary producer signatures (Matthews and Masumder 2007, 2005, Post 2002, Cabana and Rasmussen 1996). Due to this variation, the zooplankton samples from Peninsula and Harp lakes are assumed to be estimates of the true stable isotope signatures incorporated by predators over the entire summer season. The separation of zooplankton into three size fractions with different species assemblages (Appendix IV) led to no significant trends in stable isotope signatures or Hg concentrations; however, sample sizes were small. There is limited data on the variation of stable isotope values and zooplankton size fractions. A Baltic Sea study also separated zooplankton based on size into micro- (90-200 μm) and mesozooplankton (>200 μm) size classes and found no significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (Gorokhova et al. 2005). Fry and Quinones (1994) found small changes associated with different marine zooplankton size fractions; however, these were quite varied and not consistent. Most published studies typically use bulk zooplankton samples to assess zooplankton trophic position; however, separation into taxonomic and/or functional groups (e.g., copepods versus cladocerans) has resulted in clearer differentiation within zooplankton trophic relationships (Matthews and Mazumder 2005, 2003). Because there are no trends in size-fractionated zooplankton, it is recommended that stable isotope analysis be performed on bulk zooplankton or zooplankton separated into taxonomic groups.

Within a single plankton species, there may be a stronger relationship between size and trophic position. The sample sizes from Peninsula and Harp lakes were small, but *Mysis* show increased $\delta^{15}\text{N}$ values with increasing size. This has been documented in previous studies and relates to an ontogenic shift from juveniles to the more carnivorous adults (Branstrator et al. 2000, Johannsson et al. 2001). Unlike *Mysis*, *Bythotrephes* instars showed no trend of increasing trophic position with increased age. While third instars

may be able to handle a larger range of prey sizes (Muirhead and Sprules 2003), perhaps *Bythotrephes* does not have a comparatively large a diet shift as the highly omnivorous *Mysis*.

There are no published data regarding *Bythotrephes* and Hg concentrations in aquatic food webs and it is unknown whether *Bythotrephes* invasion will affect Hg concentrations. In Peninsula and Harp lakes, *Bythotrephes* had similar THg concentrations in *Mysis* and the slopes of the log-THg: $\delta^{15}\text{N}$ regressions were not significantly different from each other, indicating similar THg biomagnification power for Peninsula and Harp lakes. These slopes are also similar to published biomagnification slopes for non-invaded lakes in Quebec (Garcia and Carignan 2005) and northwestern Ontario (Kidd et al. 1995). While a change in biomagnification slope may not necessarily be seen when a food chain is lengthened, it is interesting to note that *Bythotrephes* does not appear to be affecting biomagnification in Peninsula and Harp lakes. Yet, *Bythotrephes* and *Mysis* have different fatty acid profiles (Nordin et al. *in press*) that may influence growth rates and therefore THg concentrations in fish. There is evidence that *Mysis* is consuming *Bythotrephes* (Nordin et al. *in press*), thereby possibly elevating the trophic position of *Mysis*. To determine if the interaction between *Mysis* and *Bythotrephes* is changing Hg biomagnification in invaded lakes, further research is necessary.

The bioaccumulation of Hg by *Bythotrephes* may also play an important role in determining the effects of *Bythotrephes* on Hg concentrations at higher trophic levels. In zooplankton, 57% (range 11-83, n=15) of THg is in the form of MeHg (%MeHg) and can vary substantially depending on the taxonomic, morphometric and ontogenic characteristics of the zooplankton species (Watras et al. 1998). It is unknown what the

%MeHg is for *Bythotrephes* and this study only measured THg concentrations. However, given the diet differences between *Mysis* and *Bythotrephes*, it is reasonable to assume there are differences in %MeHg burdens that could propagate through the food web to higher trophic levels.

Hg concentrations in prey may not be the only way that *Bythotrephes* could impact fish Hg burdens. Through bioaccumulation, growth rate and weight-length relationships of fish, Hg concentrations can be affected (Trudel and Rasmussen 2006). *Bythotrephes* has dramatic effects on zooplankton abundance (Strecker et al. 2006), possibly translating to decreased growth rate of planktivorous fish by reducing the amount of available prey. It is hypothesized that *Bythotrephes* can compete with larval fish and native invertebrate predators for zooplankton and may reduce the recruitment of fish to larger size classes (Vanderploeg et al. 2002). Coulas et al. (1998) found that weight-length relationships of lake herring in lakes with and without *Bythotrephes* (including Harp Lake) did not differ. However, availability of *Bythotrephes* in Lake Ontario was associated with an increase in the slope of the spring weight-length relationship for adult alewife (*Alosa pseudoharengus*), another important forage fish species, between pre- and post invasion years (1972 versus 1888) (Mills et al. 1992). This increased alewife weight in the spring did not translate to larger fish in the fall; the authors concluded that this was due to increased predation pressures on zooplankton due to a larger alewife year class in 1988. Yet, this predation pressure may also be caused by *Bythotrephes* competition for the same resource. Another possibility is that consumption of *Bythotrephes* is not as energetically valuable compared to other prey items (Nordin et al. *in press*) and may affect fish growth rates.

The examination of historical fish Hg concentration data may be a useful tool in assessing the effects of *Bythotrephes* invasion. The OME Sport Fish Contaminant Monitoring Program's data extends back to the 1970s; however, these data are inadequate to compare fish Hg concentrations from pre- and post-invasion in Harp and Peninsula lakes. Further inspection of this large database may yield invaded lakes that have a wider variety of sampling years, species and fish lengths to allow for an historical comparison of Hg concentrations in Ontario sport fish. For example, this approach has determined that THg concentrations in Lake Ontario salmon populations have changed little over the past 20 years, but does not examine *Bythotrephes* as a driver (French et al. 2006).

While *Bythotrephes* is generating changes in a lake's food webs, such as *Mysis* fatty acid composition (Nordin et al. *in press*) and zooplankton community structure (Boudreau and Yan 2003), there appears to be no major short-term changes in trophic structure or THg biomagnification of invaded lakes. This contradicts previous hypotheses that *Bythotrephes* invasion would abruptly alter food web dynamics, lengthen food chains and increase contaminant levels at higher trophic levels (Gewurtz and Diamond 2003, Vanderploeg et al. 2002, Shuter and Mason 2001, Lehman and Caceres 1993). Dramatic impacts on ecosystems by invading macroinvertebrate predators have been documented in other areas of North America (Spencer et al. 1991), yet *Bythotrephes* is not creating a new trophic position in Peninsula and Harp lakes. Instead, it is assimilating into a trophic niche already held by native macroinvertebrate predators, such as *Mysis*.

The effects of *Bythotrephes* may be more dramatic in lakes with reduced linkages to the top trophic levels. In these lakes, *Bythotrephes* could create a new trophic level, thereby

lengthening the food web and possibly increasing Hg concentrations in top predators (Vander Zanden and Rasmussen 1996, Cabana et al. 1994). Yet, the idea of *Bythotrephes* creating a new trophic level may not be realistic; while there are many lakes that do not contain *Mysis*, most lakes still contain other macroinvertebrate predators such as *Chaoborus* (Wissel et al. 2003)

Table 2-1: Physical, chemical and biological properties of Peninsula and Harp lakes. Water chemistry data from August 2006. Abundance (individuals/m³) of zooplankton in Peninsula and Harp lakes. *Bythotrephes* values were the average of 5 hauls over 5 stations in the lake. Zooplankton values from a single haul. Only presence/absence data available for *Mysis* and *Chaoborus*.

| Parameter | Peninsula | Harp |
|--------------------------------------|------------------|-------------|
| Latitude | 45'20° | 45'23° |
| Longitude | 79'06° | 79'07° |
| Year <i>Bythotrephes</i> found | 1991 | 1993 |
| Surface area (ha) | 865 | 71 |
| Watershed area (ha) | 5453 | 542 |
| Z _{max} (m) | 34 | 32.5 |
| Z _{mean} (m) | 9.7 | 13.3 |
| Secchi depth (m) | 3.5 | 3.9 |
| TP (µg/L) | 7.2 | 5.2 |
| DOC (mg/L) | 4.6 | 5.4 |
| pH | 7.2 | 6.21 |
| Conductivity (µmhos/cm) | 66.2 | 35.6 |
| Abundance (ind/m³) | | |
| Crustacean zooplankton | 16793 | 6316 |
| Cladocerans | 2450 | 2797 |
| Copepods | 14342 | 3496 |
| <i>Bythotrephes</i> | 3.84 ±5.93 | 1.81 ±2.00 |
| <i>Mysis</i> | present | present |
| <i>Chaoborus</i> | present | present |

Table 2-2: Peninsula and Harp lake-collected zooplankton and macroinvertebrate predator stable isotope ratios of carbon and nitrogen and total mercury (THg) concentrations. na – not analysed, c – composite sample.

| Species | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) | Mercury (ng/g dw) | SD |
|--|------------------------------|------------------------------|----------------------|-------|
| PENINSULA LAKE | | | | |
| <i>Bythotrephes</i> (1 st instar) | 8.20 | -27.30 | na | |
| <i>Bythotrephes</i> (2 nd instar) | 8.28 | -27.61 | 109.4 | 5.04 |
| <i>Bythotrephes</i> (3 rd instar) | 8.02 | -27.61 | 126.1 | 6.60 |
| <i>Chaoborus</i> | 9.85 | -27.94 | c | |
| <i>Mysis</i> (large) | 9.77 | -31.64 | c | |
| <i>Mysis</i> (large) | 9.70 | -30.50 | c | |
| <i>Mysis</i> (large) | 9.31 | -30.64 | c | |
| <i>Mysis</i> (large) | 9.27 | -30.40 | c | |
| <i>Mysis</i> (large) | 9.15 | -31.24 | c | |
| <i>Mysis</i> (large) | 9.39 | -31.06 | c | |
| <i>Mysis</i> (large) combined | | | 178.6 | 7.75 |
| Zooplankton (153-243 μm) | 5.72 | -29.70 | 66.7 | 3.30 |
| Zooplankton (153-243 μm) | 6.70 | -30.35 | 51.3 | 3.70 |
| Zooplankton (243-500 μm) | 6.92 | -30.06 | 68.6 | 4.85 |
| Zooplankton (243-500 μm) | 7.10 | -30.35 | 91.1 | 4.43 |
| Zooplankton (>500 μm) | 5.10 | -29.26 | 78.8 | 5.44 |
| Zooplankton (>500 μm) | 6.03 | -29.75 | 100.3 | 5.16 |
| HARP LAKE | | | | |
| <i>Bythotrephes</i> (1 st instar) | 7.806 | -27.72 | na | |
| <i>Bythotrephes</i> (2 nd instar) | 7.372 | -27.613 | 152.8 | 44.76 |
| <i>Bythotrephes</i> (3 rd instar) | 6.558 | -27.587 | na | |
| Zooplankton (153-243 μm) | 5.852 | -33.273 | na | |
| Zooplankton (153-243 μm) | 4.219 | -29.779 | 126.3 | 3.41 |
| Zooplankton (243-500 μm) | 5.377 | -34.999 | na | |
| Zooplankton (243-500 μm) | 5.772 | -35.02 | 102.8 | 4.03 |
| Zooplankton (>500 μm) | 4.261 | -32.28 | na | |
| Zooplankton (>500 μm) | 5.285 | -34.624 | 109.7 | 4.68 |
| <i>Mysis</i> (small) | 5.16 | -28.418 | 106.2 | 8.76 |
| <i>Mysis</i> (medium) | 6.66 | -30.083 | c | |
| <i>Mysis</i> (medium) | 6.703 | -30.017 | c | |
| <i>Mysis</i> (medium) combined | | | 245.5 | 9.37 |
| <i>Mysis</i> (large) | 7.618 | -28.597 | c | |
| <i>Mysis</i> (large) | 7.746 | -29.03 | c | |
| <i>Mysis</i> (large) | 7.207 | -29.147 | c | |
| <i>Mysis</i> (large) | 7.224 | -29.072 | c | |
| <i>Mysis</i> (large) | 7.31 | -29.074 | c | |
| <i>Mysis</i> (large) | 6.121 | -28.648 | c | |
| <i>Mysis</i> (large) | 6.89 | -30.144 | c | |
| <i>Mysis</i> (large) | 7.186 | -29.36 | c | |
| <i>Mysis</i> (large) | 6.736 | -29.919 | c | |
| <i>Mysis</i> (large) combined | | | 378.9 | 7.19 |

Table 2-3: Summary of data collected and analysed for fish from Harp and Peninsula lakes.

Na = Not assessed; ND = not determined; SBF = skinless boneless dorsal filet; FE = whole fish with head and organs removed; FCE = combined sample of whole fish with heads and organs removed.

| Species | OME fish number | Total length (cm) | Fork length (cm) | Weight (g) | Sex | Mercury ($\mu\text{g/g ww}$) | Trace $\frac{1}{2}$ DL | Mercury (ng/g ww) | Mercury (ng/g dw) | Portion | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|-----------------------|-----------------|-------------------|------------------|------------|-----|--------------------------------|------------------------|-------------------|-------------------|---------|---------------------------|---------------------------|
| Peninsula Lake | | | | | | | | | | | | |
| Lake trout | W3821 | 56.5 | 53.1 | 1859 | F | 0.43 | | 430 | 2150 | SBF | 14.03 | -27.58 |
| Lake trout | W3822 | 61.6 | 58.9 | 2964 | M | 0.71 | | 710 | 3550 | SBF | 14.52 | -27.51 |
| Lake trout | W3823 | 47.8 | 44.3 | 1146 | M | 0.26 | | 260 | 1300 | SBF | 15.24 | -25.50 |
| Lake trout | W3824 | 24.1 | 21.8 | 122 | F | 0.2 | | 200 | 1000 | SBF | 13.50 | -24.80 |
| Lake trout | W3825 | 26.1 | 23.9 | 118 | M | 0.2 | | 200 | 1000 | SBF | 14.07 | -25.55 |
| Lake trout | W3826 | 52.3 | 48.9 | 1404 | M | 0.51 | | 510 | 2550 | SBF | 14.30 | -27.15 |
| Lake trout | W3827 | 40.1 | 37.7 | 610 | F | 0.29 | | 290 | 1450 | SBF | 14.58 | -26.22 |
| Lake trout | W3828 | 58.3 | 54.0 | 2055 | F | 0.58 | | 580 | 2900 | SBF | 14.57 | -26.74 |
| Pumpkinseed | W3861 | 16.0 | 13.9 | 87 | ND | 0.07 | | 70 | 350 | SBF | 9.02 | -20.70 |
| Pumpkinseed | W3862 | 10.0 | 8.2 | 2.1 | ND | na | | na | na | | 9.35 | -20.82 |
| Pumpkinseed | W3863 | 17.0 | 14.9 | 109 | ND | 0.08 | | 80 | 400 | SBF | 9.11 | -21.62 |
| Pumpkinseed | W3864 | 19.5 | 16.6 | 160 | ND | 0.07 | | 70 | 350 | SBF | 8.33 | -16.82 |
| Pumpkinseed | W3865 | 20.0 | 17.8 | 174 | ND | 0.07 | | 70 | 350 | SBF | 9.11 | -19.09 |
| Pumpkinseed | W3866 | 17.4 | 14.6 | 131 | ND | 0.06 | | 60 | 300 | SBF | 8.96 | -18.30 |
| Pumpkinseed | W3867 | 16.2 | 13.4 | 98 | ND | 0.07 | | 70 | 350 | SBF | 8.86 | -19.40 |
| Pumpkinseed | W3868 | 12.9 | 10.9 | 40 | ND | 0.04 | 0.02 | 20 | 100 | SBF | 9.20 | -21.12 |
| Pumpkinseed | W3869 | 13.2 | 11.1 | 49 | ND | 0.04 | 0.02 | 20 | 100 | SBF | 8.79 | -20.15 |
| Pumpkinseed | W3870 | 13.4 | 11.4 | 46 | ND | 0.04 | 0.02 | 20 | 100 | SBF | 9.09 | -19.94 |
| Rock bass | W3871 | 17.6 | 14.5 | 106 | F | 0.19 | | 190 | 950 | SBF | 10.95 | -23.17 |
| Rock bass | W3872 | 22.5 | 17.9 | 235 | F | 0.62 | | 620 | 3100 | SBF | 13.36 | -24.87 |
| Rock bass | W3873 | 20.6 | 17.5 | 179 | M | 0.22 | | 220 | 1100 | SBF | 11.51 | -23.67 |
| Rock bass | W3874 | 20.9 | 17.4 | 163 | F | 0.31 | | 310 | 1550 | SBF | 11.54 | -22.37 |
| Rock bass | W3875 | 19.0 | 16.0 | 133 | F | 0.17 | | 170 | 850 | SBF | 11.60 | -24.17 |
| Rock bass | W3876 | 16.5 | 13.8 | 92 | F | 0.12 | | 120 | 600 | SBF | 11.22 | -23.49 |
| Rock bass | W3877 | 18.9 | 15.5 | 125 | F | 0.17 | | 170 | 850 | SBF | 10.22 | -20.57 |

Table 2-3, continued.

| Species | OME fish number | Total length (cm) | Fork length (cm) | Weight (g) | Sex | Mercury ($\mu\text{g/g ww}$) | Trace $\frac{1}{2}$ DL | Mercury (ng/g ww) | Mercury (ng/g dw) | Portion | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|-----------------|-----------------|-------------------|------------------|------------|-----|--------------------------------|------------------------|-------------------|-------------------|---------|---------------------------|---------------------------|
| Rock bass | W3878 | 18.5 | 16.4 | 126 | M | 0.28 | | 280 | 1400 | SBF | 12.10 | -25.37 |
| Rock bass | W3879 | 18.4 | 15.9 | 129 | F | 0.18 | | 180 | 900 | SBF | 10.32 | -21.20 |
| Rock bass | W3880 | 18.9 | 15.6 | 136 | F | 0.19 | | 190 | 950 | SBF | 11.38 | -23.27 |
| Rock bass | W3881 | 12.7 | 12.4 | 42 | ND | 0.14 | | 140 | 700 | FC | 9.37 | -19.97 |
| Rock bass | W3882 | 8.7 | 8.5 | 13 | ND | na | | na | na | | 8.85 | -20.15 |
| Rock bass | W3883 | 12.9 | 12.6 | 41 | ND | na | | na | na | | 9.95 | -20.26 |
| Rock bass | W3884 | 13.4 | 13.0 | 43 | ND | na | | na | na | | 10.95 | -23.63 |
| Rock bass | W3885 | 15.6 | 15.0 | 75 | ND | na | | na | na | | 11.06 | -24.14 |
| Rock bass | W3886 | 12.0 | 11.7 | 35 | ND | na | | na | na | | 9.94 | -23.32 |
| Rock bass | W3887 | 14.2 | 13.9 | 62 | ND | na | | na | na | | 11.51 | -24.95 |
| Rock bass | W3888 | 12.3 | 11.8 | 38 | ND | na | | na | na | | 10.57 | -22.44 |
| Rock bass | W3889 | 11.8 | 11.6 | 27 | ND | na | | na | na | | 10.85 | -24.95 |
| Rock bass | W3890 | 13.6 | 13.2 | 45 | ND | na | | na | na | | 9.70 | -22.09 |
| Rainbow smelt | W3851-60 | 12.1 | 10.3 | 9 | ND | 0.04 | 0.02 | 20 | 100 | FCE | 11.38 | -27.56 |
| Rainbow smelt | W3851 | 12.2 | 10.4 | 9 | ND | c | | c | na | | 10.76 | -26.98 |
| Rainbow smelt | W3852 | 11.0 | 9.3 | 6 | ND | c | | c | na | | 11.57 | -27.63 |
| Rainbow smelt | W3853 | 12.1 | 10.3 | 10 | ND | c | | c | na | | 11.35 | -27.08 |
| Rainbow smelt | W3854 | 12.4 | 10.8 | 10 | ND | c | | c | na | | 11.51 | -27.21 |
| Rainbow smelt | W3855 | 12.6 | 10.7 | 10 | ND | c | | c | na | | 11.81 | -27.19 |
| Rainbow smelt | W3856 | 12.3 | 10.6 | 10 | ND | c | | c | na | | 10.99 | -27.29 |
| Rainbow smelt | W3857 | 10.3 | 8.9 | 5 | ND | c | | c | na | | 10.73 | -27.30 |
| Rainbow smelt | W3858 | 13.4 | 11.6 | 12 | ND | c | | c | na | | 11.51 | -27.87 |
| Rainbow smelt | W3859 | 12.3 | 10.4 | 9 | ND | c | | c | na | | 11.53 | -27.90 |
| Rainbow smelt | W3860 | 12.0 | 10.3 | 9 | ND | c | | c | na | | 8.93 | -24.09 |
| Smallmouth bass | W3891 | 9.0 | 8.7 | 5 | ND | 0.04 | 0.02 | 20 | 100 | FC | 8.57 | -23.56 |
| Smallmouth bass | W3892 | 6.3 | 6.1 | 3 | ND | na | | na | na | | 9.47 | -22.62 |
| Smallmouth bass | W3893 | 6.8 | 6.5 | 3 | ND | na | | na | na | | 10.41 | -24.31 |
| Smallmouth bass | W3894 | 8.6 | 8.3 | 6 | ND | na | | na | na | | 9.17 | -22.67 |
| Smallmouth bass | W3895 | 6.9 | 6.5 | 4 | ND | na | | na | na | | 11.94 | -25.26 |

Table 2-3, continued.

| Species | OME fish number | Total length (cm) | Fork length (cm) | Weight (g) | Sex | Mercury ($\mu\text{g/g ww}$) | Trace $\frac{1}{2}$ DL | Mercury (ng/g ww) | Mercury (ng/g dw) | Portion | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|-----------------|-----------------|-------------------|------------------|------------|-----|--------------------------------|------------------------|-------------------|-------------------|---------|---------------------------|---------------------------|
| Smallmouth bass | W3896 | 8.0 | 7.6 | 5 | ND | Na | | na | na | | 11.48 | -24.03 |
| Smallmouth bass | W3897 | 38.6 | 36.3 | 637 | F | 0.32 | | 320 | 1600 | SBF | 12.33 | -25.51 |
| Smallmouth bass | W3898 | 48.3 | 46.3 | 1750 | F | 0.95 | | 950 | 4750 | SBF | 11.79 | -26.10 |
| Smallmouth bass | W3899 | 32.8 | 31.0 | 485 | M | 0.21 | | 210 | 1050 | SBF | 11.38 | -24.00 |
| Smallmouth bass | W3900 | 26.5 | 25.6 | 261 | M | 0.19 | | 190 | 950 | SBF | 11.97 | -25.66 |
| Smallmouth bass | W3901 | 46.7 | 44.4 | 1444 | M | 0.79 | | 790 | 3950 | SBF | 10.78 | -24.51 |
| Smallmouth bass | W3902 | 28.5 | 27.4 | 296 | M | 0.16 | | 160 | 800 | SBF | 11.26 | -24.15 |
| Smallmouth bass | W3903 | 34.8 | 33.1 | 625 | M | 0.25 | | 250 | 1250 | SBF | 11.48 | -24.08 |
| Smallmouth bass | W3904 | 30.4 | 28.8 | 441 | M | 0.27 | | 270 | 1350 | SBF | 11.78 | -24.47 |
| Smallmouth bass | W3905 | 36.3 | 34.0 | 701 | M | 0.43 | | 430 | 2150 | SBF | 11.81 | -23.65 |
| Smallmouth bass | W3906 | 48.1 | 45.7 | 1251 | F | 1.2 | | 1200 | 6000 | SBF | 10.75 | -25.94 |
| Yellow perch | W3829 | 21.8 | 18.9 | 133 | M | 0.15 | | 150 | 750 | SBF | 10.34 | -23.43 |
| Yellow perch | W3830 | 22.1 | 18.5 | 140 | F | 0.1 | | 100 | 500 | SBF | 10.31 | -22.21 |
| Yellow perch | W3831 | 23.7 | 20.2 | 161 | F | 0.24 | | 240 | 1200 | SBF | 10.69 | -24.53 |
| Yellow perch | W3832 | 21.0 | 17.4 | 124 | F | 0.11 | | 110 | 550 | SBF | 10.64 | -26.52 |
| Yellow perch | W3833 | 21.4 | 18.0 | 121 | F | 0.09 | | 90 | 450 | SBF | 10.72 | -24.02 |
| Yellow perch | W3834 | 22.6 | 19.0 | 122 | F | 0.14 | | 140 | 700 | SBF | 10.87 | -25.46 |
| Yellow perch | W3835 | 20.8 | 18.4 | 98 | F | 0.14 | | 140 | 700 | SBF | 10.27 | -23.93 |
| Yellow perch | W3836 | 19.8 | 18.1 | 94 | ND | na | | na | na | | 10.84 | -24.58 |
| Yellow perch | W3837 | 16.0 | 13.5 | 40 | ND | na | | na | na | | 10.99 | -23.40 |
| Yellow perch | W3838 | 18.6 | 16.0 | 76 | M | 0.16 | | 160 | 800 | SBF | 11.07 | -23.76 |
| Yellow perch | W3839 | 23.9 | 20.5 | 130 | F | 0.2 | | 200 | 1000 | SBF | 10.85 | -24.58 |
| Yellow perch | W3840 | 21.3 | 17.9 | 122 | M | 0.18 | | 180 | 900 | SBF | 9.25 | -24.06 |
| Yellow perch | W3841 | 12.0 | 9.8 | 20 | ND | 0.07 | | 70 | 350 | FC | 10.01 | -23.57 |
| Yellow perch | W3842 | 11.9 | 9.9 | 20 | ND | na | | na | na | | 8.90 | -22.28 |
| Yellow perch | W3843 | 11.1 | 9.1 | 14 | ND | na | | na | na | | 8.92 | -21.66 |
| Yellow perch | W3844 | 13.8 | 11.0 | 28 | ND | na | | na | na | | 9.07 | -20.38 |
| Yellow perch | W3845 | 12.5 | 10.5 | 21 | ND | na | | na | na | | 10.16 | -22.35 |
| Yellow perch | W3846 | 12.3 | 10.6 | 17 | ND | na | | na | na | | 10.53 | -24.44 |

Table 2-3, continued.

| Species | OME fish number | Total length (cm) | Fork length (cm) | Weight (g) | Sex | Mercury ($\mu\text{g/g ww}$) | Trace $\frac{1}{2}$ DL | Mercury (ng/g ww) | Mercury (ng/g dw) | Portion | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|------------------|-----------------|-------------------|------------------|------------|-----|--------------------------------|------------------------|-------------------|-------------------|---------|---------------------------|---------------------------|
| Yellow perch | W3847 | 13.5 | 11.7 | 25 | ND | na | | na | na | | 10.97 | -24.22 |
| Yellow perch | W3848 | 14.8 | 12.7 | 34 | ND | na | | na | na | | 10.51 | -24.41 |
| Yellow perch | W3849 | 15.6 | 13.8 | 44 | ND | na | | na | na | | 9.81 | -23.30 |
| Harp Lake | | | | | | | | | | | | |
| Lake trout | W5121 | 51.1 | 46.9 | 1260 | M | 1.1 | | 1100 | 5500 | SBF | 11.84 | -28.25 |
| Lake trout | W5122 | 47.9 | 44.6 | 984 | M | 0.39 | | 390 | 1950 | SBF | 10.98 | -27.73 |
| Lake trout | W5123 | 41.1 | 38 | 588 | F | 0.44 | | 440 | 2200 | SBF | 10.86 | -27.35 |
| Lake trout | W5124 | 41.0 | 37.6 | 557 | F | 0.44 | | 440 | 2200 | SBF | 11.69 | -27.05 |
| Lake trout | W5125 | 37.8 | 34.7 | 392 | F | 0.38 | | 380 | 1900 | SBF | 11.64 | -27.15 |
| Lake trout | W5126 | 39.0 | 36.5 | 456 | F | 0.67 | | 670 | 3350 | SBF | 12.31 | -26.55 |
| Lake trout | W5127 | 29.6 | 27.1 | 214 | F | 0.27 | | 270 | 1350 | SBF | 11.228 | -25.28 |
| Lake trout | W5128 | 30.2 | 27.9 | 219 | F | 0.3 | | 300 | 1500 | SBF | 11.23 | -25.94 |
| Lake trout | W5129 | 26.9 | 24.9 | 165 | F | 0.31 | | 310 | 1550 | SBF | 11.51 | -26.36 |
| Lake trout | W5130 | 28.2 | 26.4 | 165 | F | 0.28 | | 280 | 1400 | SBF | 11.11 | -25.94 |
| Lake trout | W5131 | 23.5 | 21.7 | 102 | M | 0.31 | | 310 | 1550 | SBF | 10.98 | -27.25 |
| Lake trout | W5132 | 25.1 | 23.1 | 118 | F | 0.3 | | 300 | 1500 | SBF | 11.17 | -26.14 |
| Lake trout | W5133 | 26.7 | 24.7 | 161 | M | 0.28 | | 280 | 1400 | SBF | 11.32 | -25.45 |
| Lake trout | W5134 | 25.2 | 23 | 127 | F | 0.3 | | 300 | 1500 | SBF | 11.44 | -26.24 |
| Lake herring | W5135 | 34.1 | 30.6 | 350 | F | 0.58 | | 580 | 2900 | SBF | 9.62 | -25.94 |
| Lake herring | W5136 | 36.5 | 31.6 | 489 | M | 0.34 | | 340 | 1700 | SBF | 9.734 | -26.52 |
| Lake herring | W5137 | 35.1 | 32.9 | 452 | F | 0.42 | | 420 | 2100 | SBF | 9.61 | -27.12 |
| Lake herring | W5138 | 33.4 | 30.2 | 374 | F | 0.54 | | 540 | 2700 | SBF | 9.43 | -29.27 |
| Lake herring | W5139 | 37.7 | 34.9 | 492 | F | 0.4 | | 400 | 2000 | SBF | 9.06 | -28.16 |
| Lake herring | W5140 | 34.1 | 32.6 | 356 | M | 0.19 | | 190 | 950 | SBF | 9.05 | -28.24 |
| Lake herring | W5141 | 38.0 | 36.3 | 560 | F | 0.4 | | 400 | 2000 | SBF | 9.46 | -27.91 |
| Lake herring | W5142 | 36.1 | 33.7 | 523 | F | 0.35 | | 350 | 1750 | SBF | 9.51 | -28.57 |
| Lake herring | W5143 | 32.1 | 29.8 | 350 | M | 0.14 | | 140 | 700 | SBF | 8.99 | -28.00 |
| Lake herring | W5144 | 36.9 | 34.2 | 361 | F | 0.38 | | 380 | 1900 | SBF | 9.61 | -27.09 |

Table 2-4: Lake trout length, weight and age (assessed using scales). Age includes an edge assessment, with “+” indicating new growth. A confidence level in brackets is included, with 9 being the most confident and 1 being the lowest.

| Fish # | Length (cm) | Weight (g) | Age |
|-----------------------|--------------------|-------------------|------------|
| Peninsula Lake | | | |
| W3821 | 56.5 | 1859 | 5+(7) |
| W3822 | 61.6 | 2964 | 9+(5) |
| W3823 | 47.8 | 1146 | 7+(5) |
| W3824 | 24.1 | 122 | 2+(9) |
| W3825 | 26.1 | 118 | 2+(5) |
| W3826 | 52.3 | 1404 | 6*(7) |
| W3827 | 40.1 | 610 | 4+(7) |
| W3828 | 58.3 | 2055 | 7+(5) |
| Harp Lake | | | |
| W5121 | 51.1 | 1260 | 6+(5) |
| W5122 | 47.9 | 984 | 5+(7) |
| W5123 | 41.1 | 588 | 4+(9) |
| W5124 | 41 | 557 | 4+(9) |
| W5125 | 37.8 | 392 | 4+(7) |
| W5126 | 39 | 456 | 4+(7) |
| W5127 | 29.6 | 214 | 3+(7) |
| W5128 | 30.2 | 219 | 4+(5) |
| W5129 | 26.9 | 165 | 2+(7) |
| W5130 | 28.2 | 165 | 3+(7) |
| W5131 | 23.5 | 102 | 2+(9) |
| W5132 | 25.1 | 118 | 2+(9) |
| W5133 | 26.7 | 161 | 3+(9) |
| W5134 | 25.2 | 127 | 2+(7) |

Table 2-5: The mean proportion (MP) of stomach mass and percent occurrence (PO) of prey items among species in Peninsula and Harp lakes expressed as percentages. Due to variable prey occurrence within stomachs, totals can exceed 100%.

| Peninsula Lake | Lake trout | | Yellow perch | | Pumpkinseed | | Smallmouth bass | | Rock bass | |
|---------------------|------------|----------|--------------|----------|-------------|----------|-----------------|----------|------------|-----------|
| | MP | PO | MP | OP | MP | PO | MP | PO | MP | PO |
| | ± SD | (% of 2) | ± SD | (% of 4) | ± SD | (% of 4) | ± SD | (% of 5) | ± SD | (% of 14) |
| Fish | 58.8 ±58.3 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 28.5 ±23.3 | 21 |
| Fish eggs | 82.4 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bivalve | 0 | 0 | 1.5 ±0.7 | 50 | 0 | 0 | 0 | 0 | 0 | 0 |
| Amphipod | 0 | 0 | 17.0 ±2.8 | 50 | 0 | 0 | 73.0 | 20 | 28.0 ±24.2 | 29 |
| Isopod | 0 | 0 | 80.0 ±4.2 | 50 | 0 | 0 | 27.0 | 20 | 36.8 ±3.0 | 29 |
| Ephemeropteran | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 47.7 ±30.7 | 29 |
| Chironomid | 0 | 0 | 3.0 | 25 | 100 | 25 | 0 | 0 | 0 | 0 |
| Zooplankton | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Mysis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Chaoborus</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Bythotrephes</i> | 0 | 0 | 100 | 75 | 0 | 0 | 0 | 0 | 76.0 ±38.7 | 43 |
| spines | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 75.0 ±35.4 | 14 |
| Unknown | 0 | 0 | 0 | 25 | 100 | 75 | 100 | 80 | 97.0 ±6.0 | 36 |
| Harp Lake | Lake trout | | Lake herring | | | | | | | |
| | | (% of 8) | | (% of 4) | | | | | | |
| Fish | 9.0 | 13 | 0 | 0 | | | | | | |
| Fish eggs | 0 | 0 | 2.0 | 25 | | | | | | |
| Bivalve | 1.0 | 13 | 0 | 0 | | | | | | |
| Amphipod | 0 | 0 | 0 | 0 | | | | | | |
| Isopod | 0 | 0 | 0 | 0 | | | | | | |
| Ephemeropteran | 0 | 0 | 0 | 0 | | | | | | |
| Chironomid | 0 | 0 | 0 | 0 | | | | | | |
| Zooplankton | 0 | 0 | 41.3 ±45.2 | 75 | | | | | | |
| <i>Mysis</i> | 23.0 ±22.9 | 88 | 87.7 ±11.6 | 75 | | | | | | |
| <i>Chaoborus</i> | 0 | 0 | 2.0 | 50 | | | | | | |
| <i>Bythotrephes</i> | 45.2 ±19.9 | 88 | 2.0 | 50 | | | | | | |
| spines | 48.0 ±24.2 | 50 | 1.0 | 25 | | | | | | |
| Unknown | 0 | 0 | 6.0 | 25 | | | | | | |

Table 2-6: Mean contributions (% and ranges in brackets) of each prey item to the diet of each consumer group, calculated by IsoSource (1% increment, 0.5 tolerance). All values are from lake samples, except Benthos which is from stomach content SIA. For Harp Lake, benthos values were approximated from Peninsula Lake values. nm - not included in scenario.

| Consumer | <i>Mysis</i> | <i>Bythotrephes</i> | Zooplankton | <i>Chaoborus</i> | Benthic fish | Smelt | Benthos |
|------------------|--------------|---------------------|-------------|------------------|--------------|--------------|-------------|
| Peninsula | | | | | | | |
| Smelt | 8.1 (0-35) | 22.2 (0-100) | 31.9 (0-73) | 9.7 (0-49) | 9.7 (0-40) | nm | 18.4 (0-68) |
| Lake trout | 4.4 (0-29) | 3.6 (0-24) | 1.9 (0-14) | 8.1 (0-51) | 15.4 (0-42) | 62.1 (33-97) | 4.5 (0-27) |
| | <i>Mysis</i> | <i>Bythotrephes</i> | Zooplankton | Lake herring | Benthos | | |
| Harp | | | | | | | |
| Lake herring | 20.7 (0-65) | 17.8 (0-56) | 23.6 (4-46) | nm | 37.9 (17-64) | | |
| Lake trout | 8.3 (0-39) | 17.9 (0-84) | 3.1 (0-16) | 45.6 (12-75) | 25.2 (4-48) | | |

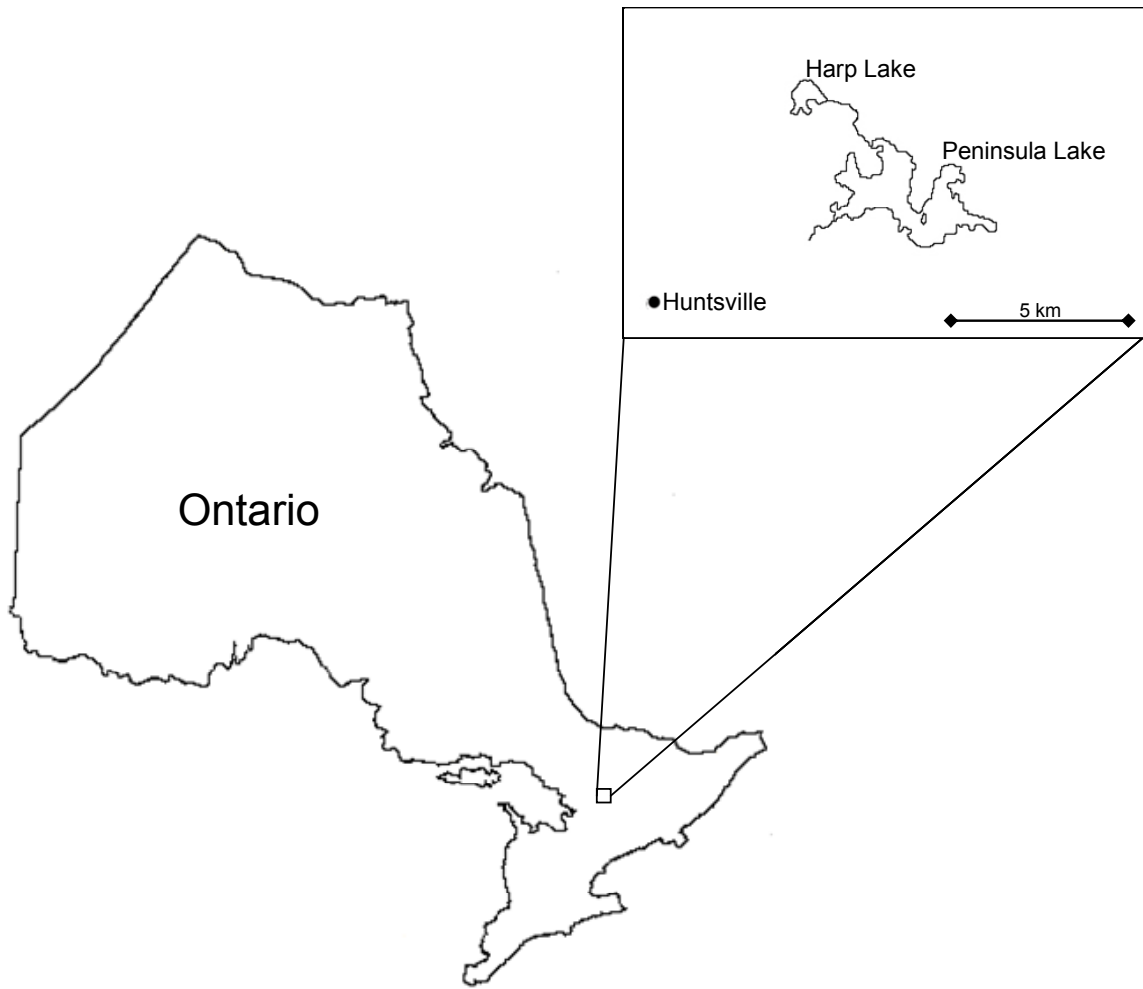


Figure 2-1: Map of study area.

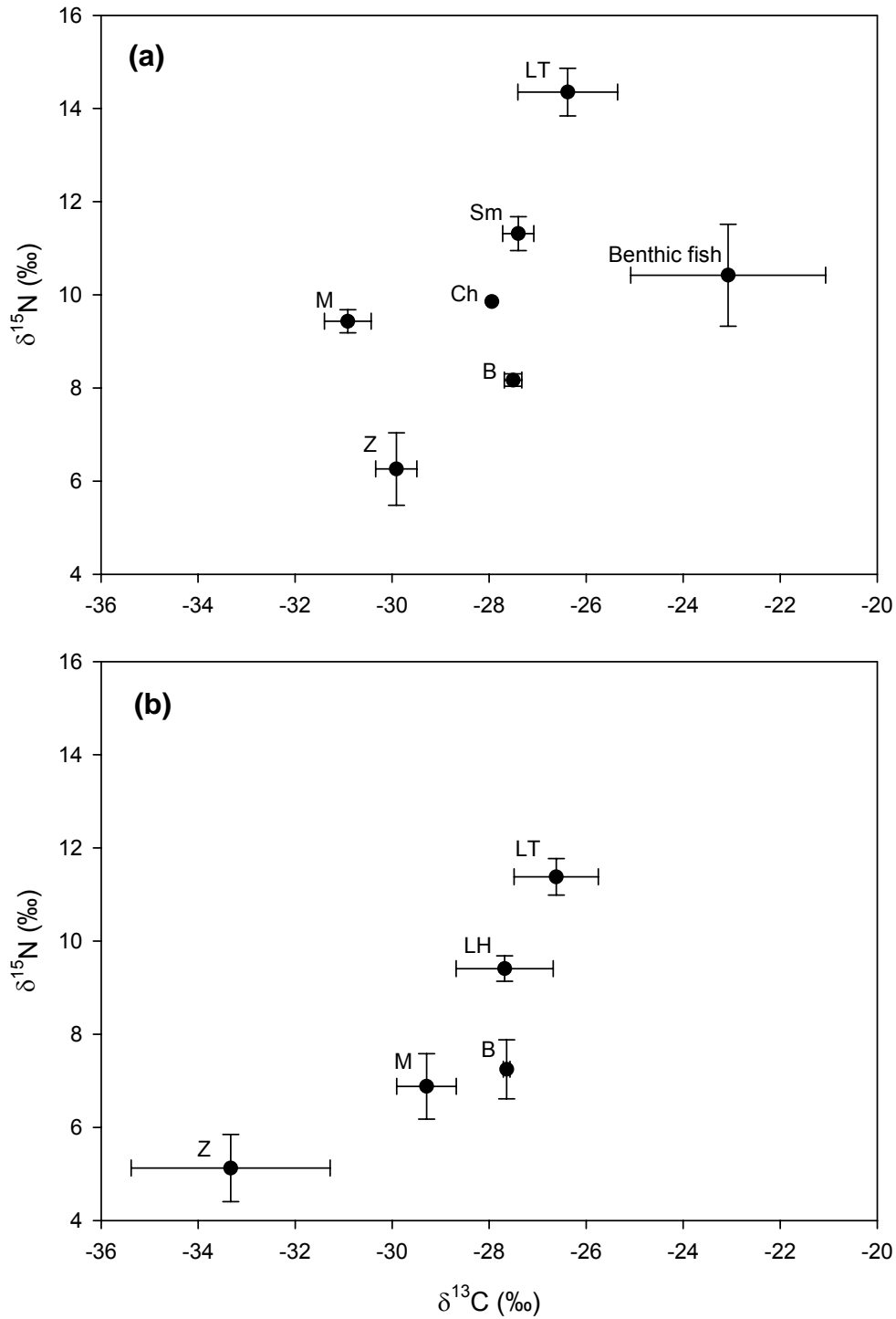


Figure 2-2: Trophic structure of Peninsula (a) and Harp (b) lake food webs as determined by stable nitrogen and carbon isotope ratios. The mean and standard deviation of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ are presented for zooplankton (Z), *Bythotrephes* (B), *Mysis* (M), *Chaoborus* (Ch), rainbow smelt (Sm), lake herring (LH), lake trout (LT) and benthic fish (yellow perch, rock bass, smallmouth bass and pumpkinseed).

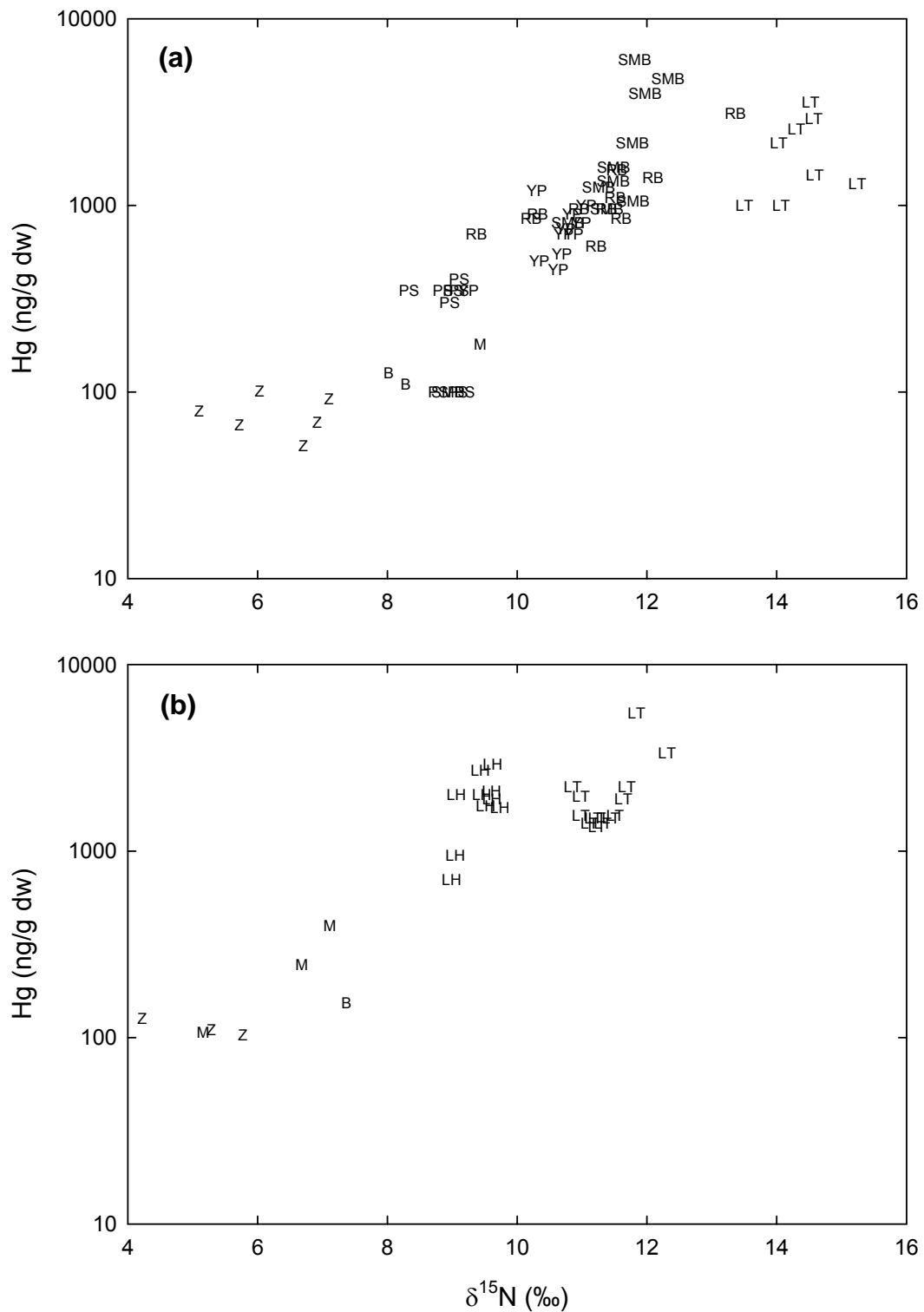


Figure 2-3: Trophic structure and total mercury concentrations for Peninsula (a) and Harp (b) lake food webs. Samples include: **Z**, zooplankton; **M**, *Mysis*; **B**, *Bythotrephes*; **LH**, lake herring; **LT**, lake trout; **SMB**, smallmouth bass; **YP**, yellow perch; **RB**, rock bass; and **PS**, pumpkinseed.

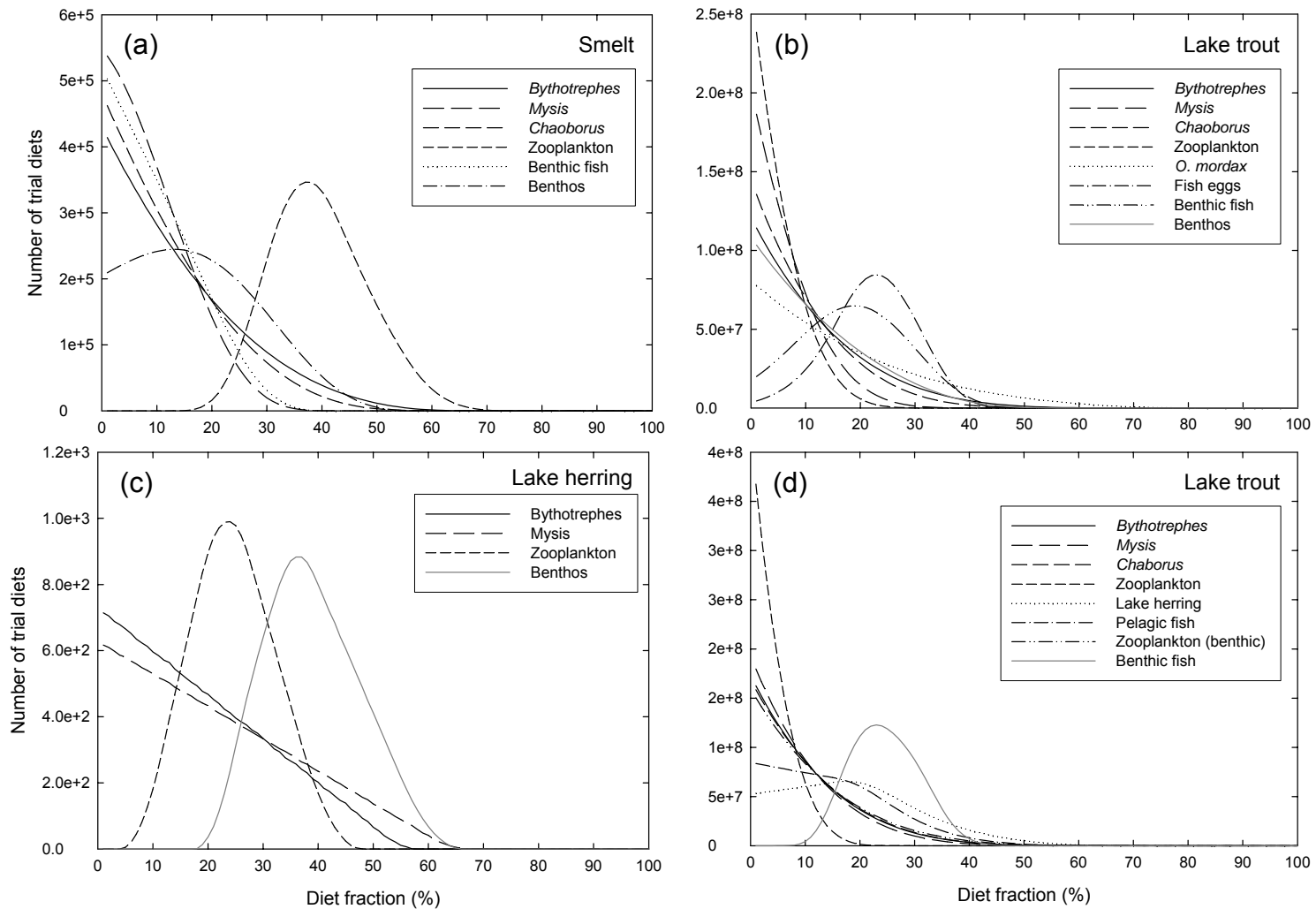


Figure 2-4: Results from the mixing model for rainbow smelt and lake trout for Peninsula Lake (a, b) and lake herring and lake trout for Harp Lake (c,d). The graphs show the frequency of a given percentage contribution of the overall diet for each dietary item.

Chapter 3:
General Discussion and Future Directions

Using a combination of stable isotope and THg analyses, the role of *Bythotrephes* in lake food webs was investigated. The results of both approaches are consistent and places *Bythotrephes* in a similar position to that of native macroinvertebrate predators found in Peninsula and Harp lakes. *Bythotrephes* assimilates itself into a trophic level previously occupied by other organisms, thus it may not be affecting pelagic food web length. Fish, both pelagic and benthic, are positively selecting *Bythotrephes* as a prey item.

In order to fully understand the role of *Bythotrephes* in lake food webs, a more complete food web study investigating the relationship and competition between native zooplanktivores and *Bythotrephes* as well as possible molecular or growth rate effects on *Bythotrephes* predators (i.e., *Mysis*, zooplanktivorous fish) is required. Additional research will aid in further understanding the bottom-up food web effects of *Bythotrephes* invasion and its effects on zooplankton communities on aquatic ecosystems. Future research should focus on:

1. A comparison of aquatic food webs in lakes with and without *Bythotrephes*. A direct comparison between similar food webs in different lakes may help to tease apart if *Bythotrephes* is elevating the macroinvertebrate trophic guild.
2. The competition between *Bythotrephes* and zooplanktivores for resources. When *Bythotrephes* invades, the total macroinvertebrate predator demand increases by 25% (Foster 2007, Ph.D. thesis); however, no changes in *Mysis* and *Chaoborus* abundances were attributed to this increased competition. Yet, effects have been seen in changes to the fatty acid composition of *Mysis* in lakes with *Bythotrephes* (Nordin et al. 2007).
3. Alteration of contaminant transfer. *Bythotrephes*-mediated changes in the food web may have important implications for energy transfer and mercury

biomagnification. For example, biodilution of Hg may occur in lakes with *Bythotrephes* due to its effects on lower trophic levels, such as an increased abundance of primary producers (Pickhardt et al. 2002). Any alteration of the Hg concentrations in fish consumed by humans and other fish predators (e.g., loons) could have health and regulatory implications.

4. The integration of the pelagic and benthic food webs. From stomach content analysis, yellow perch and rock bass consume *Bythotrephes*. Higher Hg burdens in pelagic food webs (Power et al. 2002) could be transferred to the benthic food web.

Summary

1. *Bythotrephes* inserts itself into the pelagic food web at a similar trophic position to that of native macroinvertebrate predators found in Peninsula and Harp lakes.
2. *Bythotrephes* is positively selected as a prey item by both pelagic and benthic zooplanktivorous fish.

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Appendix I: Fish presence and absence data from the 10 original study lakes. Fish caught (C), as well as present (P) and absent (A) in each lake as communicated by local fishers and cottagers.

| Lake | Latitude | Longitude | County | Township | Dates sampled | Lake herring | Rainbow smelt | Lake whitefish | Lake trout | Burbot | Walleye | Northern pike | White sucker | Yellow perch | Smallmouth bass | Rock bass | Brown bullhead | Pumpkinseed |
|---------------------|----------|-----------|-------------|------------|-----------------|--------------|---------------|----------------|------------|--------|---------|---------------|--------------|--------------|-----------------|-----------|----------------|-------------|
| Invaded | | | | | | | | | | | | | | | | | | |
| Harp | 4523 | 7907 | Muskoka | Chaffey | 29-Aug-06 | C | | | C | | | | | P | P | | | |
| Kashagawigamog West | 4459 | 7836 | Haliburton | Minden | 30-Aug-06 | C | | | C | | C | | | C | C | | C | |
| Kashagawigamog East | 4459 | 7836 | Haliburton | Minden | 26-Aug-06 | C | C | | | | C | | | C | C | | C | |
| Mary | 4515 | 7915 | Muskoka | Stephenson | 25-Aug-06 | | | | C | | | | | | C | | C | |
| Peninsula | 4520 | 7906 | Muskoka | Chaffey | 24-Aug-06 | | C | | C | | | | | C | C | C | C | C |
| Non-invaded | | | | | | | | | | | | | | | | | | |
| Blue Chalk | 4512 | 7856 | Muskoka | Ridout | 12/15/31-Aug-06 | | | | C | | | | C | C | | | C | C |
| Echo | 4511 | 7904 | Muskoka | McLean | 16-Aug-06 | | | | A | | | | C | C | C | C | C | |
| Red Chalk Main | 4511 | 7856 | Muskoka | Ridout | 09/10/31-Aug-06 | | | | C | C | | | C | C | | | C | |
| Round | 4528 | 7924 | Parry Sound | McMurrich | 18-Aug-06 | C | | C | A | | C | C | | | C | | C | |
| Waseosa | 4524 | 7917 | Muskoka | Chaffey | 23-Aug-06 | C | | | | | | | C | C | C | | C | |

Appendix II: Stable isotope ratios for fish stomach contents from Peninsula and Harp lakes. LH, lake herring; SM, rainbow smelt; LT, lake trout; SMB, smallmouth bass; YP, yellow perch; RB, rock bass; and PS, pumpkinseed.

| Species | Fish stomach | Fish/sample Code | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|---------------------------|--------------|------------------|------------------------------|------------------------------|
| Peninsula Lake | | | | |
| Amphipods | YP | 3829AMPH | 9.05 | -28.75 |
| Amphipods | YP | 3838AMPH | 8.69 | -27.02 |
| Amphipods | PS | 3876AMPH | 7.63 | -24.18 |
| Amphipods | RB | 3887AMPH | 8.20 | -25.51 |
| Bivalve | YP | 3829CLAM | 8.96 | -23.20 |
| <i>Bythotrephes</i> | LT | 3825B | 11.87 | -26.44 |
| <i>Bythotrephes</i> | YP | 3834B1 | 9.81 | -25.82 |
| <i>Bythotrephes</i> | YP | 3834B2 | 9.65 | -25.91 |
| <i>Bythotrephes</i> | PS | 3873BF | 9.51 | -22.93 |
| <i>Bythotrephes</i> | PS | 3875B | 8.19 | -24.08 |
| <i>Bythotrephes</i> | PS | 3880B | 7.60 | -24.13 |
| <i>Bythotrephes</i> | RB | 3883B | 8.17 | -22.97 |
| <i>Bythotrephes</i> | RB | 3884B | 8.25 | -23.88 |
| Chironomids | YP | 3829CHIRON | 9.39 | -28.57 |
| Chironomids | YP | 3838CHIRON | 8.99 | -27.74 |
| Ephemoptera | PS | 3868EPHEM | 5.24 | -21.07 |
| Ephemoptera | PS | 3875ephem | 7.88 | -26.18 |
| Ephemoptera | RB | 3887EPHEM | 7.52 | -24.53 |
| Fish | LT | 3824F | 11.65 | -27.17 |
| Fish | PS | 3874F | 8.22 | -22.48 |
| Fish | RB | 3884F | 9.87 | -24.53 |
| Fish eggs | LT | 3824FEGG | 15.09 | -28.38 |
| Isopods | YP | 3829ISO | 9.42 | -29.20 |
| Isopods | YP | 3838ISO | 9.26 | -27.29 |
| Isopods | PS | 3876iso | 8.28 | -24.37 |
| Isopods | RB | 3884ISO | 8.23 | -24.05 |
| Isopods | RB | 3887ISO | 8.37 | -25.25 |
| Zooplankton (cladocerans) | YP | 3833Z | 9.32 | -25.07 |

Appendix II, continued.

| Species | Fish stomach | Fish/sample Code | $\delta^{15}\text{N}$ (‰) | $\delta^{13}\text{C}$ (‰) |
|---------------------------|--------------|------------------|------------------------------|------------------------------|
| Harp Lake | | | | |
| <i>Bythotrephes</i> | LT | 5121B1 | 7.30 | -27.96 |
| <i>Bythotrephes</i> | LT | 5122B | 6.86 | -28.13 |
| <i>Bythotrephes</i> | LT | 5123B | 6.80 | -28.12 |
| <i>Bythotrephes</i> | LT | 5124B1 | 6.41 | -28.54 |
| <i>Bythotrephes</i> | LT | 5125B | 7.50 | -28.77 |
| <i>Bythotrephes</i> | LT | 5128B1 | 7.78 | -29.88 |
| <i>Bythotrephes</i> | LT | 5130B | 6.98 | -28.64 |
| <i>Chaoborus</i> | LH | 5138CH | 6.83 | -27.68 |
| Fish | LT | 5126F | 10.72 | -29.02 |
| Fish | LT | 5128F | 8.00 | -28.53 |
| <i>Mysis</i> | LT | 5121ML | 8.11 | -28.31 |
| <i>Mysis</i> | LT | 5125M | 7.26 | -29.15 |
| <i>Mysis</i> | LT | 5130M | 7.50 | -29.38 |
| <i>Mysis</i> | LH | 5140M2 | 7.76 | -29.24 |
| <i>Mysis</i> | LH | 5140M3 | 7.27 | -27.77 |
| <i>Mysis</i> | LH | 5140ML | 7.82 | -28.95 |
| <i>Mysis</i> | LH | 5143ML | 7.81 | -29.14 |
| Zooplankton (cladocerans) | LH | 5138Z | 6.59 | -28.19 |
| Zooplankton (cladocerans) | LH | 5143Z | 7.74 | -27.87 |

Appendix III: Summary of C:N and lipid normalized $\delta^{13}\text{C}$ values (using a revised model from Kiljunen et al. (2006)) for Peninsula (PA) and Harp (HP) lakes.

| Species | Lake | n | C:N | | Untreated $\delta^{13}\text{C}$ | | Normalized $\delta^{13}\text{C}$ | | $\delta^{13}\text{C}$ Difference | |
|------------------|-------|-----|------|------|---------------------------------|------|----------------------------------|------|----------------------------------|------|
| | | | mean | SD | mean | SD | mean | SD | mean | SD |
| Lake trout | PA/HP | 22 | 3.54 | 0.30 | -26.76 | 3.03 | -25.48 | 0.66 | 1.06 | 0.48 |
| | PA | 8 | 3.70 | 0.37 | -26.38 | 1.03 | -25.08 | 0.58 | 1.30 | 0.57 |
| | HP | 14 | 3.46 | 0.23 | -26.62 | 0.87 | -25.70 | 0.61 | 0.92 | 0.37 |
| Cisco | HP | 10 | 3.93 | 0.30 | -27.68 | 2.36 | -26.02 | 0.62 | 1.66 | 0.44 |
| Smelt | PA | 10 | 3.65 | 0.09 | -21.21 | 2.20 | -26.14 | 0.23 | 1.26 | 0.23 |
| Yellow perch | PA | 22 | 3.33 | 0.06 | -24.41 | 3.21 | -23.07 | 1.42 | 0.70 | 0.12 |
| Pumpkinseed | PA | 10 | 3.32 | 0.12 | -22.27 | 2.06 | -19.12 | 1.37 | 0.68 | 0.22 |
| Rock bass | PA | 20 | 3.20 | 0.05 | -24.11 | 1.15 | -22.47 | 1.74 | 0.43 | 0.11 |
| Smallmouth bass | PA | 16 | 3.31 | 0.10 | -27.78 | 2.93 | -23.67 | 1.01 | 0.66 | 0.20 |
| All fish | PA/HP | 110 | 3.43 | 0.27 | -25.41 | 3.65 | -23.72 | 2.30 | 0.85 | 0.45 |
| | PA | 86 | 3.36 | 0.21 | -23.88 | 2.43 | -23.13 | 2.25 | 0.75 | 0.36 |
| | HP | 24 | 3.65 | 0.35 | -27.06 | 1.05 | -25.83 | 0.63 | 1.23 | 0.54 |
| Invertebrates | PA/HP | 36 | 4.81 | 0.88 | -27.40 | 2.15 | -27.56 | 1.44 | 2.55 | 0.78 |
| | PA | 15 | 5.09 | 0.56 | -29.97 | 1.27 | -27.08 | 0.97 | 2.89 | 0.43 |
| | HP | 21 | 4.60 | 1.01 | -30.21 | 1.01 | -27.91 | 1.63 | 2.30 | 0.88 |
| Stomach contents | PA/HP | 47 | 5.38 | 1.41 | -26.57 | 2.49 | -23.75 | 2.98 | 2.93 | 0.91 |
| | PA | 28 | 5.93 | 1.60 | -25.38 | 2.08 | -22.12 | 2.86 | 3.26 | 1.02 |
| | HP | 19 | 4.58 | 0.35 | -28.59 | 0.61 | -26.15 | 0.43 | 2.44 | 0.37 |

Kiljunen M, Grey J, Sinisalo T, Harrod C, Immonen H and RI Jones. 2006. A revised model for lipid-normalizing $\delta^{13}\text{C}$ values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology* 43:1213-1222.

Appendix IV: Zooplankton size fraction summary.

Table AIV-I: Qualitative zooplankton analysis for zooplankton fractions by mesh size used in stable isotope analysis.

| Lake | mesh size | Daphniidae | Holopediidae | Bozminidae | Calanoida | Cyclopoida | Nauplii |
|-----------|-------------------|------------|--------------|------------|-----------|------------|---------|
| Peninsula | 153 μm | x | x | present | present | present | present |
| | 243 μm | present | x | present | present | present | x |
| | 500 μm | present | present | present | present | present | x |
| Harp | 153 μm | x | x | x | present | present | present |
| | 243 μm | x | x | x | present | present | x |
| | 500 μm | present | present | x | present | present | x |

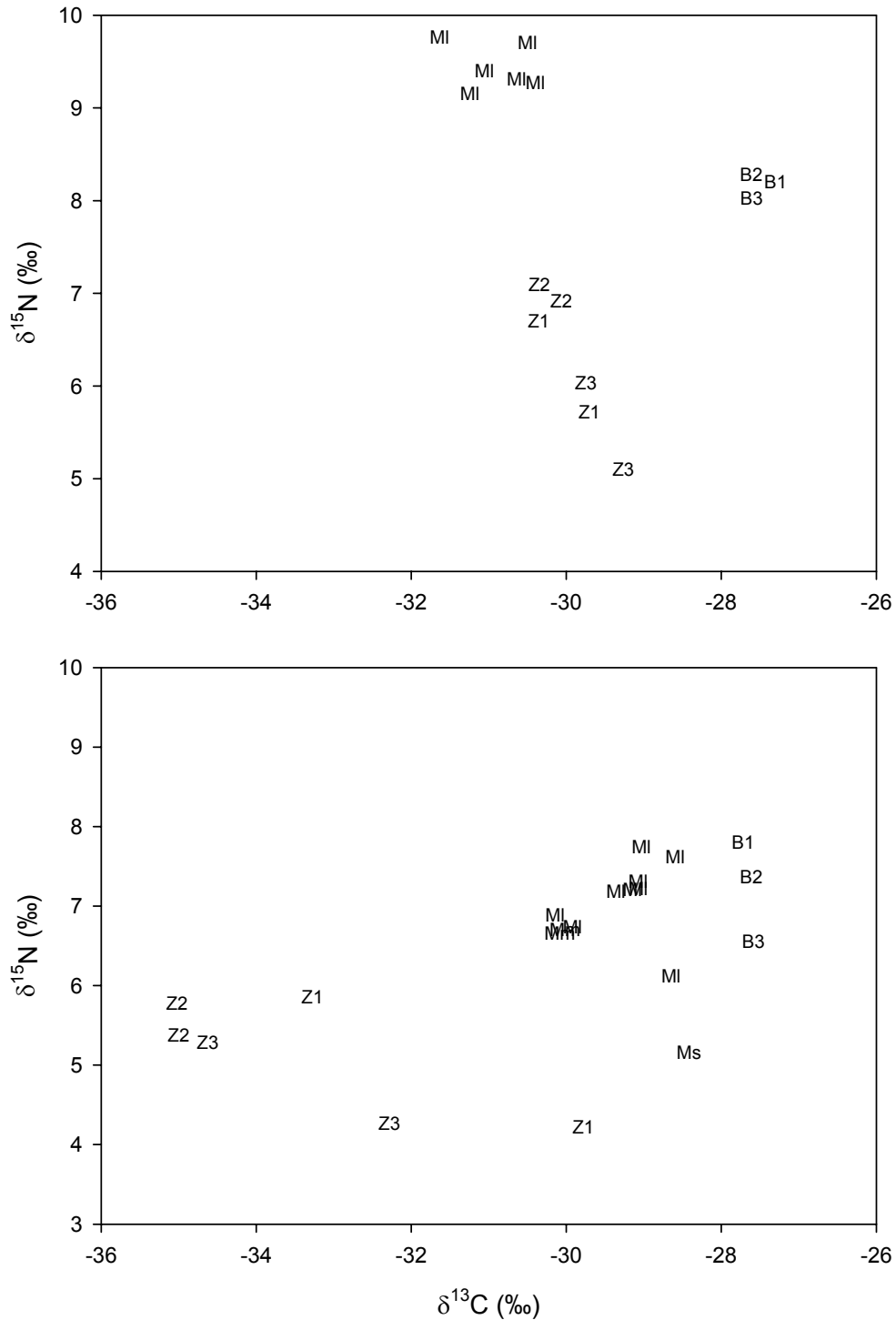


Figure AIV-1: Plankton trophic structure of Peninsula (a) and Harp (b) lakes as determined by stable carbon and nitrogen isotopes. Zooplankton was separated into three size classes: 153-243 μm (Z1), 243-500 μm (Z2) and $>500 \mu\text{m}$ (Z3). *Mysis* were separated into three size classes: small (Ms), medium (Mm) and large (MI). *Bythotrephes* were separated into first (B1), second (B2) and third (B3) instars.

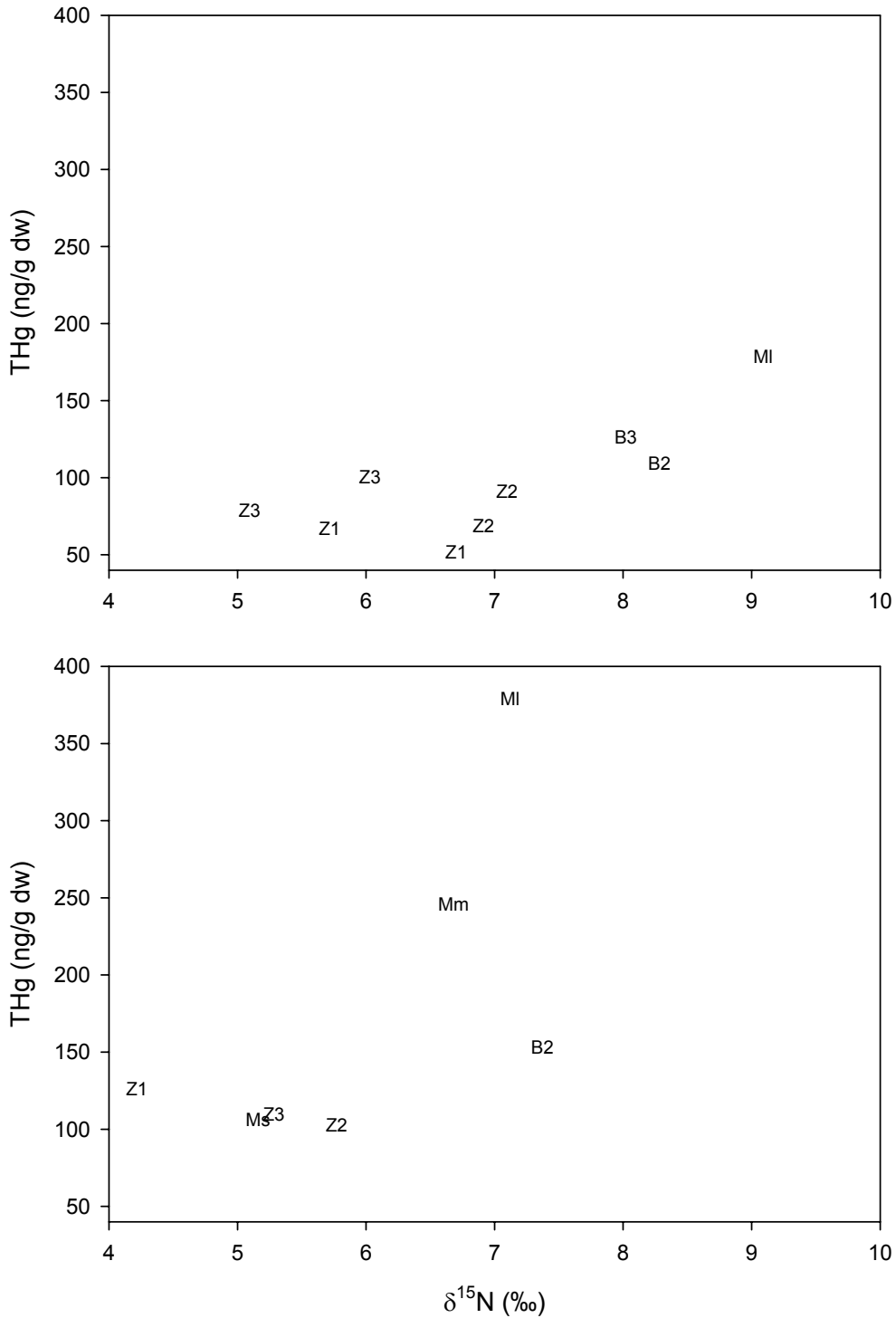


Figure AIV-2: Plankton trophic structure of Peninsula (a) and Harp (b) lakes as determined by nitrogen stable isotope ratio and total mercury (THg). Zooplankton was separated into three size classes: 153-243 μm (Z1), 243-500 μm (Z2) and >500 μm (Z3). *Mysis* were separated into three size classes: small (Ms), medium (Mm) and large (MI). *Bythotrephes* were separated into first (B1), second (B2) and third (B3) instars.