Impacts of *Mysis diluviana* extirpation on a whole lake ecosystem

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ANDREW MILLING

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ABSTRACT

Understanding species interactions is critical to predicting the ecosystem-level impacts of anthropogenically-caused species extirpations. In particular, the loss of keystone species - those that have exceptionally large effects on their environment relative to their abundance - would be expected to have dramatic and cascading effects throughout an ecosystem. Following the experimental acidification of Lake 223 at the IISD-ELA in the early 1980's, all species that had been extirpated due to the experiment have since returned to the lake with the notable exception of the freshwater shrimp, *Mysis diluviana*. *Mysis* have had dramatic impacts when introduced into non-native habitats, but the role *Mysis* in structuring ecosystems in their native habitat is unclear. Through this unique opportunity to study and compare two different time periods where the only difference was the presence and absence of *Mysis* in the same ecosystem, I evaluated the impacts of *Mysis* on the community structure of Lake 223. My results suggest that *Mysis* may serve as a keystone species in their native environment, having significant impacts on the biomass of both fish and zooplankton species; in the absence of *Mysis*, zooplankton species composition shifted towards dominance of *Chaoborus* and large cladocerans. Stable isotope analysis suggests that *Chaoborus*, the largest predatory zooplankton species in the absence of *Mysis*, represent an energetic bottleneck that has resulted in lake trout having slower growth rates, lower total biomass, and lower recruitment compared to when *Mysis* were present in the lake. This work demonstrates the importance of *Mysis diluviana* in structuring aquatic food webs in which they are native and provides support for considering re-establishment of *Mysis* into atmospherically acid-damaged lakes as a potential recovery strategy.

LAY SUMMARY

Mysis diluviana are a relatively large (<20mm) freshwater shrimp species that is an important zooplankton predator in Canada's freshwater ecosystems and are considered important members of aquatic food webs. However, direct tests of their importance in ecosystems have not been conducted. In the late 1970's and early 1980's, Lake 223 at the IISD-Experimental Lakes Area was experimentally acidified to evaluate the effects of acid-rain on lake ecosystems. While the acid additions caused many species to die-off and lake trout to starve, the lake has since been chemically recovered for >20 years and all species have since returned to the lake, except *Mysis* shrimp. Here I examined the Lake 223 ecosystem before and after acidification, where the only difference in the ecosystem was the presence/absence of *Mysis*. This comparison provided a test of their importance in the Lake 223 ecosystem. My results suggest that *Mysis* are play a strong role in structuring aquatic ecosystems by facilitating the flow of energy from lower to higher trophic levels, with their extirpation correlated with decreases in the biomass of several species. In the absence of *Mysis*, lake trout and white sucker in Lake 223 have increased pelagic reliance, potentially feeding on another large predatory zooplankton. As a consequence, there are fewer, slower-growing lake trout, and fewer of them survive to adulthood. These results suggest that acid damaged lakes in which *Mysis* have been extirpated may benefit from the re-establishment of *Mysis* to lakes where they were previously present.

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GENERAL INTRODUCTION

The keystone species concept was first introduced by Robert Paine in 1969 (Paine 1969), after noticing that some species have an overwhelming impact on ecosystem processes relative to other species. He identified the two hallmarks of a keystone species as: (1) the presence of the species being crucial in maintaining the organization and diversity of the ecological communities to which they belong, and (2) that a keystone species is exceptionally important compared to the rest of the community. This definition suggests that keystone species are essential in regulating other species that would otherwise dominate the community, such as sea otters maintaining kelp forests by feeding on potentially destructive sea urchins (Estes et al. 1978). Changes in the abundance of dominant species within the ecosystem will undoubtedly have a large impact on ecosystem processes and structure, but a less abundant keystone species can also have a large impact. Power et al. (1996) updated the definition of keystone species, as "*a species whose effect is large, and disproportionately large relative to its abundance*". Losing keystone species would then, by definition, cause disruptions to the organization of the ecological communities to which they belonged.

Mysis diluviana—formerly *Mysis relicta* (Audzijonyte and Väinölä 2005) —are a relatively large (<20mm) omnivorous freshwater shrimp that occupies an important intermediate trophic position in aquatic communities. *Mysis* participate in daily vertical migrations, feeding on detritus and benthos during the day and rising to feed on phytoplankton and smaller zooplankton at night below the thermocline (Beeton 1960). *Mysis* are also implicated in controlling the relative abundance of other zooplankters

(Ramcharan et al. 1985, Nero and Sprules 1986b). Omnivores, such as *Mysis*, play a unique role in ecological communities due to their direct and indirect interactions with other species (Diehl 1993). An analysis of 515 lakes across northeastern North America indicated that lakes containing *Mysis* have more trophic levels and a greater incidence of omnivory than lakes without *Mysis* and are positively correlated with fish species richness (Sprules and Bowerman 1988; Vander Zanden et al. 1999). Omnivory has also been shown to have keystone stabilizing effects in ecological communities, adding complexity to food webs in microcosm, observational, and modeling studies (Polis 1991, Lawler and Morin 1993, McCann et al. 1998, Holyoak and Sachdev 1998, Petchey et al. 2004).

The demonstrated ability of *Mysis* to regulate ecosystems from an intermediate trophic position supports the ecological significance of this predatory zooplankton species as a potential keystone species (Rieman and Falter 1981, Lasenby et al. 1986, Spencer et al. 1991). Chipps and Bennett (2000) found that introduced *Mysis* consumed four times as many cladocerans as kokanee salmon, and have been shown to alter fish communities when introduced into non-native habitats (Richards et al. 1975, Lasenby et al. 1986, Spencer et al. 1991). Daily vertical migrations allow *Mysis* to avoid significant predation by spatially segregating themselves from planktivorous predators (Beeton 1960, Teraguchi et al. 1975, Beeton and Bowers 1982), resulting in *Mysis* being weakly linked to top-down control. As well, *Mysis* are opportunistic omnivores and feed on detritus, phytoplankton, and zooplankton (Grossnickle 1982); this omnivorous lifestyle and ability to switch between a variety of distinct energy channels has the effect of also reducing controls from bottom-up regulation. The omnivorous feeding behaviour and vertical migrations hold strong implications for benthic-pelagic coupling, and their intermediate trophic position allows them to play a key role in transferring energy from lower trophic levels to higher levels.

Although native to large, deep lakes across North America (Lasenby et al. 1986), *Mysis diluviana* have a long history during the last half of the 20th century of being introduced into lakes and reservoirs to boost fish production in an effort to provide a new food source for intermediate-sized salmonids (Gosho 1975, Fürst 1981). The first introduction of *Mysis* to boost salmonid production came in 1949, when *Mysis* were experimentally introduced from Waterton Lake into Kootenay Lake with the intention of boosting rainbow trout populations by providing an intermediate food source larger than the native zooplankton (Larkin 1951). Following the introduction, the growth rate of rainbow trout did not increase; rather, the growth and mean size of another native salmonid, the kokanee salmon increased dramatically, and this was used as a rationale for future introductions elsewhere (Northcote 1970). *Mysis* have since been introduced into more than 100 lakes in northwestern USA and Canada (Lasenby et al. 1986, Martinez and Bergersen 1989).

Few of these introductions had the intended result. As one of several examples of these introductions gone wrong, *Mysis* were introduced between 1968 and 1975 into three lakes of the Flathead catchment of Northwest Montana, and by 1981 had made their way downstream into Flathead Lake where they outcompeted kokanee salmon for cladoceran prey (Spencer et al. 1991). Lake trout, which were introduced 70 years prior in Flathead Lake, were the unexpected beneficiary of the *Mysis* introduction (Spencer et al. 1991, Ellis et al. 2011) and their population abundance increased dramatically (Stafford et al.

2002). *Mysis* were also introduced into Lake Tahoe, California where they were responsible for the disappearance of various cladoceran species. Lake trout fed heavily on *Mysis*, which reduced the mean weight and abundance of kokanee salmon as a result of increased competition (Richards et al. 1975, Morgan et al. 1978, Goldman et al. 1979). As the practice of introducing *Mysis* became common across western North America and Europe, and more and more examples emerged of unanticipated negative consequences to native ecosystems and fisheries, a moratorium was eventually issued on *Mysis* introductions by the Mysid Research Group (Morgan 1982).

Mysis have demonstrated keystone-like impacts throughout their range, but it is possible that their impacts are greater in non-native than in native environments. Studies of *Mysis* in native environments show differences in zooplankton distribution in the water column between lakes with and without *Mysis* (Nero and Sprules 1986a, 1986b), but no significant differences in zooplankton community composition as a whole (Almond et al. 1996). By contrast, *Mysis* introductions into non-native environments have significantly altered zooplankton communities and impact ecosystems through what have been described as middle-out trophic cascades (Richards et al. 1975, Spencer et al. 1991). This difference in degree of effects between native and non-native environments seems to agree with the common assumption that invasive species occur at greater abundances and have greater impacts in their introduced range than in their native environments (Crawley 1987, Noble 1989, Blossey and Notzold 1995). Although ‘impacts’ in native ecosystems (assessed through comparisons of systems with and without *Mysis*) do not appear as drastic as those seen in many of the western North American introductions, comparative studies are unable to reveal the extent to which *Mysis* regulate and structure ecosystems in their native environments.

While studying the ecological effects of a species introduction is relatively straightforward (assuming the availability of background data), there have been very few species-removal studies in aquatic environments. Ideally, experimentally removing one species would be the best way to determine direct and indirect effects of species within an ecosystem, but the logistical challenges of such an experiment are daunting and often impossible (Power et al. 1996). In the late 1970's, an acidification experiment took place to evaluate the effects of acid rain on the aquatic ecosystem of Lake 223 at the Experimental Lakes Area (ELA) near Kenora, Ontario (Schindler et al. 1980). The pH of the entire lake was lowered to a maximum acidity of 5.1 pH from 1978 to 1981 and was slowly returned to pre-acidification levels by the mid 1990's. Over the course of the experiment, many species were extirpated from the lake as the pH surpassed species' thresholds (fathead minnows *Pimaphales promelas* (5.9pH), slimy sculpin *Cottus cognatus* (5.6-5.9pH; (Mills et al. 2000), and *Mysis diluviana* (5.9-5.6pH, Nero 1981). These extirpations had drastic impacts on the resident lake trout population, such as a decline in abundance due to low recruitment, as virtually all their food sources were removed from the lake (Mills et al. 2000). In the decades since this experiment, the pH of the lake was returned to normal, and all extirpated species returned to the lake with the notable exception of *Mysis diluviana*. As such, comparing the ecosystem in Lake 223 before and after acidification provides a rare and unique opportunity to evaluate the impacts of removing *Mysis* from its native habitat.

This thesis describes my research comparing the aquatic community structure of Lake 223 during a period when *Mysis diluviana* was present, before the impacts of whole-lake acidification, to the community structure of Lake 223 following recovery from acidification when *Mysis* were absent. Historically, poor knowledge of the structure and dynamics of ecosystems prior to human impacts has limited our ability to understand change (Power et al. 1996). All previous studies of the ecological 'impacts' of *Mysis* in their native range have compared different lakes that have *Mysis* present or absent, or they have compared the same lake before and after the introduction/invasion of *Mysis* into systems where they are non-native. The main objective of this study was to evaluate the ecological impact of the removal of *Mysis diluviana* on the aquatic community structure of Lake 223.

My first objective was to use changes in the Lake 223 ecosystem to evaluate whether *Mysis diluviana* fit the definition of a "keystone species" in their native environment. Zooplankton density and biomass, as well as biomass of other ecosystem constituents was estimated before the extirpation of *Mysis* and compared to similar data after the lake had been chemically recovered for >20 years. I hypothesized that, based on only subtle differences previously reported between lakes with and without *Mysis* (Almond et al. 1996), that *Mysis* would not fit the definition of a keystone species within their native environment. As *Mysis* have been known to prey on *Chaoborus* (Nordin et al. 2008), and *Chaoborus* are positively correlated with non-*Mysis* lakes (Nero and Sprules 1986b), in the absence of *Mysis*, *Chaoborus* frequently become a dominant invert predator. Thus, I further predict that *Chaoborus* will have replaced *Mysis* in Lake 223, and that because *Chaoborus* larvae have similar diet and vertical migration behaviour as *Mysis* that there is high functional redundancy between these two invertebrate predators.

My second objective was to determine changes in energy pathways to lake trout and white sucker due to the loss of *Mysis* using stable isotope analysis, and resultant impacts of *Mysis* loss on lake trout. A two-source-mixing-model using nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) signatures was used to determine changes to the tropic position and littoral energy use (France 1995, Hecky and Hesslein 1995, Post 2002). Acidifying Lake 223 disrupted the isotopic equilibrium of carbon fractionation, requiring the use of baseline signatures to tease apart food web changes due to the loss of *Mysis* from the effects of acidification. Following the extirpation of *Mysis*, a major biomass compartment of the pelagic food chain, I hypothesized that lake trout and white sucker will have become more dependent on littoral resources such as crayfish and minnow species, resulting in increased trophic position and increased littoral reliance. I predicted that the extirpation of *Mysis* from a lake already depauperate of prey choices in the pelagic environment will have resulted in the lake trout population being less abundant, in poorer condition, and growing at a slower rate that with *Mysis* present. Reference systems with long-term data at the IISD-ELA will help to tease apart effects due to regional change (e.g. climate change) from effects due to the loss of *Mysis*.

CHAPTER 1

Keystone species or misnomer? The ecological role of *Mysis diluviana* in an Ontario lake

ABSTRACT

Mysis diluviana have been introduced into many freshwater lakes across western North America and northern Europe, often with significant impacts to native ecosystems. *Mysis* are widely regarded as a keystone species in both their introduced and native range due to their omnivorous feeding behaviour, diel vertical migrations, and trophic position as a meso-predator. However, it remains debatable as to whether *Mysis* are a 'true' keystone in their native environment, or if this reputation is a result of *Mysis* having keystone-like effects in introduced ranges, as "keystone-aliens". To determine if *Mysis* are a keystone species in their native environment, I compared the ecosystem of a lake before and after the extirpation of *Mysis diluviana*. I hypothesized that if *Mysis* were indeed a keystone species, their extirpation from the lake would result in dramatic changes in the zooplankton community that would have cascading impacts to other ecosystem components. Results from multivariate analyses revealed significant changes to the zooplankton community following the loss of *Mysis*, including increased biomass of large cladocerans (*Daphnia*) and *Holopedium* that were not observed in reference lakes. *Chaoborus* replaced *Mysis* as a predatory pelagic planktivore in Lake 223, but currently represent only 20% of the invertebrate predator biomass previously represented by *Mysis*. The current standing biomass of the lake trout population is lower relative to years prior to *Mysis* extirpation, apparently as a result of decreased recruitment. My results demonstrate large changes in the Lake 223 aquatic community associated with the loss of *Mysis diluviana*, potentially supporting the designation of *Mysis* as a keystone species in their native environment.

INTRODUCTION

The keystone species concept describes species whose presence disproportionately dictates ecological structure and function based on the observation that removal of *Pisaster* sea stars in intertidal zones decreased species diversity (Paine 1969). Later, this definition was redefined as being a strong interactor in food webs, and "*one whose removal would produce a dramatic effect*" (MacArthur 1972). Since these original definitions, the term "keystone species" has been broadly used and frequently applied out of its original context (Mills et al. 1993). A more recent definition of keystone species that seeks to clarify the concept is "*a species whose effect is large, and disproportionately large relative to its abundance*" (Power et al. 1996). By any of these definitions, the removal of a keystone species should lead to major disruptions in ecosystem function through major changes in both direct and indirect interactions among remaining species.

The keystone species concept was originally described for natural community assemblages but has been argued to be incorrectly applied to cases of introduced species with large impacts on non-native environments. These introduced species with a large impact are more accurately described as "keystone alien" species (Clout 2006). For example, the often-ensuing disruptions to aquatic food webs following the wide-spread introduction of *Mysis diluviana* (hereafter referred to as *Mysis*) across western North America where they are not native (e.g. Goldman et al. 1979, Rieman and Falter 1981, Lasenby et al. 1986) has played a large part in their description as a keystone species throughout both their native and non-native range. As a relatively large (up to 20mm) freshwater zooplankton species, *Mysis* play a key role in transferring energy from lower to higher trophic levels (Covich et al. 1999), often facilitated by their propensity to undergo daily vertical migrations (DVMs) where they spend daylight hours avoiding predation at the sediment/water interface, and rising to just below the thermocline at night to feed on zooplankton. Their high oxygen and cold-water requirements restrict *Mysis* to the well-oxygenated, hypolimnetic layer of lakes during summer stratification. *Mysis* are omnivorous, feeding on detritus, phytoplankton, and zooplankton (Grossnickle 1982). *Mysis* stomachs collected from the Laurentian Great Lakes have been found to contain the remains of algae, detritus, cladocerans, copepods, and other small *Mysis* (McWilliam 1970, Bowers and Vanderploeg 1982, Nordin et al. 2008). As an opportunistic omnivore, the diets of *Mysis* can vary widely, depending on available food sources (Grossnickle 1982). *Mysis* are visual predators (Ramcharan and Sprules 1986) with larger *Mysis* feeding on larger prey (Cooper and Goldman 1980, Branstrator et al. 2000, Whall and Lasenby 2009). Their wide-ranging omnivorous diet and DVMs (which minimize predation by fish) lead to *Mysis* being weakly-regulated by either top-down or bottom-up processes. By contrast, and largely because of these qualities, *Mysis* also have the capacity to regulate both lower and higher trophic levels in a “middle-out” fashion (Stein et al. 1995).

Within their native range, *Mysis* are hypothesized to regulate the abundance and distribution of prey species that co-habit the hypolimnion through predation (Nero and Sprules 1986b). Large hypolimnetic zooplankton species have reduced densities in the presence of *Mysis* (Nero and Sprules 1986b, Nowicki et al. 2017). Also, species such as *Daphnia longiremis, Eubosmina longispina*, *Cyclops scutifer* and *Diaptomus sanguineus* are rare in lakes with *Mysis* (Nero and Sprules 1986b, Almond et al. 1996). Comparatively, the impacts of *Mysis* to zooplankton communities following introduction to lakes where they are non-native tend to be much more dramatic. The unintended increase in the Kokanee salmon population following the first introduction of *Mysis* in 1949 into Kootenay Lake, B.C. (Larkin 1951) was used as rationale for further *Mysis* introductions with the intention of providing an additional food source for salmonid populations. However, not all introductions had this desired effect. For example, *Mysis* were introduced into the headwaters of Flathead Lake, Montana and dramatically reduced native cladoceran and copepod populations (Spencer et al. 1991), consumed 3-4 times as much energy than they did in their native habitat (Chess and Stanford 1998), and out-competed kokanee salmon for shared zooplankton prey (Ellis et al. 2011). *Mysis* have since been introduced with similar unanticipated negative ecosystem impacts in over 100 waterbodies in western North America, leading to the Mysid Research Group recommending a global moratorium for further *Mysis* introductions (Morgan 1982).

It is unclear whether *Mysis* are a true keystone species in their native range or are better classified as a keystone-alien. *Mysis* have been labelled a keystone species in the Laurentian Great Lakes based on both ecosystem modelling results (e.g. Boscarino et al. 2007, Stewart and Sprules 2011, Rogers et al. 2014), and from gut content analysis (e.g. Gamble et al. 2011, O'Malley and Bunnell 2014). However, most studies actually describing the role of *Mysis* within the zooplankton community in their native range have been based primarily on comparisons between lakes with and without *Mysis* (e.g. Roff et al. 1981, Nero and Sprules 1986b, Almond et al. 1996); these assessments have found that while *Mysis* affect the abundance of certain species at certain depths, these differences arguably fall short of giving *Mysis* keystone status. Before-after introduction comparisons (e.g. Richards et al. 1975, Lasenby et al. 1986, Spencer et al. 1991, Ellis et al. 2011) have shown that *Mysis* are a strong interactor in their introduced range, and have explicitly been labelled as a keystone species in a Colorado reservoir where they were introduced (Johnson and Martinez 2012). Despite these dramatic community-level impacts appearing to be limited to their introduced range, *Mysis* are frequently referred to as a keystone species, regardless of whether *Mysis* are native or introduced to the ecosystem.

Experimentally testing the “keystone-ness” of a species is difficult. The experimental removal of a species (sensu Paine 1969) is the best way to identify both the direct and indirect interactions of a species in their native habitat; further, removal experiments need to persist long enough to allow indirect effects to manifest (Power et al. 1996). From 1976 to 1983, Lake 223 at the International Institute for Sustainable Development’s – Experimental Lakes Area (IISD-ELA) was experimentally acidified to evaluate the effects of acid rain on aquatic ecosystems. A maximum acidity of 5.1 pH was reached in 1981 before being allowed to incrementally recover to pre-acidification levels in 1996. Over the course of the experiment, many species were extirpated from the lake as the pH surpassed species' thresholds, including fathead minnows (5.9 pH), crayfish (5.6 pH), slimy sculpin (5.6-5.9 pH), and *Mysis diluviana* (5.6-5.9 pH; Nero 1981, Davies 1989, Mills et al. 2000). In the years since Lake 223 has chemically recovered, and all species that had been previously extirpated have since returned (Colvin and Rennie, unpublished data), except for *Mysis*. This dataset provides a unique opportunity to evaluate the role of *Mysis* in Lake 223 by comparing the zooplankton community and aquatic ecosystem during two different time periods (pre-acidification vs post-recovery), with the main difference being the absence of *Mysis*.

To evaluate the impact of *Mysis* on an ecosystem where they are a native species, I observed changes in the zooplankton community and other ecosystem components before and after the extirpation of *Mysis* from Lake 223. I hypothesized that the removal of *Mysis* from Lake 223 would result in increases in large zooplankton species such as large cladocerans, similar to findings from studies comparing lakes with and without *Mysis* across their native range (e.g. Roff et al. 1981, Nero and Sprules 1986b, Almond et al. 1996). I also predicted that *Chaoborus spp*. would have effectively replaced the trophic niche left void by *Mysis*, because *Chaoborus* are also a daily-vertical-migrating, pelagic, meso-predator species known to occur in the lake (pers. obs.). Lastly, I predicted that the standing biomass of the lake trout population would be lower following the extirpation of *Mysis*, an important prey species. An unmanipulated reference lake was also examined to represent regional-scale changes to these systems (e.g., climate), allowing the effects of the *Mysis* extirpation to be isolated from regional changes in measured response variables.

METHODS

Study sites

Founded in 1969, the IISD-ELA is a collection of 58 lakes designated for whole-lake ecosystem experiments. During acidification, the *Mysis* population in Lake 223 was extirpated by October 1979, being undetectable after having declined from an estimated

6.7 million in August 1978 (Nero and Schindler 1983). Lake 224 is located immediately upstream of Lake 223 and served as an un-impacted reference lake and a potential source of extirpated species to Lake 223 during recovery from acidification. Both lakes have fish communities typical of small (<40ha) ELA lake trout lakes (Beamish et al. 1976), but differ from typical northwestern Ontario lakes that also contain *Cisco spp*. (Trippel and Beamish 1989, 1993). Lakes 223 and 224 are deep, oligotrophic lakes at the IISD-ELA (Table 1.1) connected by a small stream that allows downstream flow of water from Lake 224 to 223. Fish species present in both lakes include lake trout (*Salvelinus namaycush*), white sucker (*Catastomus commersoni*), fathead minnow (*Pimephales promelas*), pearl dace (*Semotilus margarita)*, finescale dace (*Phoxinus neogaeus*), brook stickleback (*Culaea inconstans*), and slimy sculpin (*Cottus cognatus;* (Mills et al. 1987)).

During 1974, zooplankton abundance and species composition data were collected from Lake 223 and 224 at the deep station using a transparent 28.7L Schindler-Patalas trap during the day (Schindler 1969). Zooplankton were collected at 1m intervals throughout the water column. After 1975, barrel closing nets were used (Table 1.2). In 1977, a 4-barrel closing net was hauled vertically through the water column at the deep station from <2m above the lake bottom to the surface. From 1978 to 1995, a two-barrel closing net was used to sample discrete thermal strata (epilimnion, metalimnion, hypolimnion) at the deep station (Chang et al. 1980, Chang and Malley 1987). After 1995, zooplankton were collected using the two-barrel net, but without sampling of discrete thermal layers. All samplers used a 53um mesh and data from different depths were combined for all years to generate the equivalent of whole-water column densities at the deep station. Zooplankton densities were available for most years since 1974 from

Lake 223 and reference Lake 224, with noticeable gaps in the sampling record. Samples were collected during daylight hours, typically every two weeks during the open-water season of the year from early May to mid-November. Abundance data were converted to numbers of individuals/L to make data comparable using different collection methods. More detailed sampling and processing methods are described in detail by Chang and Malley (1987). Net efficiencies were assumed to be equivalent since all methods used 53µm mesh size, and other research has shown efficiencies of Schindler-Patalas traps and zooplankton nets to be similar (Levine et al. 2014). However, other evidence suggests that Schindler-Patalas traps tend to catch larger, more motile zooplankton in higher abundances relative to vertically hauled nets (Devries and Stein 1991, Johannsson et al. 1992). The average dry-mass of each species was applied to abundance density data, to yield a biomass density estimate for each sampling event. Using species' average biomass in analyses of the zooplankton community therefore masks any changes to the size of individual species through time.

The zooplankton community in 1974 were used to represent the community when *Mysis* were present, and communities in the years 2015-2017 were used to represent the community after recovery from acidification and *Mysis* extirpation. These years were chosen for comparisons because 1976 is when the lake started receiving acid additions (Schindler et al. 1985), and years after 2014 were chosen because of the availability of consistent data on both lakes, as well as there being the greatest amount of time allowing recolonization of lost species back into Lake 223 (Colvin and Rennie, unpublished). Due to the unforeseen buffering capacity of the lake, there was no significant change in the pH of the lake in 1976 despite sulfuric acid additions during that year (Schindler et al. 1980).

Thus, up until and including 1977, all species were still within the range of natural variation compared to reference lakes in the region (Schindler et al. 1985). Lake 223 has maintained neutral pH, at or above pre-acidification pH levels since the mid-1990's (Fig. 1.1).

Due to some inconsistencies in the identification of copepodites, as well as some groups of *Daphnia* between time periods, some taxa were grouped together to permit meaningful comparisons between time periods. Specifically, *Daphnia catawba, D. pulex, and D. schoedleri* are part of a complex that may or may not represent different taxa and consequently, these species were grouped together in both datasets. Similarly, *D. galeata mendotae and D. dubia* were also grouped together for both time periods. Other details of taxonomic groupings are provided in the Appendix.

Macro-zooplankton predators

During 2014-2017, nine fixed sampling stations were used for zooplankton predator (*Mysis* and *Chaoborus*) collections in Lake 223 and Lake 224. These stations were located along a gradient of depths within each lake. Samples were collected monthly at least 1 hour after sunset, throughout the open water season, using a 0.75m diameter net and a 500 μm cod end, hauled from approximately 1.5 m above the lake bottom to the surface. Nero and Schindler (1983) also used nine fixed sampling stations along a depth gradient and found non-significant differences in *Mysis* densities among transects within Lake 223. This suggests that although the transect locations differed between 1978 and 2014-2017, comparisons can be made between time periods. In 2002 the 1.13m diameter net used by Nero and Schindler (1983) was tested alongside the 0.75 m diameter net currently used for *Mysis* collections, and was found to be not statistically different in *Mysis* capture efficiencies using raw *Mysis* counts (paired *t*-test, $t_7 = 0.18$, *p*-value = 0.86). Therefore, net efficiencies were corrected to 80% to make *Mysis* abundance counts from 2014-2017 comparable to densities estimated in Nero and Schindler (1983).

At night during summer stratification, *Mysis* migrate from the bottom of the lake and concentrate just below the thermocline (Bowers and Grossnickle 1978). As such, if an evenly distributed population were concentrated below the thermocline, the population would appear to decline with depth if densities were calculated volumetrically. Therefore, *Mysis* abundance and biomass were calculated based on area instead of by lake volume. During daylight hours in the summer, *Mysis* are restricted to areas of the lake with acceptable temperature and oxygen conditions (Nero and Davies 1982, Paterson et al. 2011). When *Mysis* vertically migrate at night to just below the thermocline, there is not a homogenous distribution throughout the lake since *Mysis* only appear to horizontally migrate <50m from the lake bottom they inhabited during the day (Nero and Davies 1982).

The predator abundances in Lake 223 (*Chaoborus*) and Lake 224 (*Mysis*) were estimated using two different methods. The first method was conducted to make estimates comparable to historical estimates from Nero and Schindler (1983), where I averaged predator densities across all sampling stations to get a comparable density estimate. As Nero and Schindler (1983) reported the Lake 224 *Mysis* density in July 1981, and the Lake 223 *Mysis* density in August 1978 before becoming extirpated, comparing predator densities within these respective months is the best way to investigate if predator densities have decreased. Counts of *Mysis* in Lake 224 collected in July 2014-

2017 were averaged, corrected for an 80% net efficiency, corrected to density per m^2, and compared against the July 1981 Lake 224 *Mysis* density in Nero and Schindler (1983). In Lake 223, counts of *Chaoborus* collected in August 2014-2017 were averaged, corrected to density per m^2, and compared against the August 1978 Lake 223 *Mysis* density in Nero and Schindler (1983).

The second method of estimating predator abundances was calculated following the method of Paterson et al. (2011) using weighted average densities within depth intervals to represent contemporary lake-wide densities and abundances. Although this method does not permit comparisons with historical estimates, it likely provides a more accurate estimate of lake-wide abundances since it explicitly considers the temperature requirements of *Mysis* as well as comparative estimates to other ELA lakes (Paterson et al. 2011). Depth strata were separated into 5 m intervals after the first interval, which ranged from 0 m to the depth of 12^{o}C, the second interval being depth of 12^{o}C to 10 m, then 10 m - 15 m, etc. The depth of 12^{o}C was set as a limit to the first strata because *Mysis* rarely enter water greater than 12^{o}C (Boscarino et al. 2007, Paterson et al. 2011), therefore setting a strata boundary that captures the "ring" distribution of *Mysis* around a lake (Nero and Davies 1982, Sellers 1995). Densities of *Mysis* (#/m^2) caught from multiple stations at night within each depth interval were averaged within each interval, and then multiplied by the area represented by each respective depth interval. The totals from each depth interval were then summed to estimate lake-wide abundance. Lake-wide weighted average densities were also calculated by summing the densities within each depth interval and multiplying them by the proportion represented by each depth interval in the lake. Since depth intervals fluctuated based on the observed depth of the 12^{o}C isocline, weighted average densities were calculated separately for each of the 14 sample periods.

Contemporary *Chaoborus* population estimates were calculated using depth intervals in the same way as *Mysis*, except that the intervals did not include an upper thermal boundary; since *Chaoborus* do not have the same temperature restrictions as *Mysis* (Moore 1988). Instead, the intervals used for *Chaoborus* were 0-5 m, 5-10 m, and 10-14 m (max depth in Lake 223). Depth intervals were used in estimating lake-wide weighted average densities for *Chaoborus* to incorporate heterogenous distribution across lake depth, and to have a comparable method to the contemporary Lake 224 *Mysis* population estimate.

To estimate biomass from length for invertebrate predators, a camera-mounted dissecting microscope was used to capture images of both *Chaoborus* sampled from Lake 223 and *Mysis* from Lake 224 and were measured using "ImageJ" computer software. *Mysis* were measured from the tip of the rostrum to the end of the last abdominal segment. The segmented line tool in ImageJ was used to measure body lengths, calibrated to a known distance of 1mm. The equation used to estimate the dry-weight for *Mysis* in mg was:

(eq. 1) $$\text{Dry-weight(mg)}=10^{-0.677+(0.592*\text{antennal length (mm)}}$$

The equation was generated using *Mysis* ($R^2 = 0.90$, p-value < 0.0001) collected from L373 at the ELA (M. Paterson, pers. comm, n=24). Frozen animals had antennal lengths measured, after which the whole animal was dried in a drying oven at ~50^{o}C for ~24hr and weighed.

Body length measurements were converted to antennal length (mm) ($n = 599$, $R^2 = 0.95$), using the equation from Nero (1981):

(eq. 2) *Mysis* body length(mm) = antennal length(mm) x 6.50

Chaoborus dry-weight biomass from body length was calculated using the formula:

(eq. 3) $\log(\text{dry-weight in mg}) = -5.4 + (5.01 * \text{body length in mm})$

This equation was derived from *Chaoborus* collected from L240 and L658 at the ELA (M. Paterson, pers. coms, $n = 44$ $R^2=0.95$, $p<0.0001$). *Chaoborus* body lengths were measured using a line or segmented line tool in ImageJ, and body length was measured from the base of the head capsule to the end of the body (Chimney et al. 2007).

Lake trout food webs

Biomass estimates of species within the lake trout food web (e.g. components of the food web that likely deliver a significant proportion of energy to lake trout in these ecosystems, excluding benthic invertebrates (Martin 1954, 1970)), and comparisons of these species before and after *Mysis* extirpation were made to determine the impact that *Mysis* may have had on the broader ecosystem. Benthic invertebrates were excluded because of a lack of available samples. Lake trout abundance and total standing biomass was calculated using mark recapture data. Lake trout from Lake 223 and 224 were captured using trap nets, gill nets, and angling each fall, before being sampled and released (Mills et al. 1987). The population was surveyed almost every year through the more than 4-decade long sampling history, with 39 sampling occasions (1973 – 2017).

The method of individually tagging fish with unique fish numbers changed throughout the years of this study (Mills et al. 2002a). Individuals >30cm were historically tagged with modified Carlin tags (White and Beamish 1972), and/or with visible implant tags commencing in 1988 (Mills et al. 2000). Starting in 1977, each fish ≥ age 1 (14cm) was also given a unique batch mark for each fall sampling period by nicking fin rays (Welch and Mills 1981). In order to construct capture histories for mark-recapture analysis (see below), fish that were missing a tag but had already been captured before as indicated by the presence of fin nicks were assumed to have either lost their original tag or not been given a tag; these fish were assigned to a fish with a similar mark in a previous sampling effort that had a matching capture history.

Abundance of lake trout in Lake 223 were calculated using the mark-recapture POPAN Jolly-Seber fully time-dependent model (Jolly 1965, Seber 1982, Schwarz and Arnason 1996) for an open population in Program MARK (White and Cooch 2012). QAIC (quasi-Akaike Information Criterion) was used to determine that the fully time-dependent model had the greatest support among other models (ΔQAIC for next best model = 129.1). The same model was used by Mills et al. (2000) in Lake 223 to evaluate fish populations in the first eight years of the acidification experiment. Although there was some evidence of overdispersion in the data ($\hat{c} = 1.45$), a $\hat{c}$ value of ≤3 is generally acceptable (Lebreton et al. 1992) and was accounted for with a $\hat{c}$ adjustment (White and Cooch 2012). Lake trout abundance in Lake 224 was also calculated using a POPAN Jolly-Seber model with QAIC indicating that the model with constant survival and entry into the population, but with time dependent capture probability had the greatest support. There was some evidence of overdispersion in the Lake 224 model ($\hat{c} = 1.93$) which was accounted for with a $\hat{c}$ adjustment.

To estimate lake trout biomass in both lakes, the yearly weight distribution of captured fishes was sampled with replacement based on the abundance estimate for that year. For example, the 1975 abundance estimate in Lake 223 was 173 individuals; thus, the weights of the 29 lake trout captured in that year were sampled with replacement 173 times and then summed to estimate biomass for that year. Lake 223 data from 1973 and 2017 were excluded because estimates from the first and last tagging occasions in a fully time-dependent model are non-identifiable parameters (White and Cooch 2012).

Lake 223 lake trout captured in 1977 and 1978 were used to represent the population prior to *Mysis* extirpation from Lake 223, while individuals from the years 2011-2016 represented the time period without *Mysis*. Meanwhile, 1976-1981, and 2011-2016 in the control Lake 224 represented abundance in similar time periods as in Lake 223. Although the abundances of species within Lake 223 began to be affected by 1978 (Schindler et al. 1985), *Mysis* were still present in the lake (Nero and Schindler 1983) and the food-web effects of the acidification had not begun to adversely affect the lake trout population (Mills et al. 1987); lake trout abundance in Lake 223 did not decline until 1982 (Mills et al. 1987).

Lake trout recruitment between time periods was also examined for Lake 223, and in Lake 224 as a reference of trends in an unimpacted lake. Since nearly all lake trout captured in both lakes have had their age determined, I used the total number of fish caught during all years of sampling that were born in a particular year as an index of recruitment (Mills et al. 2000). Recaptured fish were excluded from recruitment estimates (i.e., only fish aged on first capture were included). Year-classes 1970-1977 were selected to represent the time period with *Mysis* present before effects of acidification, and 2003-2011 to represent years post-chemical recovery of the lake where *Mysis* were extirpated. 2011 was chosen as the final year to be included in the later time period because fish born after this time have not fully recruited to the population.

The standing biomass of the fathead minnow population was also estimated, as minnows make up a significant portion of lake trout diet (Martin 1970, Guzzo and Blanchfield 2017). Fathead minnow catch per unit effort (CPUE) data from trap nets in Lake 223 were used to estimate abundance as:

$$\text{Abundance} = 3.50*\text{CPUE} + 0.11 \quad \text{(eq. 4)}$$

(Guzzo et al. 2014). Catches of fathead minnows began to decline in 1978 (Mills et al. 1987), so the years 1976 and 1977 were selected to represent of the time period when *Mysis* were present in Lake 223. Although fathead minnow catches were not recorded in 1977, CPUE for fathead minnows in 1977 was assumed to be the same as in 1976, as per Mills et al. (2000). The years 2014-2017 represented the time period with *Mysis* extirpated from Lake 223 and were used to compare against the 1976/1977 average.

To estimate fathead minnow biomass from abundance estimates, I randomly sampled with replacement from the fork length distribution presented in August of 1976 (reported in Mills et al. 1987) by the abundance estimate for each year. For example, in 1977 the fathead minnow abundance estimate was 8018 individuals, therefore lengths were sampled 8018 times with replacement from the 1976 length distribution. To estimate mass from fathead minnow fork lengths, we used a sample of fathead minnow lengths and weights (n=237) from Lakes 223 and 442 collected in the spring and summer of 2003 to generate a weight-length equation ($R^2 = 0.80$, $p < 0.0001$; P, Blanchfield unpublished data). The weights of individual fathead minnows in grams were estimated as:

(eq. 5) $$\text{Weight} = 10^{-4.5} * FL^{2.7}$$

Where FL is the fork length in mm. Weights from all individuals in the bootstrap procedure (above) were summed within each year.

To examine if results could be explained by changes in physical characteristics of Lake 223, I looked for changes in the temperature and oxygen profiles of the lake, total dissolved phosphorous, and chlorophyll *a* concentrations. Chlorophyll *a* concentrations were examined between time periods to see if the extirpation of *Mysis* had impacted the standing biomass of phytoplankton. *Mysis* have an omnivorous diet and *Mysis* abundance has been shown to be positively correlated with phytoplankton biomass in Lake 373 at the ELA (Paterson et al. 2011). Years 1974-1977 were used as the time period before *Mysis* extirpation, and since 2016 was the most recent data available, 2012-2016 were used as the time period with *Mysis* extirpated from Lake 223. Epilimnetic chlorophyll *a* concentrations were used to compare between time periods because it was the only region of the lake sampled consistently between the two time periods. Temperature profiles were analyzed for the month of August using the "rLakeAnalyzer" package in R (Winslow et al. 2019). Oxygen profiles were analyzed in the late summer months (August and September) when summer stratification would be at its maximum, resulting in the maximum oxygen depleted area at the bottom of the lake. Lake trout also require highly oxygenated waters, with 75-90% of fish at >6mg dissolved O_2/L at the ELA (Sellers et al. 1998). As such, I evaluated changes in the depth of 6mg/L O_2 over time.

Statistical analysis

I used Principle Coordinate Analysis (PCoA) to evaluate zooplankton community change, before and after *Mysis* extirpation in Lake 223, and compared to changes over the same time period in neighbouring reference Lake 224. PCoA is a flexible tool that can handle several different distance measures, depending on which is most appropriate for the data being analyzed. Rare species may have an unduly large influence on the analysis because differences in the abundance values in common species have less of an effect on the distance measure than abundance differences of rare species (Smith and Greig-Smith 1983, Legendre and Legendre 1998, ter Braak and Smilauer 1998). However, Poos and Jackson (2012) demonstrated that the choice to remove very rare species (species with single occurrences, and species with <5% occurrences) had little effect on ordination results when using PCoA. I chose chord distance, also called the Relative Euclidean Distance (RED), on normalized species data. Chord distance does not give high weights to rare species (Legendre and Gallagher 2001), and emphasizes proportional changes. Rare species observed sporadically as a result of “sampling error” are common in environments when sampling is done blindly – as is done in aquatic ecology – and should not be given high weight in community analysis (Legendre and Gallagher 2001). Therefore, very rare species that comprised less than 1% of total biomass in at least one time period were removed to not drastically impact the ordination results. The coefficients of the eigenvectors were then used to plot points relative to one another on a two-dimensional plot. PCoA analyses were performed on both lake data sets separately to emphasize seasonal shifts in each zooplankton community within each lake, as well as with Lake 223 and Lake 224 biomass densities combined to emphasize differences between lakes. To make the analyses comparable, the same groups of species from the

Lake 223 analysis were included for the analysis of the reference Lake 224, regardless of whether a species composed 1% of the Lake 224 community in either time-period.

A canonical discriminant analysis (CDA) was conducted to test the *a priori* hypothesis that zooplankton communities differed between the time periods of pre-acidification to post-recovery in Lake 223, and no similar change in Lake 224. The CDA's from each of these lakes were compared to address the null hypothesis of no change in Lake 223 species biomass relative to Lake 224. A CDA is a constrained ordination technique that is designed to identify differences among groups in multivariate quantitative data. This approach differs from PCoA in that an unconstrained approach aims to explain the highest amount of variation in the data matrix with eigenvectors, while CDA generates axes from the original quantitative variables that best separates groups, while minimizing within group variation. The CDA generates axes, or canonical discriminant functions (CDFs) that explain lower and lower proportions of the variance in the analysis. The first CDF captures the most variation between groups, and the second CDF describes variation between groups that is independent of the first, and so on for the third, fourth, etc., discriminant functions, depending on the number of constrained variables (Quinn and Keough 2002).

Analysis of zooplankton communities in the PCoA suggested differences in the location of the group centroids between the two time periods (see results), so a time-period factor with two groups ("*Mysis* present" and "*Mysis* absent") was created for the CDA, as well as a lake factor (Lake 223 and Lake 224) and an interaction term between the two, and all were used as constraints in the analysis. A CDA was also run for Lake

223 and Lake 224 separately with only a time period factor to better emphasize how species densities have changed in each lake.

A CDA is mathematically identical to a single factor multivariate analysis of variance (MANOVA) with the same assumptions, with the most important of these assumptions being homogeneity of the within-group variance–covariance matrices (Quinn and Keough 2002). This assumption is very difficult to test formally. Tabachnick and Fidell (1996) suggest plotting the scores for each observation for the first two discriminant functions and checking if the spread of points is similar among the groups (Quinn and Keough 2002). Since there was only one discriminant function in my analysis of Lake 223 and Lake 224 separately, the spread of the points along the first and only discriminant function axis was evaluated and did not appear to be clearly heterogenous between groups. A generalized Shapiro-Wilk test for multivariate normality indicated non-normal multivariate data ($p < 0.0001$), however, multivariate tests are relatively robust to deviations from multivariate normality (Quinn and Keough 2002), and several transformations were unable to correct non-normality. Collinearity among taxa was detected by plotting all taxa in a correlation plot and dealt with by grouping taxa together that were highly correlated (>0.80), resulting in several rotifer species being condensed into a single rotifer group.

Since the assumption of multivariate normality was not met, a semi-parametric approach was also used to interpret community differences between Lakes 223 and 224. A permutational multivariate analysis (PERMANOVA) is a method of partitioning variation across a multivariate data cloud, in the space of a chosen dissimilarity measure, in response to one or more factors in an analysis of variance design (Anderson 2017).

Again, chord distances were used. Like the CDA, lake and time-period were chosen as factors, allowing the PERMANOVA to generate distributions of pseudo *F*-values for these main effects, where the *p*-value is generated by comparing the observed *F*-value with the ordered distribution of the pseudo *F*-statistic. The PERMANOVA tests the null hypothesis of no difference in the location of the group centroids in the given dissimilarity measure. Since this method uses permutations, there is no reliance on parametric distributions, and it allows one to make distribution-free inferences with no assumption of multivariate normality (Anderson 2017). An assumption of the PERMANOVA test is homogeneity of multivariate dispersions between the groups in the space of the chosen dissimilarity measure. There was homogenous variance between time-period groups in Lake 223 (Permutation tests for homogeneity of multivariate dispersions, permutations = 999, Pr =0.19), but there was heterogenous variance in Lake 224 (No. permutations = 999, Pr = 0.014). Heterogeneity of time period group dispersion in Lake 224 could be because of a relatively larger number of observations in the recent time period when compared to Lake 223 (Table 1.3). I used 4999 permutations for to generate *p*-values of main effects, as generally at least 1000 permutations should be done for tests with an α-level of 0.05 and at least 5000 permutations should be done for tests with an α-level of 0.01 (Manly 2006).

Both *t*-tests and one-sample Wilcoxon rank-sum tests were used to test if lake trout standing biomass from the time period without *Mysis* (2011-2016) was significantly different than the mean standing biomass from 1977/78, and a regular Wilcoxon rank-sum test to test for differences in the standing biomass of lake trout in Lake 224 between time periods. Because these temporal data points from the lake trout population are not truly independent, resulting probabilities should probably be regarded with caution. Also, a Wilcoxon-rank sum test was also used to test the differences in chlorophyll *a* concentrations between time periods. Temperature and oxygen concentrations were analyzed between pre-extirpation to the post-recovered time periods using Wilcoxon rank-sum tests.

RESULTS

PCoA on the zooplankton communities in Lake 223 and 224 showed each lake changing over time but in different directions (Fig. 1.2). Following acidification and the subsequent extirpation of *Mysis* from Lake 223, the Lake 223 zooplankton community shifted primarily along axis 1 in a manner that did not mirror the shifts observed with the Lake 224 zooplankton community, primarily on axis 2. The first axis explains almost twice as much variation as the second axis, with 50.1% of the total variation explained by the first two axes. The PCoA of the Lake 223 seasonal zooplankton community biomass data explained 47.7% of the variation on the first two axes and showed separation between the time periods with and without *Mysis* (Fig. 1.3). The community appears to be similar at the start (May) and end (October/November) of the open-water season in any given year, generally transitioning seasonally in a saltatory, directional-with-return trajectory through multivariate space as described in Lamothe et al. (2018). The PCoA on the Lake 224 zooplankton community data does not show as clear of a separation between time periods with a high degree of overlap in the points in the biplot, suggesting there is more similarity between time periods than in Lake 223. The Lake 224 PCoA was able to explain 61.6% of the variance on the first two axes.

Lake-wide Lake 223 *Chaoborus* August densities in 2014-2017 were estimated to be 25.1/m^2 relative to a historical August 1978 *Mysis* density of 42.8 /m^2 (Table 1.4). Lake 224 July densities of *Mysis* were estimated to be 77.2 /m^2 relative to a historical density of 595 /m^2 in July 1981 (Nero and Schindler 1983). The density of these predaceous zooplankton in both Lake 223 and Lake 224 have dramatically declined since the experimental acidification of Lake 223.

The CDA on both the Lake 223 ($F_{17,31} = 8.33$, $p<0.0001$) and 224 ($F_{16,31} = 11.51$, $p<0.0001$) zooplankton community significantly separated the groups based on the time period constraint (Fig. 1.4). When density data was combined from Lake 223 and Lake 224, a lake factor was included with a time period factor and an interaction term, there was a significant interaction ($F_{17,64} = 4.07$, $p<0.0001$) indicating that Lake 223 responded differently between time periods than Lake 224. *Holopedium glacialis* (previously *H. gibberum* (Rowe et al. 2007)) increased significantly ($p<0.05$) in biomass in Lake 223 following *Mysis* extirpation while there was a significant decrease ($p<0.05$) in Lake 224 (Figure 1.4). Although the group of *Daphnia* species including *D. catawba, D. pulex, and D. schoedleri* were not present in Lake 224, this group of species showed a significant increase ($p<0.05$) in biomass in Lake 223 following *Mysis* extirpation. The group of *Daphnia* species including *Daphnia g. mendotae* and *D. dubia* showed a non-significant increase in biomass in Lake 223 following *Mysis* extirpation, with a non-significant decrease in Lake 224. *Mesocylops edax* and rotifer species showed a significant ($p<0.05$) biomass decrease in Lake 223 following *Mysis* extirpation, but showed a non-significant increase in Lake 224, there was a significant ($p<0.05$) biomass decrease in Lake 223 following *Mysis* extirpation.

The PERMANOVA conducted on both zooplankton communities supported the CDA results, indicating that the location of the time-period group centroids were significantly different (PERMANOVA, $p<0.0001$) for both Lake 223, and Lake 224 (PERMANOVA, $p<0.0001$).

The biomass of lake trout in Lake 223 have decreased by roughly 39% relative to the average biomass prior to the extirpation of *Mysis*, relative to a 12% decline in Lake 224 (Fig. 1.5). A one-sample Wilcoxon rank test found the that the 2011-2016 lake trout standing biomass (of 284 kg) was marginally different than the average 1977/78 lake trout standing biomass of 463kg (p-value = 0.06). Meanwhile the annual standing biomass of the lake trout population in Lake 224 remained much more consistent throughout time, and has significantly decreased in biomass but only by 12% over the same time period using a (Wilcoxon rank test, p-value = 0.019) from an average biomass of 260kg in 1976-1981 to 229kg in 2011-2016. The average weight of individual lake trout in Lake 223 between time periods was similar, from an average of 676.1g in 1977/78 to 674.7g in 2014-2017. In addition to lower standing biomass in the Lake 223 lake trout, there was also decreased recruitment (>70% decline) in the time period with *Mysis* extirpated, relative to before *Mysis* were extirpated (Figure 1.6A, $t_{12}=8.3$, p-value <0.0001) from an average of 119 fish/yr before 1978 to an average of 36 fish/yr from 2000-2005. Meanwhile in Lake 224, there was no observed difference in recruitment between the same time periods (Figure 1.6B, $t_{12} = 1.7$, p-value = 0.12), and recruitment has remained relatively consistent.

The mean abundance and standing biomass of the fathead minnow population in Lake 223 has slightly decreased relative to the 1976/77 estimates (Table 1.5). A nonparametric one-sample Wilcoxon rank test found the 2014-2017 fathead minnow weights were non-significantly different than the average weight estimate from 1976/1977 (*p*-value = 0.375). Also, a one-sample *t*-test also found no significant difference in fathead minnow biomass between time periods (t_3= -1.1, *p*-value = 0.35). The total biomass of species considered within the Lake 223 lake trout food web has decreased by 28% relative to the time period prior to *Mysis* extirpation (Table 1.6). The largest absolute loss in biomass was from the lake trout population which has decreased by 179kg relative from when *Mysis* were still present in Lake 223. In the absence of *Mysis* in Lake 223, the *Chaoborus* population has increased by an assumed 3kg, since *Chaoborus* were not counted in Lake 223 prior to acidification but were not apparently abundant (Malley et al. 1982). *Chaoborus* abundance in Lake 223 is almost equivalent to the abundance of *Mysis* in Lake 224 (Figure 1.7) yet is much smaller in terms of biomass (Figure 1.8), demonstrating the difference in biomass between these two species.

Physical properties of Lake 223 have remained relatively constant throughout time. As lake trout are restricted to the hypolimnetic layer of lakes during summer stratification, if the volume of the habitable hypolimnetic layer were to be reduced either through deeper thermoclines or more shallow intrusions of low oxygen water, the habitable area of the lake would also be reduced. The August thermocline depth, representing peak stratification, has not significantly changed between time periods (1974-1976 vs. 2015-2017; $W = 8$, $p = 0.2$). However, the depth of oxygen concentrations in the late summer months below 6mg O_2/L, has become significantly more shallow from an average of 11.7 m deep in 1974-1977 to 9.4 m deep in 2015-2017 ($W = 48$, $p = 0.002$), meaning the habitable volume in Lake 223 between these time periods may have been reduced. The *p*-value of the depth of 6mg dissolved O_2/L should be regarded with caution as there were ties in the ranks. There has been no change in total dissolved phosphorous between time periods with *Mysis* (1974-1978) compared to without *Mysis* (2000-2008, t_{38} = -1.6, *p*–value = 0.13), indicating that there has been no change in the productivity of the lake. There was also no difference in epilimnetic chlorophyll-*a* concentrations between the time periods (W_{72} = 524.5, *p*-value = 0.12), suggesting there has not been any difference in standing biomass of phytoplankton.

DISCUSSION

Overall, the results of this study provide evidence that *Mysis* are potentially a strong interactor in their native ecosystem, with their absence being correlated with the "dramatic effect" predicted from the removal of a keystone species. The loss of *Mysis* in Lake 223 resulted in several ecosystem changes not observed in an unmanipulated reference lake. There have been changes to relative species abundances in the zooplankton community with contemporary estimates of *Daphnia spp.* biomass increasing while calanoid copepod populations declined. *Chaoborus spp.* now occupy the niche of pelagic zooplankton macro-predator in Lake 223 but favour different species of prey, have different vertical distribution patterns, and represent only 20% of the biomass previously occupied by *Mysis* before their extirpation. The extirpation of *Mysis* from Lake 223 has likely resulted in lower abundance, biomass, and recruitment in the lake trout population. This evidence combined with the impacts *Mysis* have had in non-native environments supports their definition as a potential keystone species and are therefore more than just “keystone aliens”.

Directional change of zooplankton communities in Lake 223 was clearly different compared to Lake 224, suggesting different drivers acting on the Lake 223 community than natural or regional changes alone. The zooplankton community of Lake 223 currently has a different composition than reference Lake 224, in part representing the effects of *Mysis* loss. Group centroids from PCoA showed the resultant location of the current communities have shifted in different directions, indicating different community composition changes with time in each lake. Given that communities are transitory (Keller and Yan 1998), this difference in direction between communities is evidence of differential drivers between systems. If only regional change was acting on communities, group centroids should shift in roughly the same manner.

As with all long-term data, consistent data collection methods are important for data integrity. As an example larger zooplankton may be overrepresented in the historical samples collected using the Schindler-Patalas trap compared to net tows (Devries and Stein 1991, Johannsson et al. 1992). *Daphnia* species and *Holopedium* increased in biomass in Lake 223 despite this possible sampling artifact, further suggesting that these changes in biomass are as a result of the extirpation of *Mysis*. Also, there have been changes to the zooplankton counting protocols; prior to 1983, only 5% of the sample was counted, and afterwards, the whole sample was scanned for larger organisms that may have been missed in the 5% subsample, meaning that more rare large organisms were more likely to be missed in the early samples, potentially contributing to the observed increase in large cladocerans (M. Paterson, pers. comm.). However, the exclusion of rare taxa in the zooplankton community analyses also likely helped mitigate this issue to some degree.

The Lake 223 CDA, when compared to the Lake 224 analysis representing natural environmental changes, indicate that large cladocerans were the groups in Lake 223 that increased in biomass following the extirpation of *Mysis*. As a predator with an active cruising strategy (Cooper et al. 1985), *Mysis* prefer slow-moving, large-bodied prey (like many cladoceran species), while fast moving copepods are not as affected by the presence of *Mysis* (Nero and Sprules 1986a). These results are supported by other studies that show that large cladocerans inhabiting the hypolimnion, tend to be most affected by the presence of *Mysis* (Grossnickle 1982, Nero and Sprules 1986b, Almond et al. 1996). Lake 375 at the ELA was the site of an aquaculture cage operation experiment that resulted in a large decline in the abundance of *Mysis* (Paterson et al. 2011). Correlated with the decline in *Mysis* abundance in Lake 375 was an increase in abundance of *Bosmina longirostris* and *Tropocyclops extensus* (Paterson et al. 2010). *Mysis* have been shown to be effective predators of *Bosmina* (Almond et al. 1996, Spencer et al. 1999) but were excluded from analysis in this study as they were a rare species in Lake 223 and Lake 224. However, Malley and Chang (1994) found that *Bosmina longirostris* in Lake 223 showed little change in abundance with acidification, and exhibited variability in abundance from 1974 to 1991 similar to this species in non-impacted Lake 239. Where I found that the group of *Daphnia* that includes *D. pulex* and *D. catawba* have significantly increased in biomass following the extirpation of *Mysis*, Nero and Sprules (1986b) also found that the presence of *D pulex* and *D. catawba* were correlated with lakes that did not contain *Mysis*. Also consistent with my observations of increases in large cladoceran biomass with the loss of *Mysis*, in microcosms with *Mysis* and natural prey assemblages, the biomass of cladocerans were most negatively affected by the presence of *Mysis* (Seckar 2009). In their natural range, large *Mysis* in Lake Michigan had cladocerans in their stomach contents (McWilliam 1970). In Lake Tahoe, California, *Mysis* predation was found to be directly responsible for the virtual disappearance of *Daphnia* and *Bosmina* (Richards et al. 1975, Goldman et al. 1979, Threlkeld et al. 1980, Morgan et al. 1981).

Along with zooplankton community changes following the loss of *Mysis*, there was also evidence of indirect effects on the zooplankton community following the extirpation of *Mysis* in Lake 223. Coinciding with the increase of large bodied cladocerans was a decrease in calanoid copepod biomass, possibly due to large bodied cladocerans outcompeting calanoids for phytoplankton (Sato and Hurlbert 1991). In enclosure experiments with natural prey assemblages, *Mysis* did not affect the abundance of calanoid biomass (Seckar 2009), which suggests that the decrease in calanoid biomass observed in Lake 223 was not due to direct interactions with *Mysis*, but rather was an indirect effect through food web processes, possibly via competition for phytoplankton as in Sato and Hurlbert (1991). Additionally, a decrease in calanoid abundance could affect the growth rates of age-0 lake trout, or be implicated in lake trout recruitment declines as calanoids have been found to make up a significant proportion of the diet of age-0 lake trout in Lake Superior (Hudson et al. 1995).

The cyclopoid copepod *Mesocyclops edax* decreased in abundance through time in Lake 223, compared to a non-significant increase in abundance in Lake 224 where it is rare. *Mesocyclops edax* is a large cyclopoid copepod species that is found in warmer water above the thermocline (Williamson and Magnien 1982), and prefer to prey on smaller zooplankters (Brandl and Fernando 1975). Following the removal of a large predatory zooplankton competitor (*Mysis*), *M. edax* might be expected to increase their abundance. With no *Mysis* to control *Daphnia* populations in Lake 223, *Daphnia* may be preventing the survival and maturation of *M. edax* nauplii. Elsewhere, high densities of *Daphnia* reduced and prevented *M. edax* nauplii maturation and survival by creating a recruitment bottleneck, as juvenile *M. edax* are inferior competitors to adult *D. pulex* for shared food resources (Toscano et al. 2016). Also, the decrease in abundance of *M. edax* is unlikely to be due to lagging effects of the acidification, as the abundance of *M. edax* showed no obvious trend with acidification and similar variability as in reference Lake 239 (Malley and Chang 1994). Taken together, these results combined with those from the Lake 223 zooplankton community suggest that in the absence of *Mysis*, *M. edax* abundance may be suppressed due to a juvenile bottleneck resulting from increased cladoceran abundance.

Though *Chaoborus* have become the dominant invertebrate predator in Lake 223, this study indicated that they represent a small fraction of the biomass previously occupied by *Mysis*. Relative to a historical *Mysis* population estimated at 6.7 million (Nero and Schindler 1983), the present-day abundance of Lake 223 *Chaoborus* is 73% the 1978 Lake 223 *Mysis* population, yet only 20% of the biomass. With a translucent body, *Chaoborus* are a sit-and-wait ambush predator that preys on active zooplankton, different than the active searching strategy of *Mysis* (Cooper et al. 1985). *Mysis* are typically about twice as large in total length as *Chaoborus* larvae (Dumont and Balvay 1979) and are capable of consuming much larger prey. Instar IV *Chaoborus* feed on zooplankton about 1mm in size (Vinyard and Menger 1980), while zooplankton greater than 1mm are less abundant in lakes with *Mysis* (Almond et al. 1996).

These differences in size and feeding behavior between *Mysis* and *Chaoborus* likely also affect the abundance of zooplankton prey species present. *Holopedium glacialis* create a gelatinous sheath around themselves as a predator avoidance tactic and have significantly increased in biomass in Lake 223 through time, despite a significant decrease observed in Lake 224. *Mysis* are able to consume *Holopedium* (O'Malley et al. 2017), with *Holopedium* being rare in lakes that have *Mysis* (Almond et al. 1996). In Lake 224 where *Mysis* make up approximately 20-50% of the total zooplankton biomass, *Holopedium* are rare. The observed increase in abundance of *Holopedium* in Lake 223 following the extirpation of *Mysis* reflects the reverse effect of a *Mysis* introduction into Lake Mesvattnet in Sweden, which resulted in decreased *Holopedium* abundance (Kinsten and Olsen 1981). Instar III and IV *Chaoborus* feed on crustaceans such as *Daphnia*, meanwhile avoiding *Holopedium* because of their protective jelly sheath (Neill 1981, Moore 1988). Although *Chaoborus* occupy a similar niche as *Mysis*, the differences between the two species has allowed *Holopedium* to increase in biomass following the extirpation of *Mysis*.

Though decreasing trends in calcium concentrations have contributed to increasing *Holopedium* dominance generally (Jeziorski et al. 2014b), data from Lake 223 indicates increases in *Holopedium* biomass is more likely related to food web changes and not changes in calcium concentrations. While calcium decline in northern temperate lakes may also be preventing daphniid recovery following acidification (Jeziorski et al. 2008, 2014a), I do not believe this is occurring in Lake 223, where *Daphnia spp*. were observed to have actually increased. Calcium concentrations <1.5 mg/L have been found to be detrimental to the growth, reproduction and survival of *Daphnia* (Hessen et al., 2000; Ashforth & Yan, 2008; Tan & Wang, 2010). Azan and Arnott (2018) showed that low calcium concentrations resulted in negative growth rates of *D. catawba*, and *D. pulex*; two of the *Daphnia* species that are part of a complex in Lake 223 that actually increased in abundance. Decreases in lake calcium concentration selects for *Holopedium* dominance within the crustacean zooplankton community because *Holopedium* require one-tenth the calcium that *Daphnia* require (Beaton and Hebert 1989). Even though calcium concentrations have been declining in ELA Lakes broadly (Jeziorski et al. 2014a), Lake 223 has remained essentially unchanged at a 2015-2016 average of 2.26mg/L, compared to the historical epilimnetic calcium concentration of 2.17mg/L in 1978-1979 (Nero 1981, Davies 1989, S. Havens pers. coms.). Despite no clear change in the calcium concentration of Lake 223*, Daphnia* species have increased in biomass in Lake 223 relative to Lake 224, suggesting that the increase has been due to food web processes and not due to changes in environmental conditions.

Although community composition changes following disturbance are the result of many factors, it is important to note that Lake 223 has been chemically recovered for more than 20 years. Malley and Chang (1994) found that during the recovery of Lake 223, the recovering community did not resemble the community at the same pH during the acidification of the lake, and predicted that the recovery zooplankton community will not be a reversal of the acidification pathway. In other lakes that were experimentally acidified to a pH of 5.7, zooplankton communities resembled that of the pre-acidification community within 10 years of chemical recovery (Yan et al. 1996), suggesting that Lake

223 should have been recovered from acidification over the time period examined here. Also, lakes in the Sudbury area that have been atmospherically acidified are moving toward communities more typical of near-neutral Precambrian Shield lakes (Keller et al. 1992). By contrast, the Lake 223 community shifted in a different direction compared to Lake 224. Lamothe et al. (2018) found that the Lake 223 crustacean zooplankton community following acidification showed gradual, directional trajectories with return, before tracking away from the historical centroid to a new endpoint. The results from my study appear to corroborate the results of Lamothe et al. (2018), indicating a new endpoint in Lake 223 that is different from pre-acidification, but based on comparisons with Lake 224, also in a different direction than that expected due to climate change alone. Invasion sequence (or in this case, the sequence of re-establishment of extirpated species or those reduced in abundance) may influence the resultant community (Robinson and Dickerson 1987), suggesting that various community endpoints are possible following an environmental disturbance. For example, *Daphnia g. mendotae* was dominant before the lake acidification, and declined in abundance throughout the acidification experiment resulting in *D. dubia* and *D. catawba* becoming present in the lake (Malley and Chang 1994). *Daphnia g. mendotae* has since increased in abundance to densities similar to those before acidification, potentially indicating that any lagging effects of the acidification experiment are no longer impacting species abundances. With more than 20 years since Lake 223 has been chemically recovered, enough time has likely passed such that lagging effects of the acidification experiment (besides the absence of *Mysis*) are unlikely.

Perhaps the most significant impact *Mysis* has on an ecosystem may be their role in transferring energy from lower to higher trophic levels. *Mysis* sequester significant quantities of epilimnetic zooplankton production and transfer this energy to the benthic environment, forcing the energy through a pathway dominated by benthic or deep-water fishes (Chess and Stanford 1999). As an omnivorous macro-invertebrate predator in an intermediate trophic position, *Mysis* also transfer energy from benthic and pelagic pathways to higher trophic levels (Covich et al. 1999). *Chaoborus* may not be as effective at transferring energy to higher trophic levels as *Mysis* were in Lake 223, creating a potential energetic bottleneck. The vertical distribution of *Chaoborus* throughout the water column is different compared to the diel vertical migrations of *Mysis*, potentially affecting availability for consumption and energy flow to higher trophic levels such as lake trout; although *Chaoborus* daily vertically migrate, only instar III and IV *Chaoborus* spend daylight hours in the hypolimnion (Moore 1988). In contrast, all age classes of *Mysis* spend daylight hours against the sediment and migrate to the thermocline at night (Grossnickle 1979). This difference in DVM dynamics between species could lead to a decrease in energy being sequestered from the epilimnion into benthic/deep-water pathways with a shift from *Mysis* to *Chaoborus* in Lake 223. Also, zooplankton communities with *Chaoborus* as the dominant zooplankton predator have a greater density of *Holopedium,* which have much lower phosphorus and calcium concentrations than their Daphniid counterparts (Andersen and Hessen 1991, Jeziorski and Yan 2006, McCarthy and Irvine 2010). It is predicted that dominance of *Holopedium* would also reduce vertical nutrient transfer throughout the food web (Ramcharan et al. 2001). Though *Chaoborus* have been assumed to have increased in abundance with the absence of *Mysis*, the biomass of *Chaoborus* is only a fraction of the historical *Mysis* biomass, meaning that a predator consuming *Chaoborus* would be less efficient in its energy acquisition. Taken together, this evidence suggests that the replacement of *Mysis* with *Chaoborus* as the dominant pelagic zooplankton meso-predator in Lake 223 is likely to have resulted in reduced benthic-pelagic coupling and less efficient transfer of energy to higher trophic levels.

Alternatively, with the removal of a significant prey source from the pelagic environment, the extirpation of *Mysis* may have caused lake trout to shift their main energy source from pelagic to littoral habitats. Lake trout in circular lakes have been found to have a higher degree of habitat coupling, as well as omnivory, when compared to reticulate lakes (Dolson et al. 2009). Lake 223 is a relatively simple, non-reticulate shaped lake (Schindler et al. 1980), providing the opportunity for a high degree of habitat coupling between pelagic and littoral environments. Isotopic analysis of the food web could provide insights into these hypotheses.

The natural recovery of the lake trout abundance in Lake 223 following acidification was predicted to take several years (Mills et al. 2002b), but even >15 years after chemical recovery, I found that the lake trout population still had a lower standing biomass and lower recruitment compared to that before the extirpation of *Mysis*. The abundance of lake trout in Lake 223 prior to acidification was abnormally high relative to abundances of other lake trout populations at ELA lakes (Mills et al. 2002a), but was comparable to the highest estimates of lake trout abundance ha^{-1} in the lakes studies by Burr (1997). The depth of 6mg dissolved O_2/L has become more shallow and was the only significant difference in Lake 223 physical characteristics between the time period before acidification compared to the lake chemically recovered. A shallower depth of acceptable O_2/L for lake trout in Lake 223 means there is less habitable volume for lake trout and may have been partly responsible for the decrease in population biomass and recruitment. A smaller hypolimnion due to the reduction in dissolved O_2 would also reduce the habitable area for hypolimnetic zooplankton species, potentially resulting in declines in species abundances in the hypolimnion. The loss of 10 kg in *Mysis* standing biomass from Lake 223 is correlated with a 179 kg (39%) decrease in average annual lake trout standing biomass. Meanwhile, in Lake 224, the mean annual biomass and production of lake trout has remained relatively consistent (Fig. 1.5b, Mills et al. 2002b). When *Mysis* have been introduced into non-native ecosystems (i.e. Flathead lake), lake trout have been shown to benefit by substantially increasing population abundance (Spencer et al. 1991, Stafford et al. 2002, Ellis et al. 2011), demonstrating the extent to which the presence of *Mysis* can positively impact lake trout populations. The decreased biomass of the Lake 223 lake trout population may also be due to a recruitment bottleneck as *Mysis* make up a large portion of the diet of juvenile lake trout (Trippel and Beamish 1993, France and Steedman 1996). Recruitment has significantly decreased between time periods by more than 70%, and recruitment failure was concluded to be the primary cause for lake trout biomass declines in Lake 223 during their response to acidification (Mills et al. 2002b). Once a juvenile is recruited to the population as an adult, survival is relatively high at over 93% annually (Mills et al. 2002a), where it is able to consume a wider variety of prey. No significant changes in lake trout recruitment from Lake 224 were observed. With so many years since the lake has been chemically recovered, it seems unlikely that the lake trout population would reach pre-acidification biomass without *Mysis* re-establishing in the lake.

TABLES AND FIGURES

Table 1.1. Physical properties of Lake 223 and 224 at the IISD-ELA. Area and max depth of Lake 223/224 from (Mills et al. 1987), Secchi disk depth data from (Cleugh and Hauser 1971).

	Lake 223	**Lake 224**
Area (ha)	27.3	25.4
Max depth (m)	14.4	26.7
Secchi disc depth (m)	5.0	7.0

Table 1.2. Zooplankton sampling methods at ELA

Years	**Device**	**Remarks**	**Reference**
1969-1975	28.7 L Schindler-Patalas trap	Vertical series at Center-buoy (CB)	(Chang and Malley 1987)
1977	4-barrel net; used only data collected with 53 µm nets	2 hauls X depth of sampling layer at CB	(Chang and Malley 1987)
1979-present	2-barrel closing sampler; 53 µm mesh; 13 cm each mouth diameter	2 hauls X depth of sampling layer at CB	(Chang and Malley 1987)

Table 1.3. Number of observations of the zooplankton community between different time periods for Lake 223 and 224, used in the PCoA, CDA, and PERMANOVA.

Time Period	**Lake 223**	**Lake 224**
"*Mysis* present" time period (1974)	n = 6	n = 6
"*Mysis* absent" time period (2015-2017)	n = 20	n = 36

Table 1.4. Densities of *Chaoborus/Mysis* in Lake 223/224, respectively. *Lake 224 density is from July 1981, Lake 223 is from August 1978 (Nero and Schindler 1983). Lake 223 *Chaoborus* densities from 2014-2017 are August averages, and Lake 224 *Mysis* densities are July averages.

Lake	**Historic *Chaoborus/Mysis* density ***	**2014-2017 average *Chaoborus/Mysis* density**	**% change in density**
Lake 223	42.8 /m^2	25.1 /m^2	-41%
Lake 224	595 /m^2	77.2 /m^2	-87%

Table 1.5. Mean Lake 223 Fathead minnow abundance and standing biomass in the periods before and after *Mysis* extirpation.

Time Period	**Abundance estimate**	**Standing Biomass (kg)**
Before *Mysis* extirpation (1976/1977)	8018	4.3
Mysis extirpated (2014-2017)	5250	3.7

Table 1.6. Average biomass of species within the lake trout food web of Lake 223. **Chaoborus* biomass is assumed to be non-existent (Malley et al. 1982). Dates over which means are generated are indicated in parentheses.

Species	**Average kg prior to *Mysis* extirpation**	**Average kg after *Mysis* extirpation**	**Difference (%)**
Lake trout	463 (1977-78)	284 (2011-16)	-39
Slimy Sculpin	370 (1977-78)	318 (2018)	-14
Bulk Zooplankton	81 (1974)	62 (2015-17)	-23
Fathead minnows	4 (1976-1977)	4 (2014-17)	0
Mysis	10 (1974-77)	0	-100
Chaoborus	0*	3 (2014-17)	+100
Total	**928**	**671**	**-28**

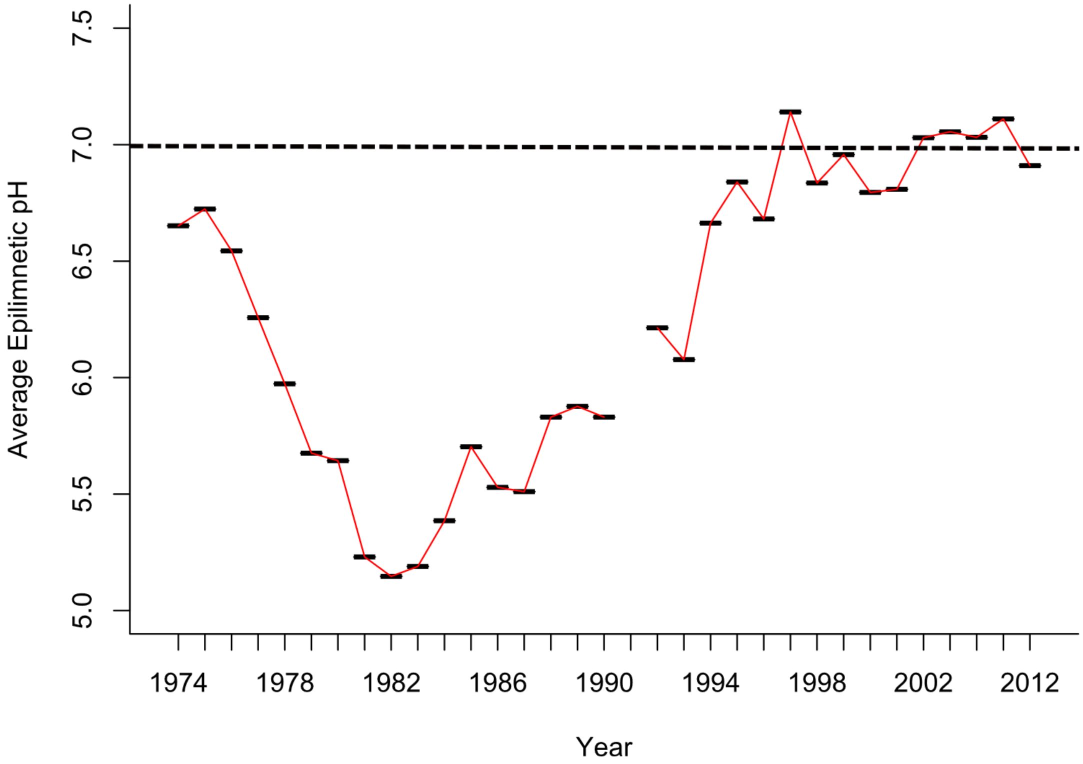

Figure 1.1. Epilimnetic pH of Lake 223 throughout acidification experiment. *Mysis* became extirpated in 1979 (Nero and Schindler 1983). Dashed line represents neutral pH.

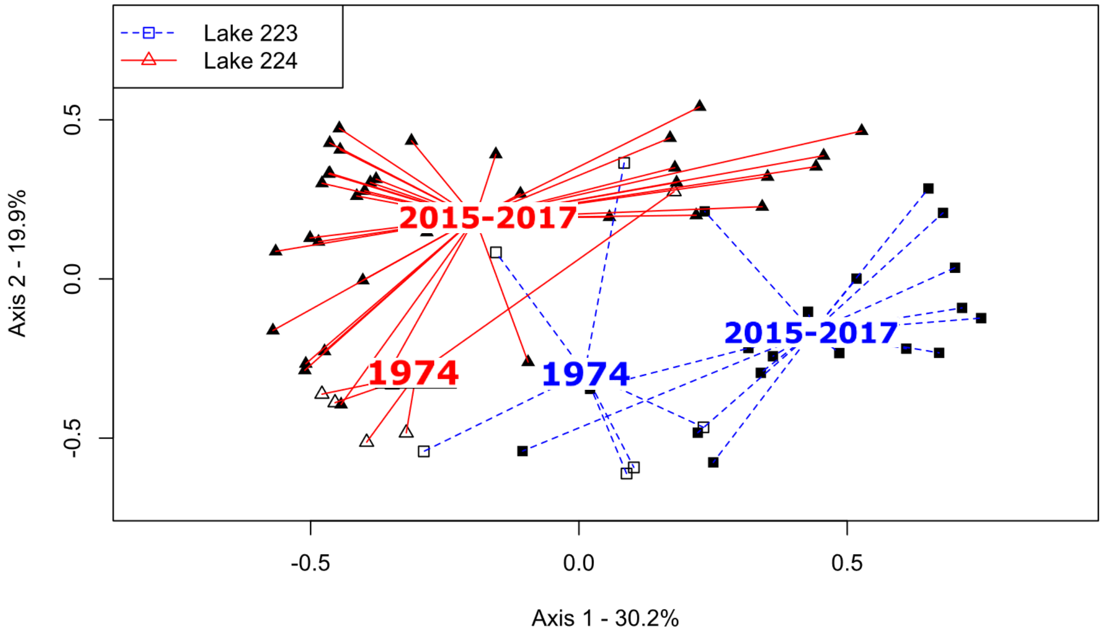

Figure 1.2. PCoA on zooplankton communities from Lake 223 and Lake 224. Centroid labels represent the different time periods from each lake with the 1974 label representing the time period before acidification, and the 2015-2017 label representing years post-recovery.

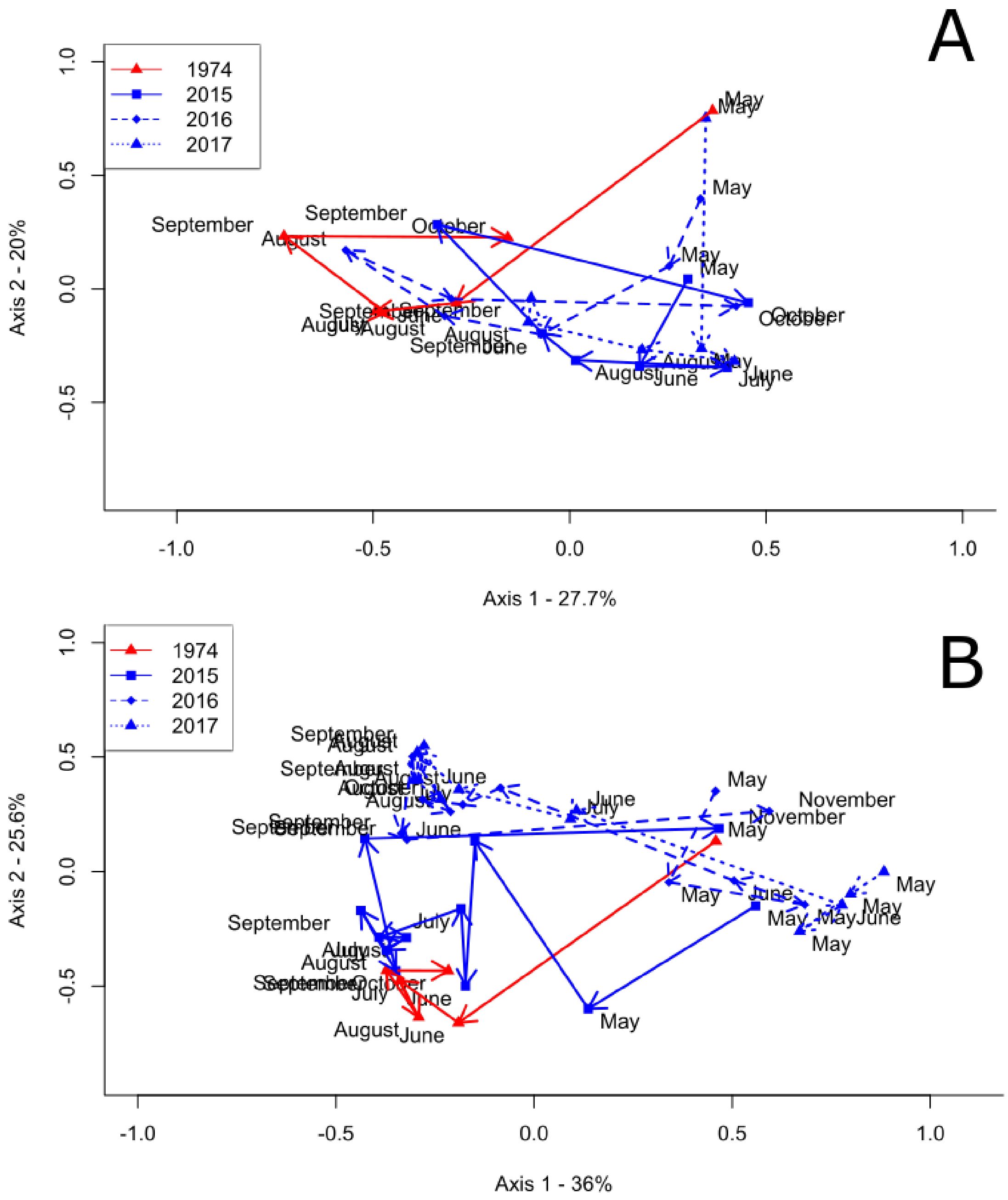

Figure 1.3. Principle Coordinates Analysis (PCoA) on Lake 223 (A) and Lake 224 (B) zooplankton community using Chord distance measure. Red and blue points and lines represent time periods with *Mysis* present and absent in Lake 223, respectively. Arrows show direction of time between points.

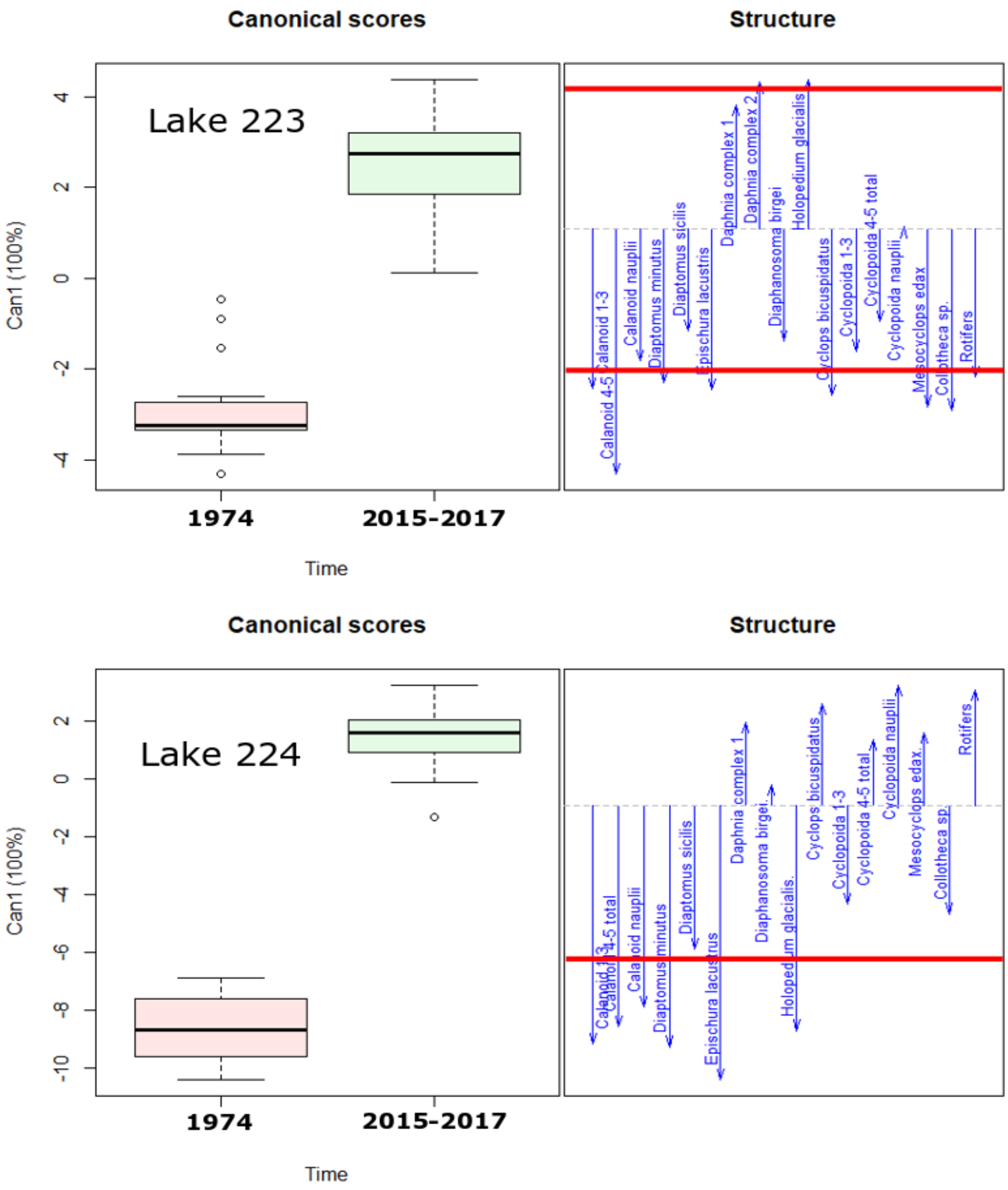

Figure 1.4. Canonical Discriminant Analysis (CDA) on the Lake 223 and Lake 224 zooplankton community using biomass density data. 1974 represents when *Mysis* were present in Lake 223, while 2015-2017 represents the time period where Lake 223 has recovered yet no *Mysis* are present. Arrows show structure scores, relating to which group each variable is more associated with. The red line approximately relates to p=0.05 level of significance, where structure scores that cross the red line signify a significant association with a time period group. *Daphnia* complex 1 included *D. g. mendotae* and *D. dubia*, complex 2 included *D. catawba, D. pulex,* and *D. schoedleri*. Rotifer group included *Gastropus hyptopus*, *Gastropus sp.*, *Gastropus. stylifer*¸ *Kellicottia longspina, Keratella cochlearis and Polyarthra vulgaris* (see Appendix).

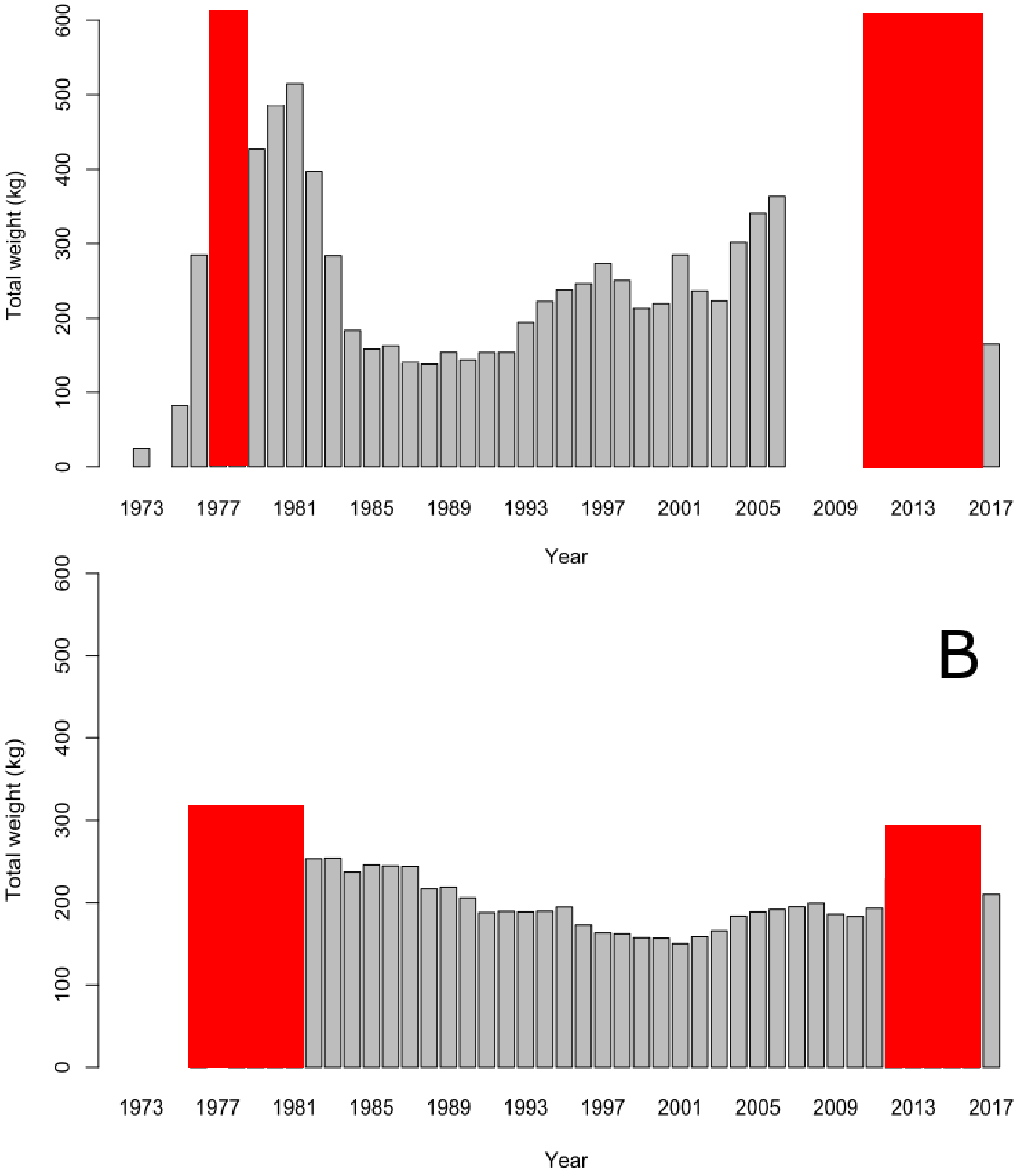

Figure 1.5. Total annual biomass of lake trout in Lake 223 (top, A) and Lake 224 (bottom, B). Red shaded areas signify time periods before and after *Mysis* extirpation. The Lake 223 time periods are 1977/1978 with a mean of 463kg, and 2011-2016 with a mean of 284kg. The Lake 224 time periods are 1976-1981 with a mean of 260kg, and 2011-2016 with a mean of 229kg.

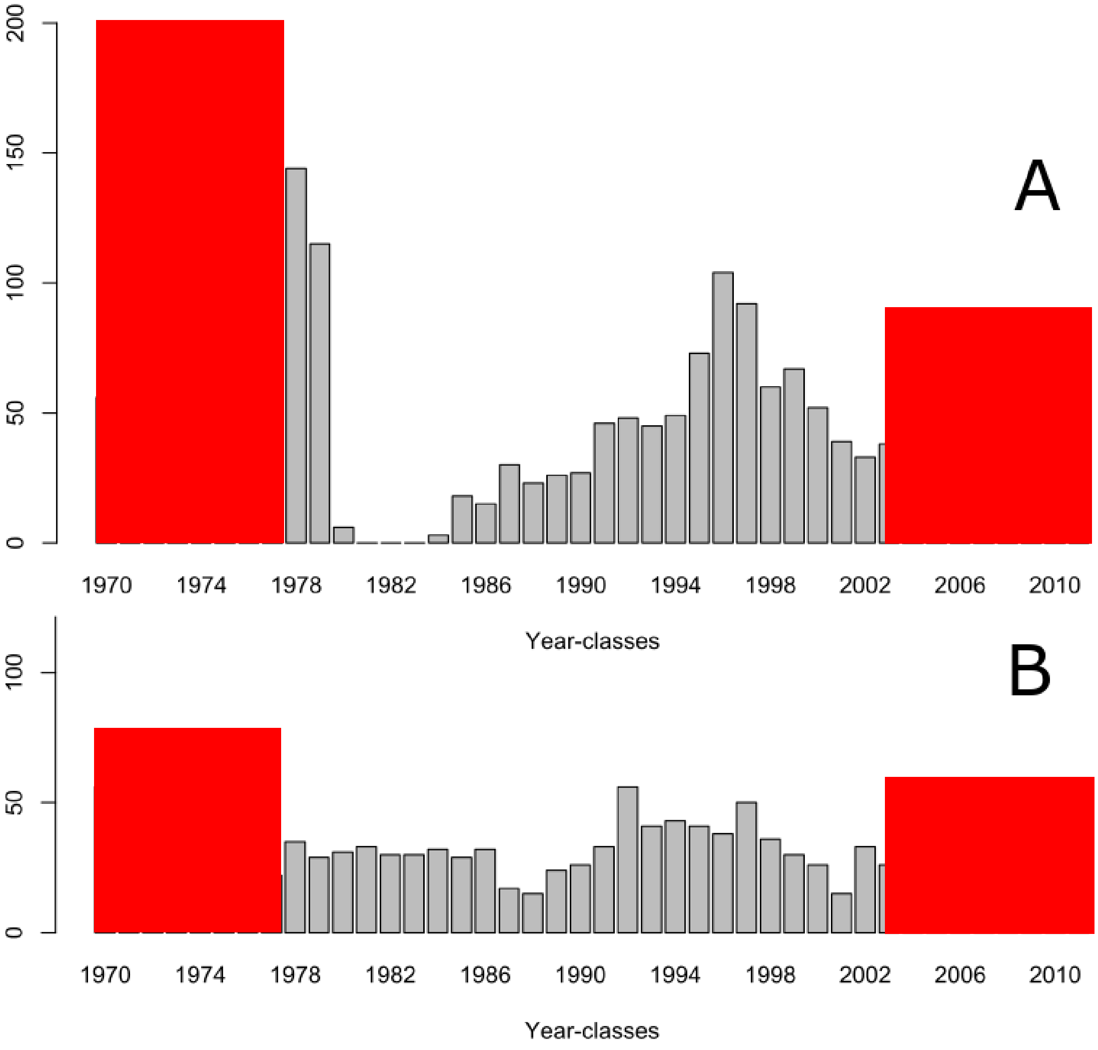

Figure 1.6. An index of lake trout recruitment (number of fish caught by year classes between 1970-2005) in Lake 223(A) and Lake 224(B). Red shaded year-classes 1970-1977 represent years with *Mysis* present, and year-classes 2003-2011 represent years where *Mysis* are extirpated in Lake 223.

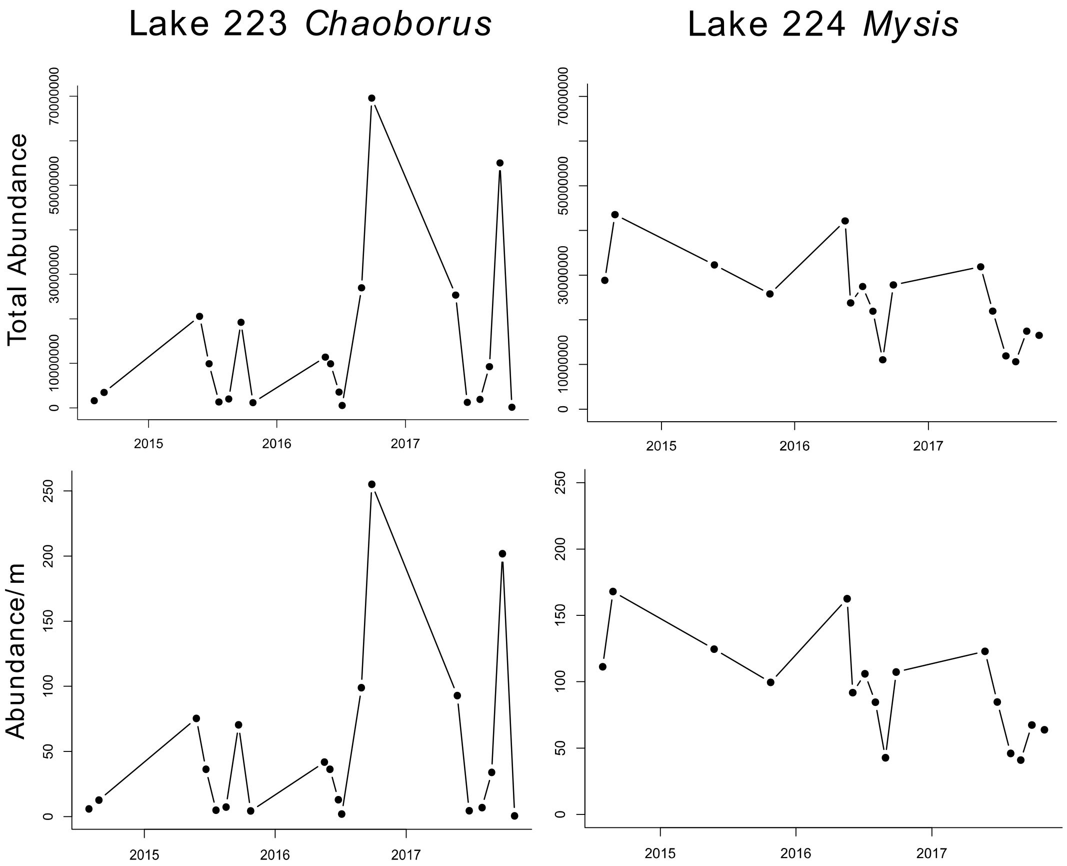

Figure 1.7. Predator abundance in Lake 223 and 224 from 2014-2017. Top row is total lake-wide abundance, and bottom row is abundance/m^2. Calculated using weighted averages among depth strata. Y-axis has been kept the same between lakes to allow comparisons.

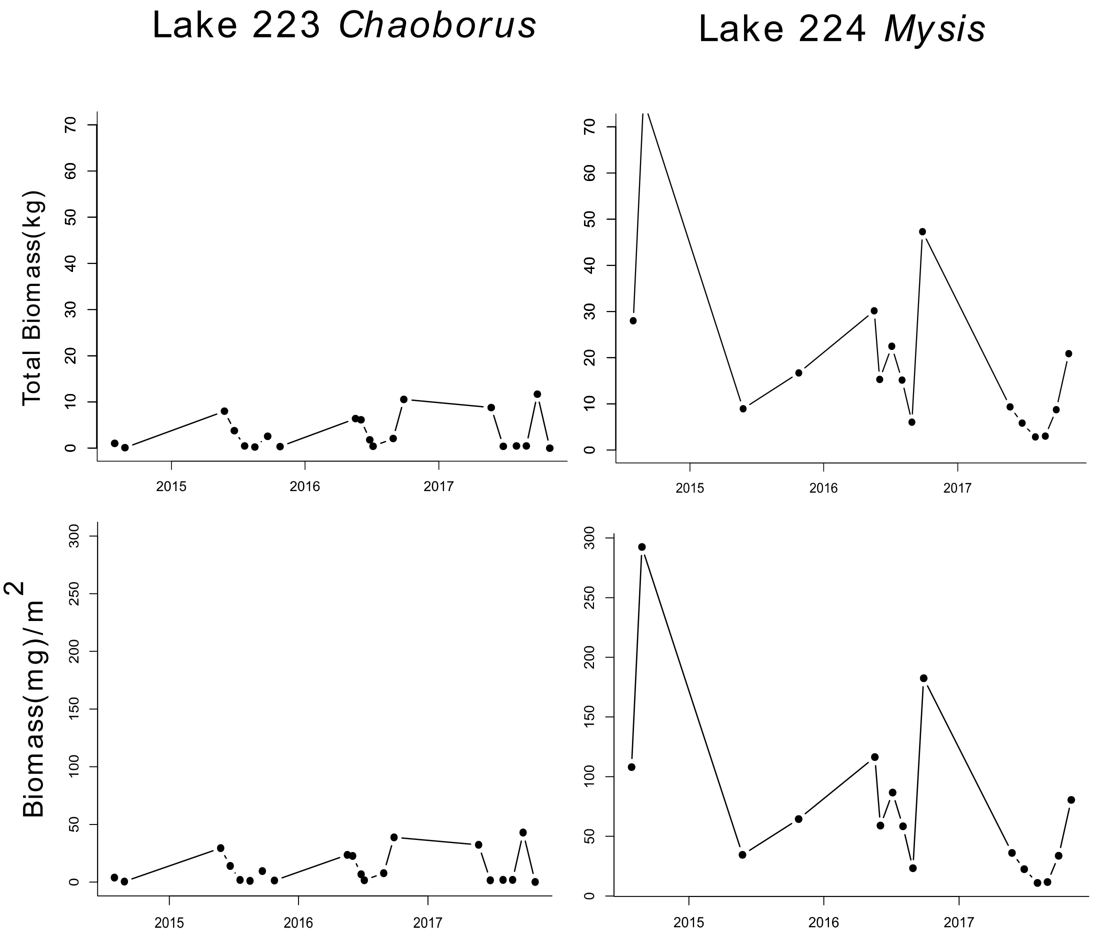

Figure 1.8. Predator biomass in Lake 223 and 224 from 2014-2017. Top row is total lake-wide biomass, and bottom row is biomass/m2. Calculated using weighted averages among depth strata. Y-axis has been kept the same between lakes to allow comparisons.

CHAPTER 2

Impaired energy pathways cause a shift towards pelagic resources by fish in recovering food webs

ABSTRACT

With thousands of Ontario lakes that have been atmospherically acidified, understanding the biological recovery of these lakes is crucial to the management of these ecosystems. More than 20 years after chemical recovery from an acidification experiment in Lake 223 at the IISD-ELA, all other extirpated species have since recolonized the lake except *Mysis*. Using stable isotope analysis (SIA) and two-source-mixing-models, I compared energy use (via stable isotopes) of lake trout and white suckers before and after *Mysis* extirpation. Growth rates of lake trout were also evaluated using length-at-age data of the most common ages and compared between Lake 223 and reference Lake 224. Contrary to my expectations, lake trout and white sucker both increased in trophic position and reliance on pelagic resources following the extirpation of *Mysis* from Lake 223. Isotopic signatures of ecosystem constituents suggest that these observed differences are due to a diet switch of lake trout and white sucker from *Mysis* to *Chaoborus*, as *Chaoborus* have become the dominant predatory zooplankton species in the absence of *Mysis*. Relative to *Mysis* in Lake 224, *Chaoborus* in Lake 223 have a greater pelagic energy reliance, and feed at a higher trophic position, appearing to prey solely on zooplankton. The lower biomass of *Chaoborus* relative to the historical Lake 223 *Mysis* population, combined with the lower spatial-temporal overlap between *Chaoborus* and lake trout, is correlated with a 5.6% slower growth rate in the Lake 223 lake trout population. Together, the results from this study suggest that *Mysis* play a significant role in the transfer of energy from lower to higher trophic levels, and that although *Chaoborus* occupy a similar ecological niche as *Mysis*, the differences between these two predatory zooplankton species results in reduced energy transfer to lake trout. This research

suggests that re-establishing *Mysis* into acid-damaged lakes may result in improved energy transfer from lower trophic levels to lake trout.

INTRODUCTION

As humanity is dependent on ecosystem services which appear to benefit from high levels of biodiversity (Daily 1997), it is important to be able to predict how ecosystem function may be affected by species loss given current projections; For example, the United Nation's Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services 2019 report outlines threats to over 1 million species. Species loss may cause ecological disruptions in food webs and potential economic disruptions from loss of ecosystem services; for example, recreational fishing contributes $8.8 billion/year to the Canadian economy (Brownscombe et al. 2014), and freshwater commercial fishing employed over 77,000 Canadians in 2017 (Statistics Canada). In 2015, trout species were listed as the second most predominant fish species harvested by the 3.2 million Canadian adult anglers (Fisheries and Oceans Canada 2019), indicating the potential economic impact of just one taxonomic group which is known to be vulnerable to several stressors, including climate change (Sharma et al. 2009). Although humans rely on ecosystem services provided through complex trophic interactions, our understanding of how biodiversity affects ecosystem function is only beginning to develop (Raffaelli et al. 2002).

Long-term experimental field research is needed to better predict the effects of species loss on ecosystem structure and function (Hooper et al. 2005). Species-removal studies have mainly concentrated on simple, single trophic level systems, while relatively few experimental studies have examined the impacts of species richness declines on ecosystem properties in multitrophic systems (Loreau et al. 2001, Duffy 2002, Ives et al.

2005). The effects of a species removal in single trophic level systems are much easier to predict due to the lack of indirect effects (Yodzis 1988, Hooper et al. 2005). While such studies can provide insight into complex ecological interactions, models of single trophic level ecosystems are insufficient to understand the functional consequences of extinctions in nature and provide little insight into how multitrophic level systems may respond (Petchey et al. 2004).

Stable isotope analysis (SIA) can be a useful tool in revealing changes in energy pathways following species extirpation. An increasing number of studies using stable isotope analysis have appeared since the early 1990's (Phillips et al. 2014), in part because SIA provides a picture of resource-use integrated over longer time periods as compared to gut content analysis (Bearhop et al. 1999, Votier et al. 2003). Large consumers have tissue turnover rates ranging from months to years (Hesslein et al. 1993), making their isotopic signatures representative of their diet over long periods of time (Post 2002). Ratios of carbon ($\delta^{13}C$ / $\delta^{12}C$) and nitrogen stable isotopes ($\delta^{15}N$ / $\delta^{14}N$) have been used to describe energy transfer in many aquatic systems (Hesslein et al. 1991, Kidd et al. 1995, Hobson et al. 2002, Gorski et al. 2003). Trophic fractionation between $\delta^{15}N$ / $\delta^{14}N$ maintains a mean of 3.4 per mill (‰), allowing for estimation of trophic position, while $\delta^{13}C$ is not appreciably changed with trophic position, allowing for the differentiation of isotopically distinct resources used by consumers (Vander Zanden et al. 2001, Post 2002). In lakes, $\delta^{13}C$ of algae and detritus in littoral and benthic regions is enriched relative to $\delta^{13}C$ of phytoplankton from pelagic regions (Hecky and Hesslein 1995). Examining isotopic signatures of ecosystem constituents before and after a species extirpation may therefore help to explain potential changes in resource use and energetic pathways of consumer species.

Although species removal experiments are not new, they are rare; it is extremely difficult to completely extirpate only one species in experimental field research. From 1976 to 1996, Lake 223 at the International Institute for Sustainable Development's – Experimental Lakes Area (IISD-ELA) was experimentally acidified to evaluate the effects of acid rain on aquatic ecosystems. A maximum acidity of 5.1 pH was reached in 1981 before being allowed to incrementally recover to pre-acidification levels in 1996. Over the course of the experiment, many species were extirpated from the lake as the pH surpassed species' thresholds, including fathead minnows (5.9 pH), crayfish (5.6 pH), slimy sculpin (5.6-5.9 pH), and *Mysis diluviana* (5.6-5.9 pH; Nero 1981, Davies 1989, Mills et al. 2000). During acidification, the lake trout population declined due to several consecutive years of recruitment failure and slow population growth during chemical recovery of the lake (Mills et al. 2000). Partial biological recovery followed chemical recovery in 1996. Crayfish returned to Lake 223 by 1998, sculpin by 2004, and fathead minnows by ~1988, although returns to historical abundance levels required an additional 10-20 years (Colvin and Rennie submitted, Mills et al. 2000). However, *Mysis* never returned to the lake. Other studies (Schindler et al. 1985, Mills et al. 1987, 2000) have previously described the effects of the acidification on the lake trout population, but none have examined changes in consumer resource use over the course of the study, nor the status of populations >20 years after the chemical recovery of the lake.

Mysis diluviana are a potential keystone species in their native range (Chapter 1), and have been especially disruptive to food webs when introduced outside their native range (Morgan et al. 1981, Chess and Stanford 1998, Ellis et al. 2011). *Mysis* are a relatively large (<20mm) freshwater zooplankton species that play a key role in transferring energy from lower to higher trophic levels (Covich et al. 1999, Ahrenstorff et al. 2011). Lake trout often rely heavily on *Mysis* as a key food source during the early years of life, especially during the ontogenetic switch from planktivory to piscivory (Eschmeyer 1956, Griest 1976, Zimmerman et al. 2009); France and Steedman (1996) found that 41% of juvenile lake trout in small Canadian shield lakes had δ^{15}N values suggesting they fed primarily on *Mysis*. Following the introduction of *Mysis* into Flathead Lake, Montana, lake trout that had previously been introduced were able to take advantage of this additional prey source and overcome a recruitment bottleneck, greatly expanding their population size (Ellis et al. 2011). The literature suggests that *Mysis* make up a significant portion of the diet of lake trout in both their introduced and native ranges, but there has not to date been a description of the impacts of *Mysis* extirpation on a lake trout population and description of energy pathways connecting them to the rest of the ecosystem.

There is a possibility that the extirpation of *Mysis* could either result in a decrease in benthic-pelagic coupling due to the loss of the transfer of energy from one habitat to the other, or an increase in benthic-pelagic coupling due to the omnivorous diet of lake trout in Lake 223. The extirpation of *Mysis* from Lake 223 may have resulted in reduced benthic-pelagic coupling, as *Mysis* have been found to effectively couple energy between benthic and pelagic environments through their DVM's (Chess and Stanford 1999). A decoupling of the benthic-pelagic pathways through the loss of *Mysis* could therefore reduce the proportion of benthic production introduced via the habitat coupling of *Mysis* in pelagic fishes. If there has been a decoupling of the two environments, organisms such as lake trout and white sucker that integrate both benthic and pelagic energy channels (Ahlgren 1996, Saint-Jacques et al. 2000, Vander Zanden and Vadeboncoeur 2002) should show changes in benthic energy reliance. It is possible that *Chaoborus* have replaced the role of *Mysis* as an integrator of energy from different sources. If lake trout have increased littoral foraging with the decline of *Mysis*, an increase in benthic/littoral C in the isotopic signatures of lake trout would be expected, reflecting an increased incorporation of energy from the littoral zone.

The purpose of this study was to evaluate changes in the energy pathways supporting lake trout and white sucker following the extirpation of a significant member of the food web (*Mysis*). Two-source-isotopic mixing-models allowed me to determine if lake trout and white sucker in Lake 223 have changed energy reliance between the pelagic and littoral environment relative to the time period before *Mysis* extirpation. I also compared the $\delta^{15}N$ and $\delta^{13}C$ isotopic signatures of all available members of the food web to make inferences about contemporary lake trout diets. With the loss of a significant pelagic food source, I predict that due to the loss of the predominantly-pelagic *Mysis*, that there should be a greater dependence on nearshore forage fish and crayfish, increasing energy coupling between the littoral and pelagic habitats. I further hypothesize that with a highly omnivorous diet (Martin 1952, Ahlgren 1996, France and Steedman 1996), in the absence of *Mysis*, lake trout will have become more dependent on minnow species in the littoral environment, therefore having an increased trophic position, while white sucker will also have an increased littoral reliance, but with a decrease in trophic position following the loss of *Mysis* from the zooplankton component of a their diet.

METHODS

Lake 223 is located in the International Institute for Sustainable Development's Experimental Lakes Area (IISD-ELA) in northwestern Ontario (Cleugh and Hauser 1971). Beginning in 1976, Lake 223 was experimentally acidified with electrolyte-grade sulfuric acid, reaching a minimum pH of 5.1 by 1981 and was maintained at that level of acidity until 1983 (Mills et al. 1987). The lake was allowed to recover in a stepwise fashion until reaching pre-acidification levels by 1996 (see Chapter 1 for details). Lake 224 is located upstream of Lake 223 and is connected by a small creek with limited flow.

The trophic position and percentage of benthic resource use for lake trout and white sucker were calculated using isotopic signatures and a two-source-mixing model from (Post et al. 2000) using representative species from the littoral and pelagic environment as respective end-members. Trophic positions were compared between time periods before *Mysis* extirpation, to after chemical recovery of the lake but without *Mysis*, using two-factor ANOVA's with lake and time period as factors. Additionally, signatures of available ecosystem constituents were also analyzed to evaluate niche space.

All SIA analyses were performed by Isotope Tracers Technologies Inc. in Waterloo Ontario. Isotopes were analyzed using a DeltaPlus Isotope Ration Mass Spectrometry (IRMS), coupled with an Elemental Analyzer EA 1110 CHN. All carbon isotope data was Suess corrected (Verburg 2007). Isotope ratios are expressed as delta values (δ) and measured as the difference (in parts per thousand, ‰) between the isotope sample and an international standard according to the formula:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \text{ x } 1{,}000$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The global isotopic standard for $\delta^{13}C$ is PeeDee limestone and for $\delta\,{}^{15}N$ is atmospheric nitrogen. Instrument error standard deviation was ±0.15‰, and ±0.3‰ for carbon and nitrogen, respectively. Sample duplicates were analyzed for roughly 10% of samples (36/339 samples); mean differences between duplicate samples closely reflected reported instrument error for $\delta^{13}C$ signatures (mean difference = -0.15‰, t_{35}= -1.10, p-value = 0.28) and was also within reported error for $\delta^{15}N$ signatures (mean difference = -0.03‰, t_{35} = -0.48, p-value = 0.64).

Acidification of water alters the isotopic carbon fractionation equilibrium by affecting the relative amounts of $CO_{2(aq)}$, carbonate, or bicarbonate in the dissolved inorganic carbon pool depending on pH (Hecky and Hesslein 1995). Additionally, the signatures of $\delta^{13}C$ or $\delta^{15}N$ alone provide very little information about an organism because there is considerable variation among ecosystems in $\delta^{13}C$ and $\delta^{15}N$ signatures at the base of the food web (Rounick and Winterbourn 1986, Vander Zanden and Rasmussen 1999, Post 2002). Therefore, baseline signatures representative of pelagic and benthic end members were needed along with target consumers to tease apart food web changes from the effects of acidification. Isotopic baselines representing the pelagic and littoral communities were created using bulk zooplankton and crayfish, respectively. During 1969-75, zooplankton abundance data were collected from Lake 223 and Lake 224 using a transparent 28.7L Schindler-Patalas trap (Schindler 1969). After 1975, barrel closing nets were used. Formalin-preserved zooplankton samples were available for almost every year since 1974 from Lake 223 and reference Lake 224. Samples were collected during daylight hours, at the deepest point in the lake, typically collected every two weeks during the open-water season of the year. More detailed sampling and processing methods are described in detail by Chang and Malley (1987). Prior to fixation, methanol was added to the zooplankton samples before being preserved with 4% formaldehyde or 4% sugared formalin. Formalin is known to enrich zooplankton in $\delta^{13}C$ relative to fresh samples by ~1‰, where methanol is known to deplete zooplankton in $\delta^{13}C$ by ~0.5‰ (Feuchtmayr and Grey 2003).

Zooplankton were used as a baseline for the pelagic environment because zooplankton samples were available throughout the time series of the acidification experiment and bulk zooplankton are better indicators of $\delta^{13}C_{base}$ for the pelagic food web than bulk seston samples (Zohary et al. 1994, del Giorgio and France 1996, Post 2002). Yearly composite samples were created by subsampling monthly zooplankton samples between 1974-2017, using 1ml aliquots. Zooplankton composites were created approximately every other year, when samples were available. Zooplankton composites were selected to represent each month in the open-water season (therefore every second sampling occasion) and were selected to maintain equal spacing between samples whenever samples were missing since zooplankton $\delta^{13}C$ signatures can vary as much as 10‰ among months in many ELA lakes (M. Paterson, pers. comm.). Lake 223 zooplankton composites spanned the entire open water season (May-November) in all years (1974-2017), except for 1974 where only samples from August, September, and November were available. Lake 224 zooplankton composites spanned the entire open water season in all years (1974-2017) except for 1974, 1976, and 1977. For Lake 224, the

1974 zooplankton composite composed samples from every open-water month except for August; the 1976 composite only included October samples, and the 1977 composite included samples from May, June, and July. Samples were composed of either the entire water column, or discrete thermal layers that were then combined to represent the entire water column. Composite samples were dried in an oven at 60ºC, homogenized, and then weighed to approximately 0.5mg into 5mm x 3.5mm tin cups.

Zooplankton composite samples acquired a buildup of preservative after being dried, which resulted in a high level of uncertainty in the nitrogen signatures. Originally, 26 out of 44 (59%) samples including duplicates, were flagged by the lab as having a high uncertainty, with a greater occurrence after the year 2000, possibly following methodological changes in sample preservation involving the addition of sugar to the formalin preservative mixture in this year and all years following. 25 out of the 26 samples that were originally flagged by the lab as having high uncertainty were re-hydrated, mixed, rinsed with DI water using a 74um sieve, and then dried once again at 60 ºC. Washed isotope samples returned from the lab without any samples flagged as having high uncertainty, suggesting that the preservative was the likely cause of uncertainty. Duplicate yearly samples were averaged, and the means were used in the zooplankton baseline. Duplicates varied by an average standard error of 1‰ with $\delta^{13}C$ signatures and varied by an average standard error of 0.47‰ with $\delta^{15}N$ signatures. Paired t-tests showed no significant differences in either the $\delta^{13}C$ zooplankton composite duplicates ($t_{13} = 1.8$, $p = 0.08$) or the $\delta^{15}N$ zooplankton composite duplicates ($t_{13} = 0.6$, $p = 0.57$). Additionally, seven of the original samples that were not flagged as having high uncertainty, that appeared suspiciously high or low in either $\delta^{15}N$ or $\delta^{13}C$, were also excluded as I suspected the preservative was still influencing the results.

Crayfish (*Orconectes virilis*) were used as a baseline for the littoral environment because of the relatively high availability of samples. As an omnivore, *O. virilis* directly link primary production and detritus-based food webs to many predatory fish species (Dorn and Mittelbach 1999, Phillips et al. 2009). Crayfish feed on a wide variety of organisms in the littoral zone, from periphyton and detritus to benthic invertebrates and vulnerable stages of vertebrates (Holomuzki 1989, Dorn and Mittelbach 2004). As a relatively long-lived species (~3.5 years; Momot and Gowing 1983), crayfish incorporate diet into tissue over long time periods with tissue turnover rates for *O. virilis* being 67 days and 77 days for $\delta^{13}C$ and $\delta^{15}N$, respectively (Glon et al. 2016). From Lake 223, crayfish were collected from 1977 – 1982, and then again in 2014-2018, but only during 2014-2018 in Lake 224.

In lieu of crayfish samples throughout the full acidification experiment, crayfish signatures were estimated from regressions between lake pH and crayfish isotopic signatures. The isotopic signatures from individual crayfish within Lake 223 were significantly correlated with the lake pH for both carbon (Fig. 2.1, $F_{6,27} = 5.06$, $R^2 = 0.42$, $p = 0.001$) and nitrogen (Fig. 2.1, $F_{6,27} = 3.6$, $R^2 = 0.33$, $p = 0.008$). Despite crayfish being extirpated in Lake 223 from 1982 to 1998 (Davies 1989, Colvin and Rennie in review), regressions were used to estimate putative crayfish carbon and nitrogen signatures in years where samples were not available. Since Lake 224 crayfish samples were not available before 2014, Lake 224 crayfish isotopic signatures were estimated from lake pH using the same regression slopes used in the Lake 223 estimates, with intercepts adjusted based on the differences in 2017/2018 mean isotope signatures between Lake 223 and Lake 224. For example, the regression equation for crayfish $\delta^{13}C$ signatures estimated from Lake 223 pH is $\delta^{13}C = 1.8 * pH - 37$ (Fig. 2.1), and the difference between Lake 223 and Lake 224 mean $\delta^{13}C$ signatures during 2017 and 2018 were -3.13 $\delta^{13}C$. The difference was subtracted from the intercept parameter to provide a Lake 224 crayfish $\delta^{13}C$ regression equation of $\delta^{13}C = 1.8 * pH – 33.87$. The same method was applied to estimate Lake 224 crayfish $\delta^{15}N$, with the Lake 223 and Lake 224 equations for estimating $\delta^{15}N$ being $\delta^{15}N = -0.9*pH +11.7$ and $\delta^{15}N = -0.9*pH +10.99$, respectively.

Lake trout and white sucker isotopic samples were collected annually from Lake 223 and Lake 224. Fish were caught during annual trap-netting on or near spawning locations for white sucker and lake trout in the spring and fall, respectively. Large fish have muscle tissue turnover rates on the order of 3 months (Hesslein et al. 1993), with fin tissue turnover rates almost identical to muscle tissue turnover rates (Suzuki et al. 2005). Therefore, the isotopic signatures of lake trout in this analysis are likely reflective of conditions during summer stratification, with white sucker signatures reflective of late winter resource use. Lake trout and white sucker lengths, weights, and aging structures were collected during this time and were used here to compare growth rates and body condition before and after *Mysis* extirpation. Generally, fins taken from fish <350mm in fork length were small enough to be completely coated in epoxy during the previous aging estimation process, rendering them un-useable for isotope analysis. Therefore, most fish selected for SIA are > 350mm, representing adults from each population. The distal tips of the fins that were not covered in epoxy were used for SIA. Fish were primarily selected based on availability of samples, and from roughly every other year of sampling. Fish fins are strongly correlated with isotope signatures from muscle tissue (Sanderson et al. 2009, Hanisch et al. 2010, Tronquart et al. 2012, Wellman et al. 2017), and lake trout signatures were converted to muscle tissue signatures using the relationships between lake trout fin and muscle $\delta^{13}C$ and $\delta^{15}N$ signatures from Wellman et al. (2017) derived from ELA lake trout. In in the absence of a published relationship between white sucker fin-muscle signatures, I used the same equation to convert white sucker fin to muscle signatures as was used for lake trout. Nitrogen input from anthropogenic sources can result in elevated $\delta^{15}N$ values (Cabana and Rasmussen 1996, Lake et al. 2001), but the lakes in this study are pristine lakes isolated from human developments.

LOESS fit to annual zooplankton and crayfish isotopic data were used to estimate annual baseline values for both the pelagic and littoral end members. These baselines were then used for estimation of trophic position and % littoral resource use for lake trout and white sucker. The fit of each point is made using neighboring points using parameter α set to 50% of the neighbouring data (19 points) in this case, and a tricubic local weighting function was used to give points closer to the focal point higher weight. An α setting of 50% of the neighbouring data is appropriate because each point already represents an annual average and deserves more weight than that allotted with using the default setting of 75%.

While the trophic level concept has been criticized as being limited by the use of discrete levels that are unable to capture complex interactions and trophic omnivory that are prevalent in many ecosystems (Paine 1988, Polis and Strong 1996, Vander Zanden and Rasmussen 1999), the trophic *position* concept does allow for the conceptualization of energy movement through all different pathways leading to an organism (Post 2002). Stable isotopes have the potential to capture both complex trophic interactions and to track energy through ecological communities (Peterson and Fry 1987, Cabana and Rasmussen 1996, Post 2002). To determine the proportion of carbon derived from the littoral environment (α), I used the equation:

$$\alpha = (\delta^{13}C_{fish} - \delta^{13}C_{zooplankton}) / (\delta^{13}C_{crayfish} - \delta^{13}C_{zooplankton})$$

Where α is the proportion of nitrogen in the consumer ultimately derived from the littoral environment. The $\delta^{13}C$ signature of the consumer fish species is designated as $\delta^{13}C_{fish}$, $\delta^{13}C_{zooplankton}$ is the $\delta^{13}C$ signature representing the pelagic environment, and $\delta^{13}C_{crayfish}$ is the $\delta^{13}C$ signature representing the littoral environment. Therefore, differences in the α value between time periods would suggest differences in the reliance on littoral resources.

Trophic position (TP) was calculated for lake trout and white sucker for both Lake 223 and Lake 224 using equations from Post (2002), for a two-end-member-mixing model that allows for the differentiation between two sources of nitrogen using the equation:

$$\text{Trophic Position(TP)} = \lambda + (\delta^{15}N_{fish} - [\delta^{15}N_{crayfish} \times \alpha + \delta^{15}N_{zooplankton} \times (1 - \alpha)])/\Delta_n$$

Where λ is the trophic position of the organisms used for the baselines from the two sources. I used a value of 2 for λ (i.e. secondary consumers) for both baselines. Trophic enrichment (Δ_n) in $\delta^{15}N$ was assumed to be 3.4‰, and 0‰ for $\delta^{13}C$ (Post 2002).

A two-factor ANOVA with time-period and lake effects was conducted, with estimated TP or % littoral resource use for individual lake trout as the response variable using a Type III sum of squares for an unbalanced design. The groups for the time period factor are "Pre-acidification" describing the time period prior to *Mysis* extirpation (1974-1977), and "post recovery" (2009-2017) and the groups for the lake factor was Lake 223 and Lake 224. Lake trout sample sizes within each lake and time period group are shown in Table 2.1 and white sucker sample sizes are shown in Table 2.2. Data from 1978 was included in Lake 224 to increase sample size. Since Lake 224 was unmanipulated, the decision to include data from this year is unlikely to have any systematic bias on the results. Sample sizes for lake trout and white sucker SIA are 5-10 individuals/year in most years. Transformations were initially unable to resolve heterogenous group variance and normality of residuals due to the large variation in the 1978 Lake 223 TP values. 1978 data were removed from the Lake 223 pre-acidification time period since Lake 223 had begun to be affected by the acidification (Schindler et al. 1985), and uneven sample sizes in the Lake 223 pre-acidification time period were violating assumptions of the ANOVA. One observation was excluded from the model that had an impossible α value that was greater than 1, as a fish cannot be >100% reliant on one habitat. I squared the TP values, after which the assumption of homogenous group variance (Levene's test, F_3 = 1.16, p-value = 0.33), and normality of residuals (Shapiro-Wilks test, W=0.98, p-value=0.13) were both satisfied.

Body condition of lake trout and white sucker from Lake 223 were estimated by calculating relative weights, expressed as a percentage of the observed weight divided by the standard weight for each species. Formulas for standard weight estimates came from

Piccolo et al. (1993) and Bister et al. (2000) for lake trout and white sucker, respectively. No lake trout with a total length less than 280mm were included in condition estimates (Piccolo et al. 1993). Lake trout and white sucker collected prior to 1979 were grouped as pre-*Mysis* extirpation, and fish from 2011-2017 represent the ecosystem post-*Mysis* extirpation. Welch's two sample *t*-tests were used to determine differences between time periods. Lake trout relative weight data were normally distributed. Two suckers with exceptionally large relative weights (164% and 173%) were excluded from the post-*Mysis* extirpation time period as outliers. After the two white sucker outliers were removed and all data were log transformed, white sucker relative weight data were normally distributed. Growth rates of the most common age classes of lake trout within Lake 223 and Lake 224 were analyzed using two-factor ANOVAs separately for each lake. Age and time period were used as factors in the ANOVAs, with age being the 3 most common ages in Lake 223 (5-7) and Lake 224 (7-11, Fig. A2.1), and time periods being defined as 1975-1978 representing the time period prior to *Mysis* extirpation, and 2011-2017 representing the time period after chemical recovery and *Mysis* extirpation.

Fork length-at-age was used as a proxy for lake trout growth in Lake 223. A frequency distribution of ages revealed that lake trout ages 5-7 were most common in the dataset and were used to best represent growth over time. Growth rates were evaluated by comparing length-at-age of the three most common aged lake trout in Lake 223 using an ANOVA with Type III Sums of Squares, with time period and lake as factors. The pre-extirpation time period for growth rates were defined as years 1975-1978, and 2011-2017 represented the post-recovered time period.

Niche space using ^{15}N and ^{13}C isotopes was visualized for Lake 223 and Lake 224 ecosystem constituents using the SIBER (Stable Isotope Baysian Elipses in R) package in R. The SIBER package fits bi-variate ellipses to stable isotope data using Bayesian inference with the aim being to describe and compare their isotopic niche (Jackson et al. 2011). Ecosystem constituents for this analysis included crayfish (*Orconectes virilis*), fathead minnows (*Pimephales promelas*), pearl dace (*Semotilus margarita*), finescale dace (*Chrosomus neogaeus*), slimy sculpin (*Cottus cognatus*), *Chaoborus spp.*, and *Mysis*. All samples were frozen upon capture and then dried in an oven at 60 ºC before being homogenized with a mortar and pestle. Samples were taken from as many years as available in archived frozen samples to best represent the pre-acidification, acidification, and recovered phases of Lake 223. The Lake 223 pre-acidification phase is defined as years earlier than 1978, since 1978 is when the effects of the acid additions began to affect species abundances in the lake (Schindler et al. 1985). The acidification phase is defined here as 1978-1996, and the recovered phase is defined as 2011-2017.

RESULTS

Lake 223 has been at pre-acidification pH levels for ~20 years (Fig. 2.2), currently similar to the pH of Lake 224. During the acidification and recovery years of Lake 223, the δ^{13}C signatures of lake trout, white sucker, zooplankton, and crayfish were highly variable (Fig. 2.3A). All δ^{13}C values for consumers and both the pelagic (zooplankton) and littoral (crayfish) baselines converged during the years following peak acidification (~1985-1990). Meanwhile, the δ^{13}C and δ^{15}N signatures of all species in

Lake 224 (Fig. 2.3B and Fig. 2.4B) were much less variable over the four-decade time series, as would be expected from an unmanipulated reference lake.

For lake trout, there was a significant interaction term for lake trout % littoral-derived energy between time period and lake (Fig. 2.5; $F_{1,85} = 7.7$, p-value = 0.007), effect of time period ($F_{1,85} = 38.6$, p-value = <0.001), and lake ($F_{1,85} = 8.7$, p-value = 0.004). Littoral reliance declined in Lake 223 between time periods, whereas lake trout in Lake 224 remained equally reliant on carbon from the littoral environment between time periods. Assumptions of homogenous variance between groups (Levene's test, $F_{3,85} = 1.89$, p-value= 0.14) and normality of residuals (Shapiro-Wilks test, W=0.98, p-value=0.27) were met.

Similarly, there was a significant interaction between the time period and lake ($F_{1,85}$= 4.3, p-value = 0.04, Fig. 2.6) on the TP of lake trout between lakes and time periods. The time period main effect was significant ($F_{1,85} = 19.4$, p-value = <0.0001), while lake had a non-significant effect on TP ($F_{1,85} = 0.3$, p-value = 0.59). The trophic position of lake trout increased in Lake 223 between the two time periods but remained fairly unchanged in Lake 224.

There was no significant interaction between time period and lake for % littoral reliance of individual white suckers ($F_{1,95} = 0.055$, p-value = 0.82) and % littoral reliance by did not differ between white sucker from either lake ($F_{1,95} = 1.2$, p-value = 0.27) or across time periods ($F_{1,95} = 1.4$, p-value = 0.24, Fig. 2.7). Assumptions of homogenous variance between groups (Levene's test, $F_{3,95} = 2.00$, p-value= 0.12) and normality of residuals (Shapiro-Wilks test, W=0.98, p-value=0.11) were met. There was a significant interaction between time period and lake for white sucker TP ($F_{1,95} = 34.7$, p-value =

<0.001), and both main effects of time period ($F_{1,95}$ = 30.2, *p*-value = <0.001), and Lake ($F_{1,95}$ =15.1, *p*-value = 0.0002) were all highly significant (Fig. 2.8). The trophic position of white sucker increased by the same magnitude as did lake trout in Lake 223 (~0.25 TP), and declined in reference Lake 224 by the same magnitude. The assumption of homogenous variance between groups was violated (Levene's test, $F_{3,95}$ = 2.80, *p*-value= 0.044), however the assumption was nearly met and I decided to proceed with the analysis. The assumption of normality of residuals (Shapiro-Wilks test, *W*=0.99, *p*-value=0.33) was met.

Though lake trout are a species with an omnivorous diet, inferences regarding diet can also be made based on available SIA data. The mean Lake 223 lake trout δ^{15}N values prior to *Mysis* extirpation before 1978 (Fig. 2.9 A) were 9.2‰ (± 0.14 SE), 9.8‰ (± 0.07) between 1978-1996 (Fig. 2.9 B), and 8.7‰ (± 0.16) since the year 2011 (Fig. 2.9 C). Contemporary lake trout populations in both Lake 223 and Lake 224 appear to be equally reliant on the littoral environments. Currently, *Chaoborus* are the species most depleted in δ^{13}C in Lake 223 (-35.9 ± 0.59‰), while crayfish are the most enriched (-25.0 ± 0.57‰). Current isotope signatures from the Lake 224 food web (Fig.2.10) indicates that the average lake trout δ^{15}N signatures are 9.3 (± 0.12‰) suggesting that the population is feeding on species with δ^{15}N values approximately similar to those of small *Mysis* (5.4 ± 0.22‰), large *Mysis* (6.3 ± 0.30‰), zooplankton (5.9 ± 0.30‰), and crayfish (4.6 ± 0.17‰), assuming a trophic fractionation of 3.4‰ δ^{15}N.

Body condition of both lake trout and white sucker from Lake 223 were not statistically different between time periods (lake trout; t_{70} = 0.18, *p*-value = 0.86, white sucker; t_{57} = -0.61, *p*-value = 0.55). Changes in growth rates of lake trout were analyzed using the most common ages of lake trout in each lake, with fish aged 5-7 being most common in Lake 223 and fish aged 7-11 most common in Lake 224 (Fig. A2.1). There was a non-significant interaction between time period and age for the lake trout growth rates ($F_{2,166} = 0.34$, p-value = 0.71), and both effects of age ($F_{2,166} = 3.6$, p-value = 0.03), and time period ($F_{1,166} = 9.7$, p-value = 0.002) were significant. Lake trout in Lake 223 are now growing slower than they were when *Mysis* were present. Between the three most common ages of lake trout in Lake 223 (5-7), fish are now 22.7mm shorter at-age (5.6%) on average, relative to before *Mysis* were extirpated from the lake. Over the same time period in reference Lake 224, an ANOVA with the same variables, using the five most common ages (7-11), and with the same time period definitions as in Lake 223, found a significant interaction effect ($F_{4,78} = 3.1$, p-value = 0.02), with non-significant main effects of age ($F_{4,78} = 1.3$, p-value = 0.29) or time period ($F_{1,78} = 0.3$, p-value = 0.58). Lake trout aged 8 and 9 in Lake 224 are larger (11.9%) in the post-recovery time period, while lake trout aged 7, 10, and 11 have remained the same length-at-age between time periods.

DISCUSSION

Contrary to my expectations, the extirpation of *Mysis* from Lake 223 resulted in lake trout deriving ~20% more energy from pelagic sources following the extirpation of *Mysis*. The loss of *Mysis* as an integrator of pelagic and littoral energy appears to be reflected in a reduction of littoral energy being transferred to lake trout as a result of the decoupling of these two environments. A possible prey alternative for lake trout and white sucker in the absence of *Mysis*, is *Chaoborus*. The $\delta^{15}N$ and $\delta^{13}C$ signatures of available ecosystem constituents suggest that *Chaoborus* has been incorporated into the diet of both lake trout and white sucker, explaining the observed changes to the trophic position in both species and % littoral reliance in lake trout, as well as the decreased growth rate of lake trout following *Mysis* extirpation.

Though both have known feeding preferences, the ability of lake trout and white sucker to demonstrate facultative diets in the face of changing environments is well documented (Martin 1952, Ahlgren 1996, France and Steedman 1996) and may have allowed them to adjust reliance to different energetic pathways following the extirpation of *Mysis* from Lake 223. It seems likely that the extirpation of an organism that integrates energy between benthic and pelagic environments would impact the proportion of littoral carbon derived by lake trout. White sucker, like lake trout, are also omnivore, feeding in the littoral zone of lakes on zoobenthos or zooplankton (Saint-Jacques et al. 2000). Among the zooplankton that white sucker are reported to consume (*Holopedium*, *Daphnia*, and *Chaoborus*), white sucker are able to specialize on the largest available prey (Saint-Jacques et al. 2000). *Chaoborus* are now the dominant meso-zooplankton predator (Chapter 1) where size-selective foraging by white sucker should select preferentially for *Chaoborus*. The observed increase in white sucker trophic position following the extirpation of *Mysis* suggests potential increased inclusion of *Chaoborus* in white sucker diets as this species has become more abundant in the lake.

The $\delta^{15}N$ signature of the Lake 223 zooplankton baseline appears to be lower in contemporary estimates following chemical recovery relative to the time period before *Mysis* extirpation, which may be in part explained by the relative decline in calanoid copepods and driving some of the changes in TP observed in fish populations. Calanoids have a higher δ^{15}N signature than *Daphnia spp*. and *Holopedium* (Matthews and Mazumder 2003), both of which have increased in biomass following the extirpation of *Mysis* (Chapter 1). However, this change in species composition does not explain the apparently depleted δ^{13}C signatures of the zooplankton baseline following chemical recovery relative to the pre-acidification signatures; nor is it driven by a sampling bias in the earliest (1974) zooplankton sample used to create a baseline. The δ^{13}C of zooplankton are known to generally peak in mid-summer before becoming more depleted in the fall months (Syväranta et al. 2006), while the 1974 zooplankton composite was composed of samples from August, September and November, potentially underestimating the annual δ^{13}C signatures of the zooplankton community. Therefore, the difference in zooplankton δ^{13}C baseline signatures between time periods is potentially slightly greater than described here, but it is unlikely that any underestimation is greatly impacting the mixing model results as this potential difference is small and that baseline estimates were derived from predicted values from LOESS fits, reducing the influence of the results from any single year on the overall baseline patterns.

In lakes where *Mysis* and pelagic forage fish are absent (Class 1 lakes, as defined by Rasmussen et al. (1990)), adult lake trout are known to feed predominantly on zooplankton and benthic organisms (Martin 1954, 1966, Cabana and Rasmussen 1994). *Chaoborus* are the largest zooplankton species found in the pelagic habitat of Lake 223, and a likely prey item for fish feeding on zooplankton. In previously-acidified lakes in the Sudbury area, yellow perch (*Perca flavescens*) trophic position and % pelagic energy were found to be positively correlated with increasing *Chaoborus* density and diet, respectively (Luek et al. 2013). Although perch are a littoral cool water species, differing from the preferred lake trout habitat of cold, well oxygenated water (Sellers et al. 1998), both species appear to respond similarly to the incorporation of *Chaoborus* in diets following acidification recovery. In another study where major changes in *Mysis* densities were observed, Kennedy et al. (2019) found that lake trout decreased in trophic position and increased littoral reliance after the collapse of *Mysis* populations following an experimental aquaculture operation in Lake 375, opposite to the results of the current study. Instead of switching to zooplankton (*Chaoborus*) or reflecting a reduction in benthic-pelagic coupling in the absence of *Mysis* as lake trout did in Lake 223, Lake 375 lake trout increased reliance on benthic littoral resources following the decline of *Mysis*. A diet of *Chaoborus* is likely the least physiologically stressful choice for lake trout as opposed to a diet of littoral invertebrates or minnows as there is more thermal overlap between vertically migrating *Chaoborus* and lake trout, than for littoral invertebrates/minnows.

Despite the fact that lake trout in lakes without *Mysis* or pelagic forage fish are known to predominantly feed on zooplankton and benthic invertebrates (Martin 1954, 1966), benthic invertebrates are likely not a predominant food item of lake trout in Lake 223. Other isotopic data from Lake 223 shows that more than 85% of littoral benthic invertebrates collected from Lake 223 were chironomids, which are too enriched in ^{13}C (-26.36 ± 4.08) and depleted in ^{15}N (2.39 ± 1.11) to explain the observed effects to the lake trout community (Tonin 2019). Following an increase in chironomid consumption, perch have shown both a decrease in trophic position and an increase in littoral reliance (Luek et al. 2013), opposite of what was observed to occur in Lake 223. Additionally, bulk zooplankton are too depleted in ^{15}N to have been incorporated in relatively higher proportions to the diet of lake trout and white sucker in Lake 223. Although minnow species make up a small portion of the diet of lake trout during the summer months (Guzzo et al. 2017), the isotopic signatures observed here do not support significant predation of littoral minnows in Lake 223. An increased reliance on *Chaoborus* appears to best explain observed effects to the trophic position and % littoral resource use by lake trout and white sucker, as well as reduced littoral/pelagic coupling in Lake 223 associated with the loss of *Mysis*.

Assuming the current (>2011) isotopic signatures of *Mysis* from Lake 224 (an unmanipulated reference lake) provide a reasonable representation of the historical isotopic signatures of *Mysis* from Lake 223, then *Chaoborus* that are currently in Lake 223 are more elevated in $\delta^{15}N$ than *Mysis* would have been historically. To make comparisons of species between lakes, each species needs to be baseline corrected to a common species. Currently, large *Mysis* in Lake 224 are only 0.44 $\Delta^{15}N$ above the zooplankton baseline on average, while Lake 223 *Chaoborus* are 3.0 $\Delta^{15}N$ above their respective zooplankton baseline on average. Also, large *Mysis* in Lake 224 are 1.7 $\Delta^{13}C$ more enriched that the zooplankton baseline on average, while the *Chaoborus* in Lake 223 are 0.9 $\Delta^{13}C$ more depleted than the zooplankton baseline. This suggests that Lake 223 *Chaoborus* signatures are enriched in ^{15}N and depleted in ^{13}C, relative to the historical Lake 223 *Mysis* signatures. As trophic position is a measure of ^{15}N enrichment relative to baselines, and % littoral reliance is a measure of ^{13}C enrichment relative to baselines, a diet switch from *Mysis* to *Chaoborus* would explain the observed increased trophic position of lake trout and white sucker and increased pelagic reliance of lake trout populations in Lake 223. These isotopic differences in *Chaoborus* and *Mysis* in Lake 223 and Lake 224 relative to their baselines differ from patterns reported from central Ontario lakes where *Chaoborus* and *Mysis* more readily coexist; there, *Chaoborus* tend to be slightly more enriched in ^{13}C relative to *Mysis* and are equally as enriched in ^{15}N as large *Mysis* (Foster and Sprules 2010). In Lake 223 however, *Chaoborus* live in the absence of *Mysis* and are likely able to express their full fundamental niche (i.e., without competition from *Mysis*), which appears to have greater pelagic energy reliance while feeding on more ^{15}N enriched prey.

The decrease in lake trout littoral/pelagic coupling following the extirpation of *Mysis* from Lake 223 could be responsible for the slower lake trout growth, as the likely prey alternative (*Chaoborus*) are much less available to predation by lake trout relative to *Mysis*. During summer stratification, the spatial overlap between lake trout and *Chaoborus* is very brief, as *Chaoborus* rise and fall in the water column during DVMs and enter the epilimnion to feed at night (Sellers et al. 1998, Weisser et al. 2018). By comparison, *Mysis* are always found in habitats susceptible to lake trout predation due to similar temperature and oxygen requirements (Sellers 1995, Boscarino et al. 2007). In addition, *Chaoborus* are highly tolerant of low oxygen conditions in the hypolimnion (Scholz and Zerbst-Boroffka 1998) and can seek refuge in low-oxygen regions which are inaccessible to lake trout which have higher oxygen requirements. Lakes at ELA typically have low oxygen regions in the hypolimnion (Guzzo and Blanchfield 2017, Rennie et al. 2019) that could provide refuge for *Chaoborus* during summer months when energy acquisition for fish is typically greatest (ref). Though both *Mysis* and *Chaoborus* spend daylight hours against the sediment and vertically migrate in the water column at night, *Chaoborus* typically migrate into the epilimnion (Teraguchi and Teraguchi 1966), whereas *Mysis* are restricted to the hypolimnion (Nero 1981, Beeton and Bowers 1982). Therefore, though lake trout can prey on *Chaoborus*, reduced spatial overlap and lower densities of *Chaoborus* compared to *Mysis* reduce foraging opportunities on this species.

As Lake 223 had been chemically recovered for more than a decade by 2011, it is unlikely that lake trout growth is still affected by density-dependent factors due to low abundance; rather, lake trout abundance appears to have reached a new equilibrium following the extirpation of *Mysis* at an abundance and associated growth rate well below pre-acidification levels. During the peak acidification years, the lake trout population ceased reproduction, resulting in a large decline in the abundance and biomass (Chapter 1). The standing biomass of the lake trout population during the 1990's was at, or near its lowest throughout its history (Fig. 1.5A). Meanwhile, there was strong and increasing reproduction (Fig. 1.6A). While Mills et al. (2002a) found no evidence of density-dependent growth in the Lake 223 lake trout population during chemical recovery of the lake, they only examined lake trout growth rates until 1993, before the large increase in recruitment in the lake, and also before prey species such as crayfish and sculpin retuned to the lake in normal densities (Colvin and Rennie in prep). Declines in lake trout growth rates since that time along with corresponding increases in population abundance suggest a role for density-dependent growth.

Through this unique opportunity to compare the resource use of lake trout and white sucker in two different time periods where the only difference was the presence/absence of *Mysis*, this analysis has provided a study of the effects experimental removal of a species from a multi-trophic level ecosystem. My hypothesis that the loss of

Mysis from Lake 223 would force lake trout and white sucker to rely more heavily on the littoral environment was rejected. While I had predicted that the extirpation of *Mysis* would result in greater energy coupling between the littoral and pelagic environment as the pelagic lake trout increased littoral reliance, the opposite pattern was found, likely due to the loss of *Mysis* which is an integrator of benthic and pelagic energy pathways, and a potential increased reliance on *Chaoborus*. The lower biomass of *Chaoborus* relative to *Mysis*, and reduced spatial overlap between *Chaoborus* and lake trout compared to that between *Mysis* and lake trout is consistent with the observed reduction in lake trout growth rates. As such, the extirpation of *Mysis* in Lake 223 appears to have had dramatic impacts to the flow of energy from lower to higher trophic levels, impacting apex fish populations.

Currently, there are *Mysis* control measures in western US states such as Idaho and Colorado to restore native ecosystems where *Mysis* have been introduced (Martinez and Bergersen 1989, Caldwell and Wilhelm 2012). In Flathead Lake, Montana, previously introduced lake trout abundance has dramatically increased following the introduction of *Mysis* (Ellis et al. 2011). Consistent with these findings, the current study demonstrates that in the absence of *Mysis*, lake trout have likely switched to a diet of *Chaoborus* resulting in the population growing slower. Based on my findings presented here, if *Mysis* were to be successfully removed or reduced from introduced water bodies either through harvesting or biological control, a reduction in the growth rate of lake trout in Flathead Lake would be predicted.

TABLES AND FIGURES

Table 2.1. Lake trout sample sizes for two-end-member-mixing model.

Lake	**Time-period**	**Sample size**
223	Pre-acidification	41
223	Post-recovery	20
224	Pre-acidification	10
224	Post-recovery	18

Table 2.2. White Sucker sample sizes for two-end-member-mixing model.

Lake	**Time-period**	**Sample size**
223	Pre-acidification	24
223	Post-recovery	34
224	Pre-acidification	11
224	Post-recovery	30

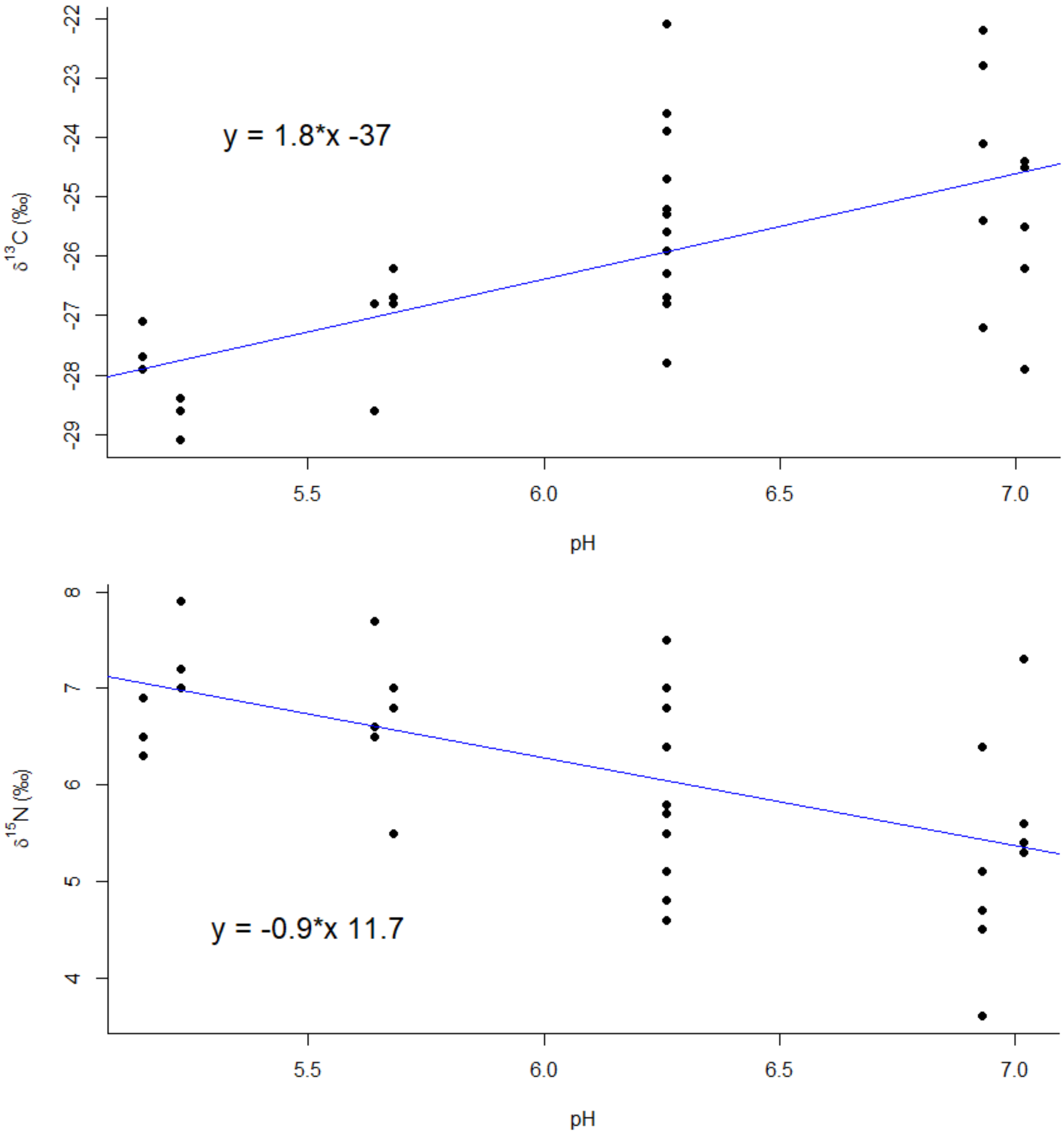

Figure 2.1. Regression analysis of Lake 223 crayfish $\delta^{13}C$ signatures (top) and $\delta^{15}N$ signatures (bottom) estimated from epilimnetic pH values between 1977-2018.

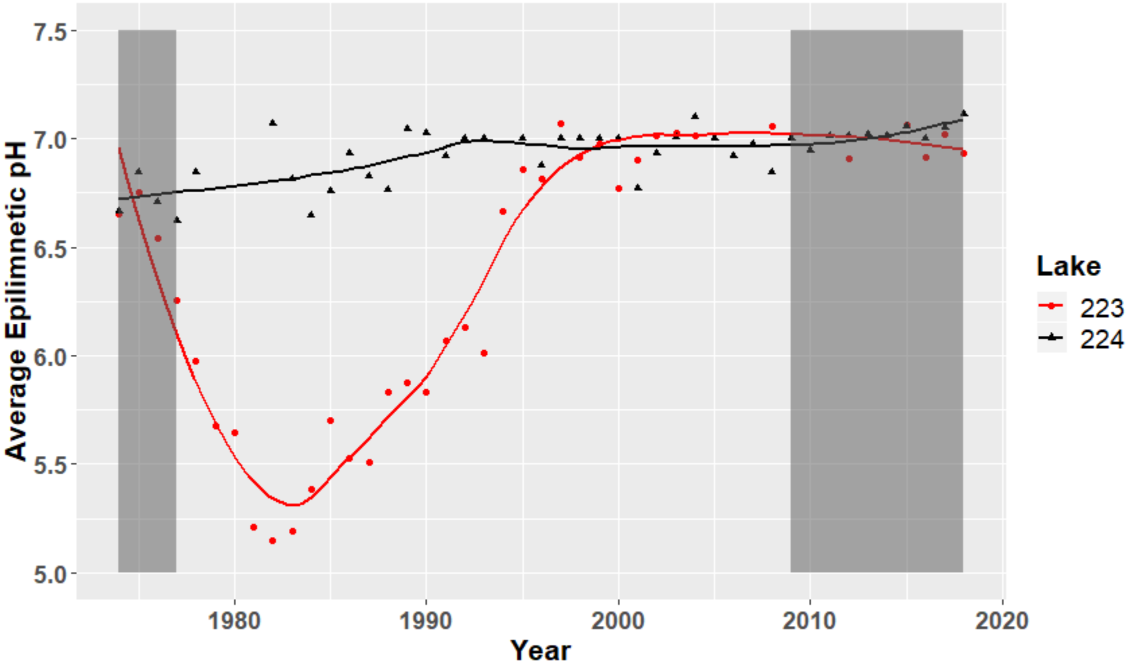

Figure 2.2. pH of Lake 223 and 224. Grey bars indicate the time periods used in mixing model ANOVAs (1974-1977, and 2009-2017). Lines are LOESS local regression fits showing the locally weighted average, using a tricubic weighting function using 50% of the neighbouring data.

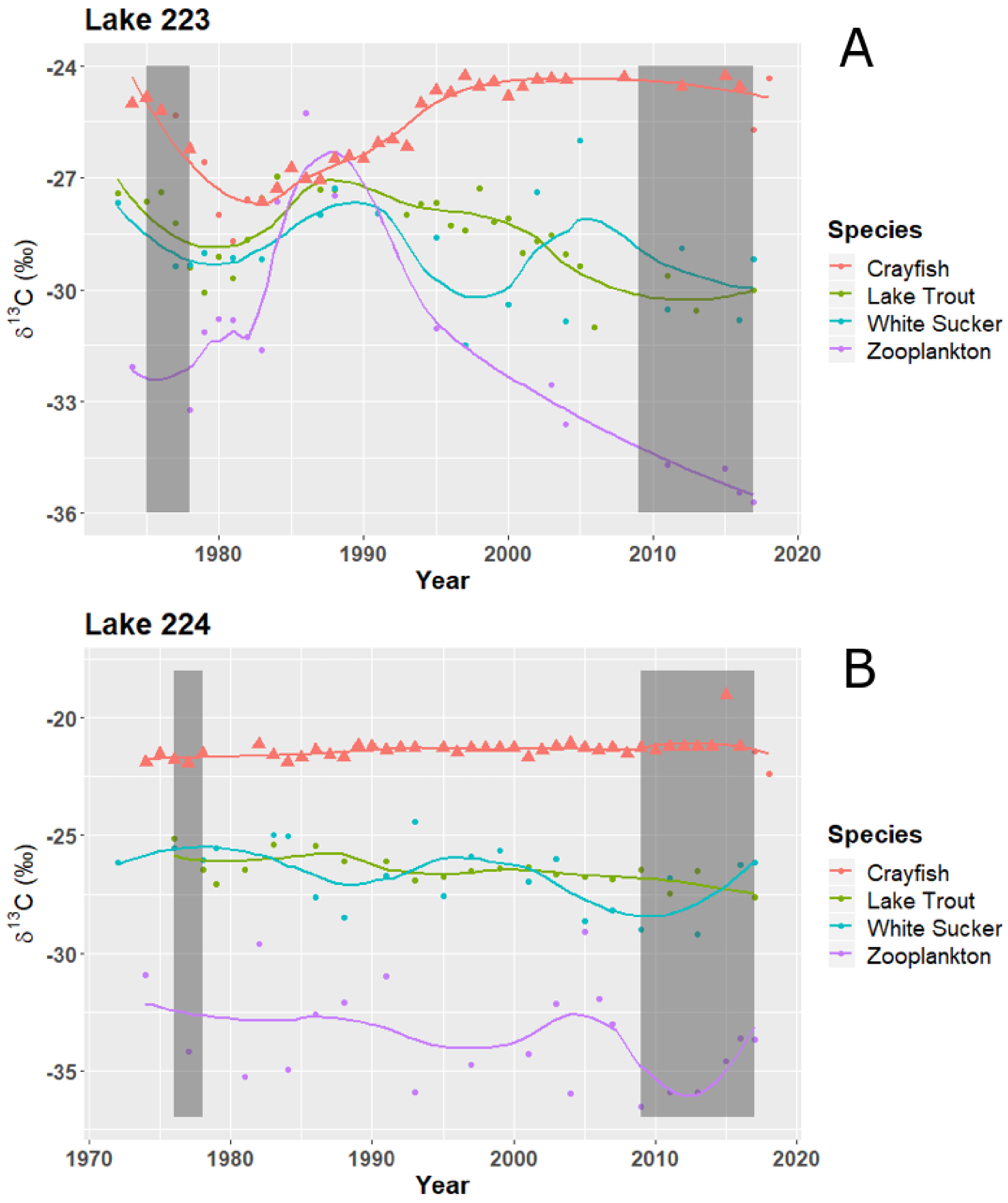

Figure 2.3. Average annual δ13C signatures from Lake 223 (A) and 224 (B), with local weighted regression lines. Grey bars indicate the time periods used in mixing model ANOVAs. Crayfish isotope signatures in years with unavailable data were estimated from lake pH based on the equations δ13C = 2.7*pH -42.3 and δ15N = -0.9*pH + 11.4 (see text). Triangles represent estimated crayfish baseline signatures. Lines are LOESS local regression fits showing the locally weighted average, using a tricubic weighting function using 50% of the neighbouring data.

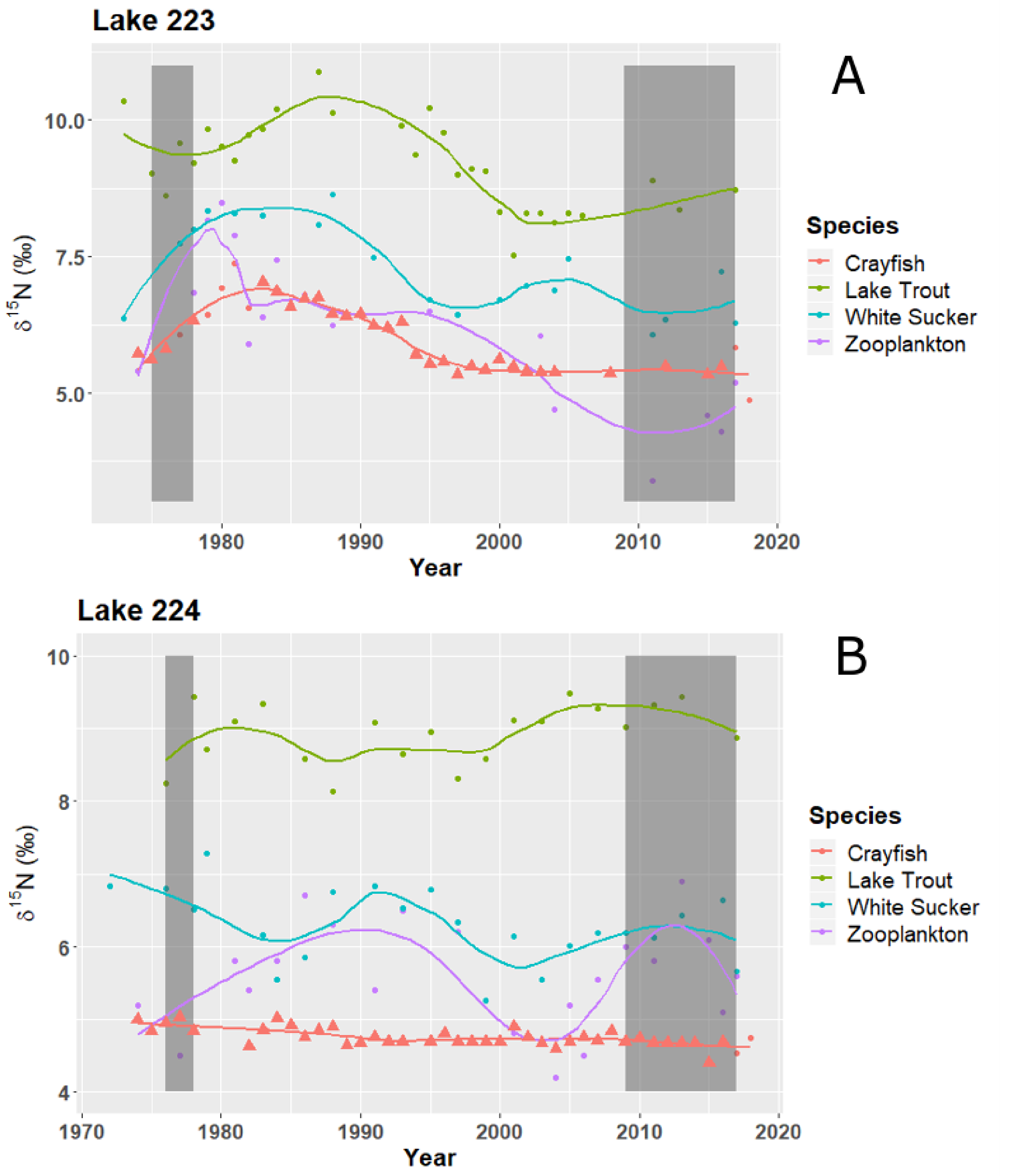

Figure 2.4. Average annual δ^{15}N signatures from Lake 223 (A) and 224 (B), with local weighted regression lines. Grey bars indicate the time periods used in mixing model ANOVAs. Crayfish isotope signatures are estimated from lake pH based on the equations $\delta^{13}C = 2.7*pH -42.3$ and $\delta^{15}N = -0.9*pH + 11.4$ (see text). Triangles represent estimated crayfish baseline signatures. Lines are LOESS local regression fits showing the locally weighted average, using a tricubic weighting function using 50% of the neighbouring data.

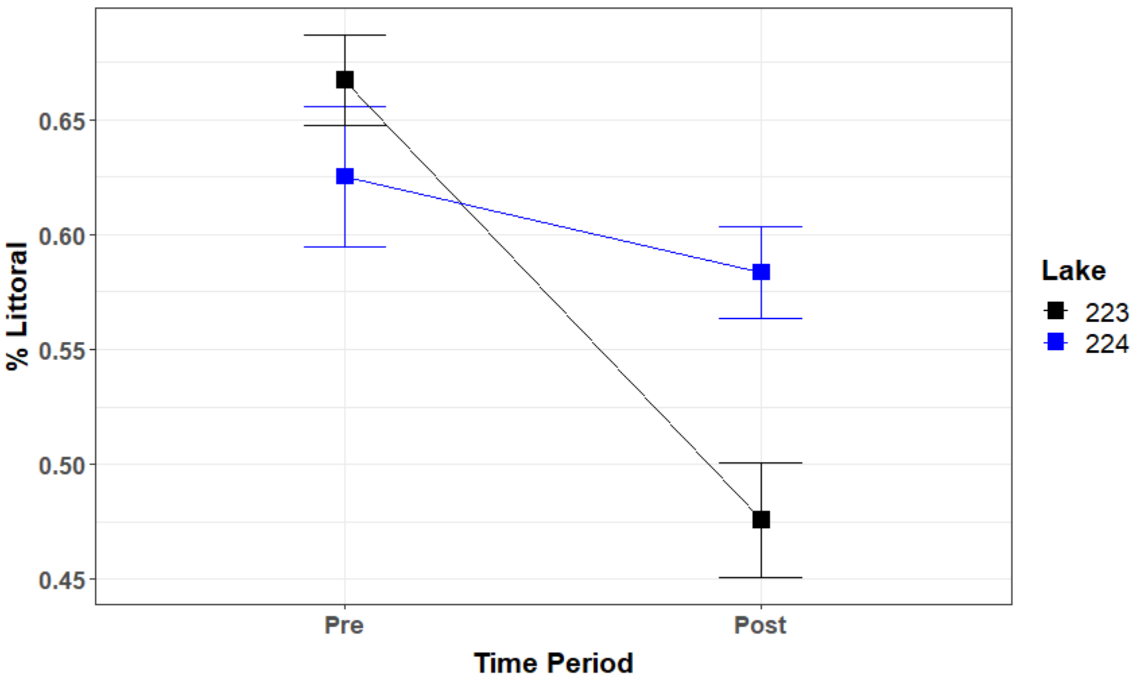

Figure 2.5. Lake trout % littoral from the two-source-mixing-model using individual fish within each time period. Bars represent standard error around the means. The time period “Pre” refers to pre-acidification (1974-1977) and “Post” refers to post chemical recovery (2009-2017).

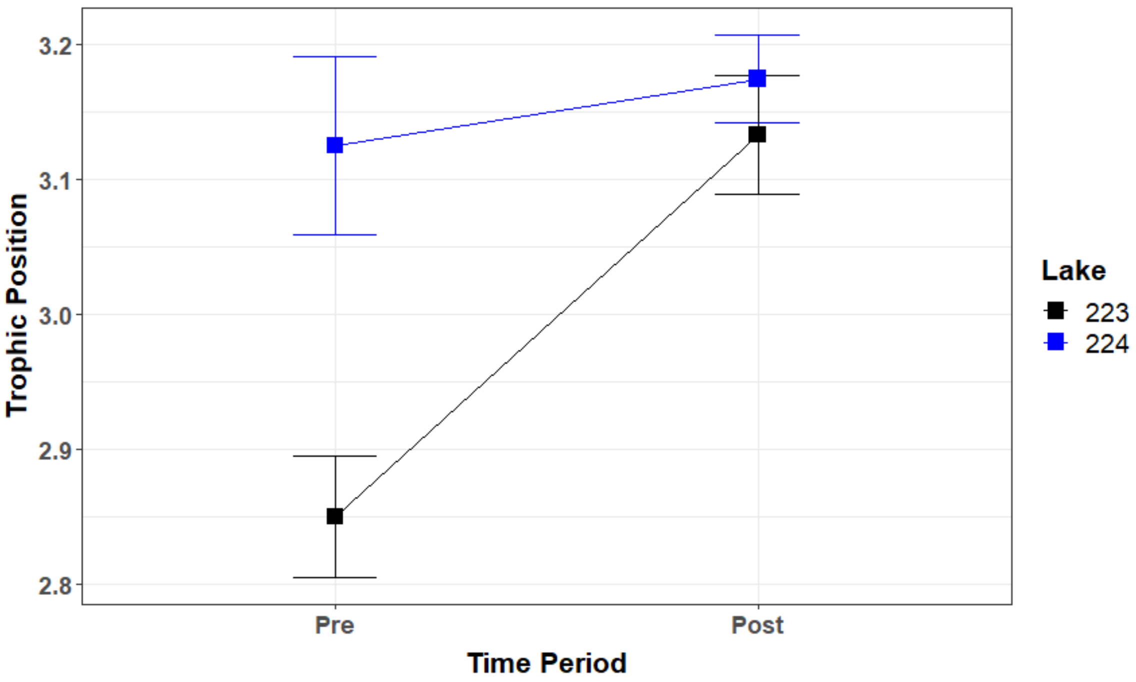

Figure 2.6. Lake trout trophic position from the two-source-mixing-model using individual fish within each time period. Bars represent standard error around the means. The time period “Pre” refers to pre-acidification (1974-1977) and “Post” refers to post chemical recovery (2009-2017). Plot is of non-transformed data, yet data was squared to satisfy assumptions of ANOVA.

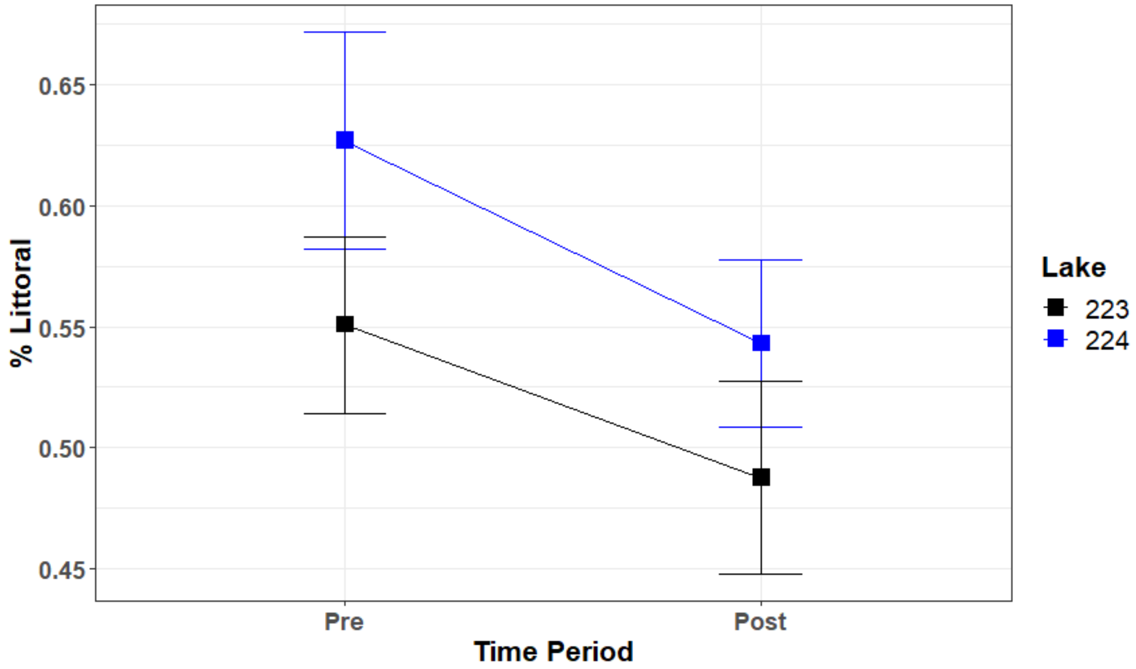

Figure 2.7. White sucker % littoral from the two-source-mixing-model using individual fish within each time period. Bars represent standard error around the means. The time period “Pre” refers to pre-acidification (1974-1977) and “Post” refers to post chemical recovery (2009-2017).

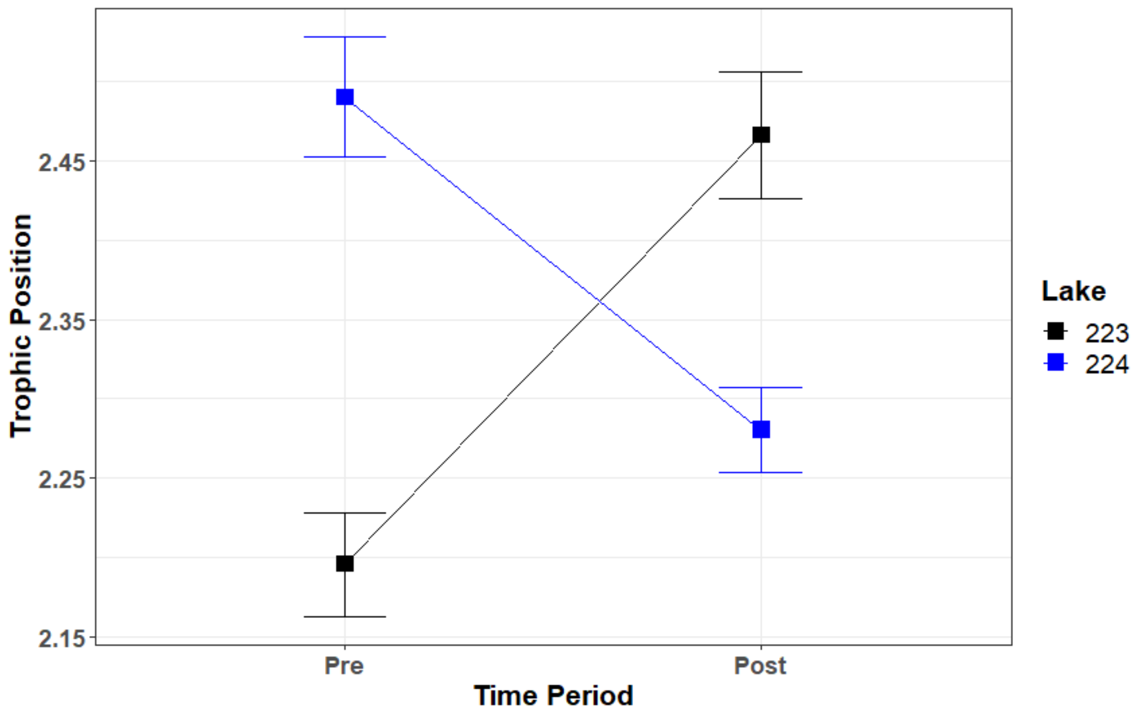

Figure 2.8. White sucker trophic position from the two-source-mixing-model using individual fish within each time period. Bars represent standard error around the means. The time period “Pre” refers to pre-acidification (1974-1977) and “Post” refers to post chemical recovery (2009-2017). Plot is of non-transformed data, yet data was squared to satisfy assumptions of ANOVA.

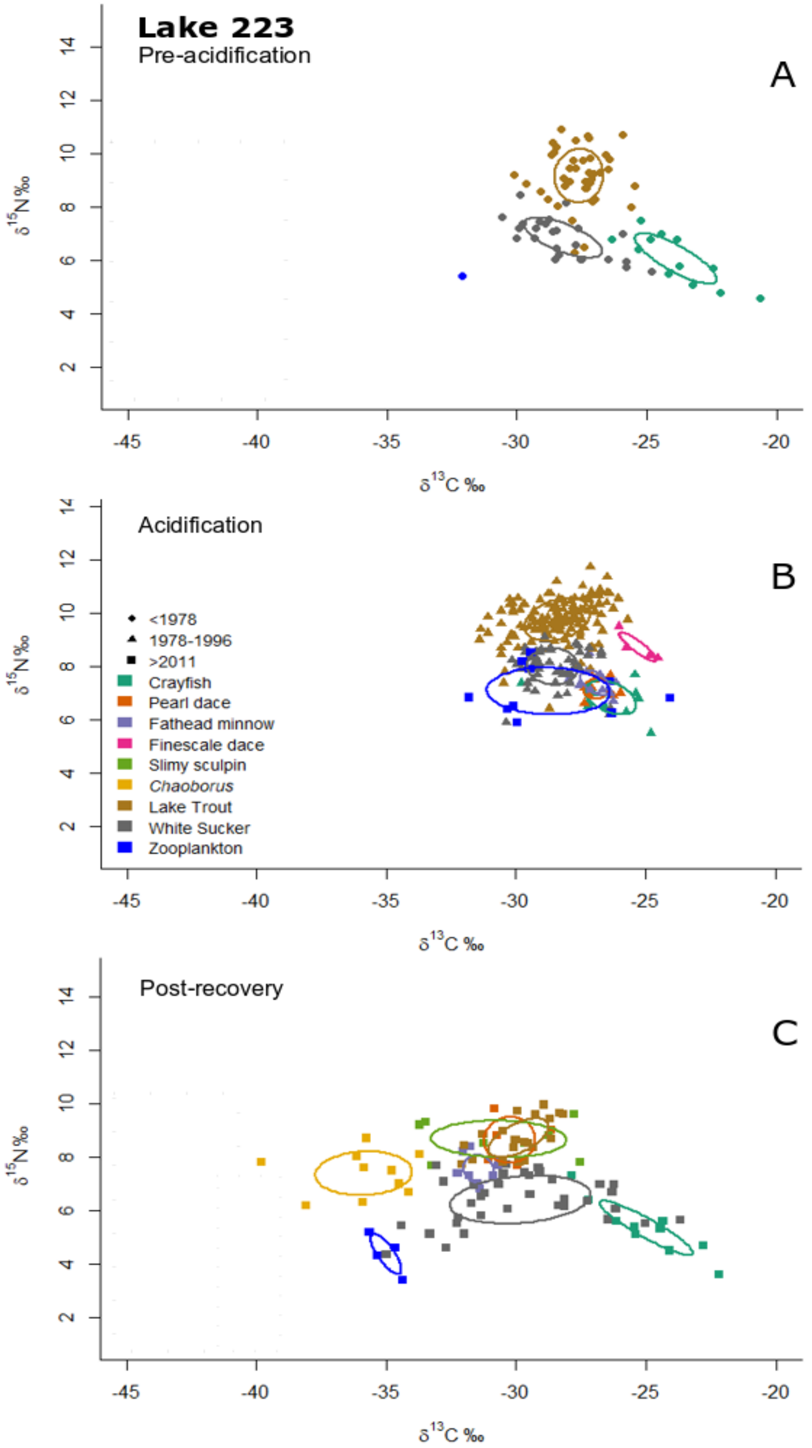

Figure 2.9. Lake 223 $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures of lake trout and other ecosystem constituents within the three time periods of the acidification experiment. Pre acidification (<1978, A), acidification (1978-1996, B), and post recovery (>2011, C) time periods have all available species included within each time period. Ellipses are maximum likelihood standard ellipses, containing roughly 40% of the data.

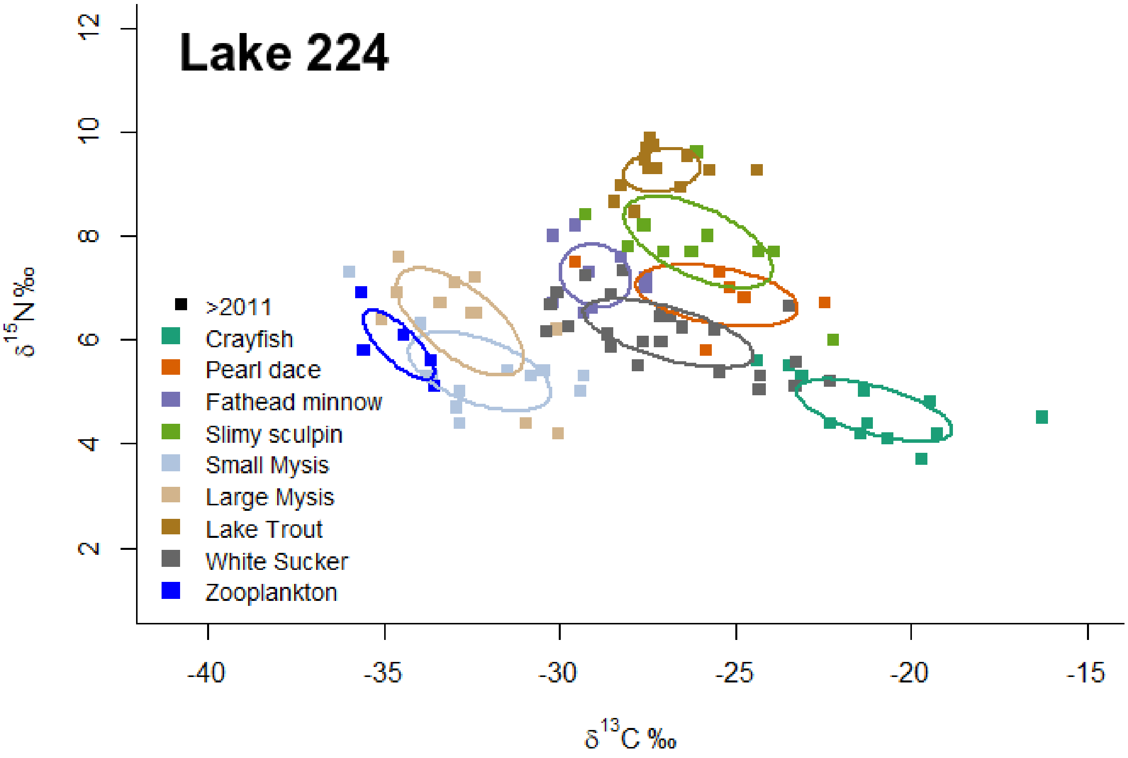

Figure 2.10. Lake 224 δ13C and δ15N isotopic signatures of lake trout and other ecosystem constituents since 2011. All available species are included. Ellipses are maximum likelihood standard ellipses, containing roughly 40% of the data.

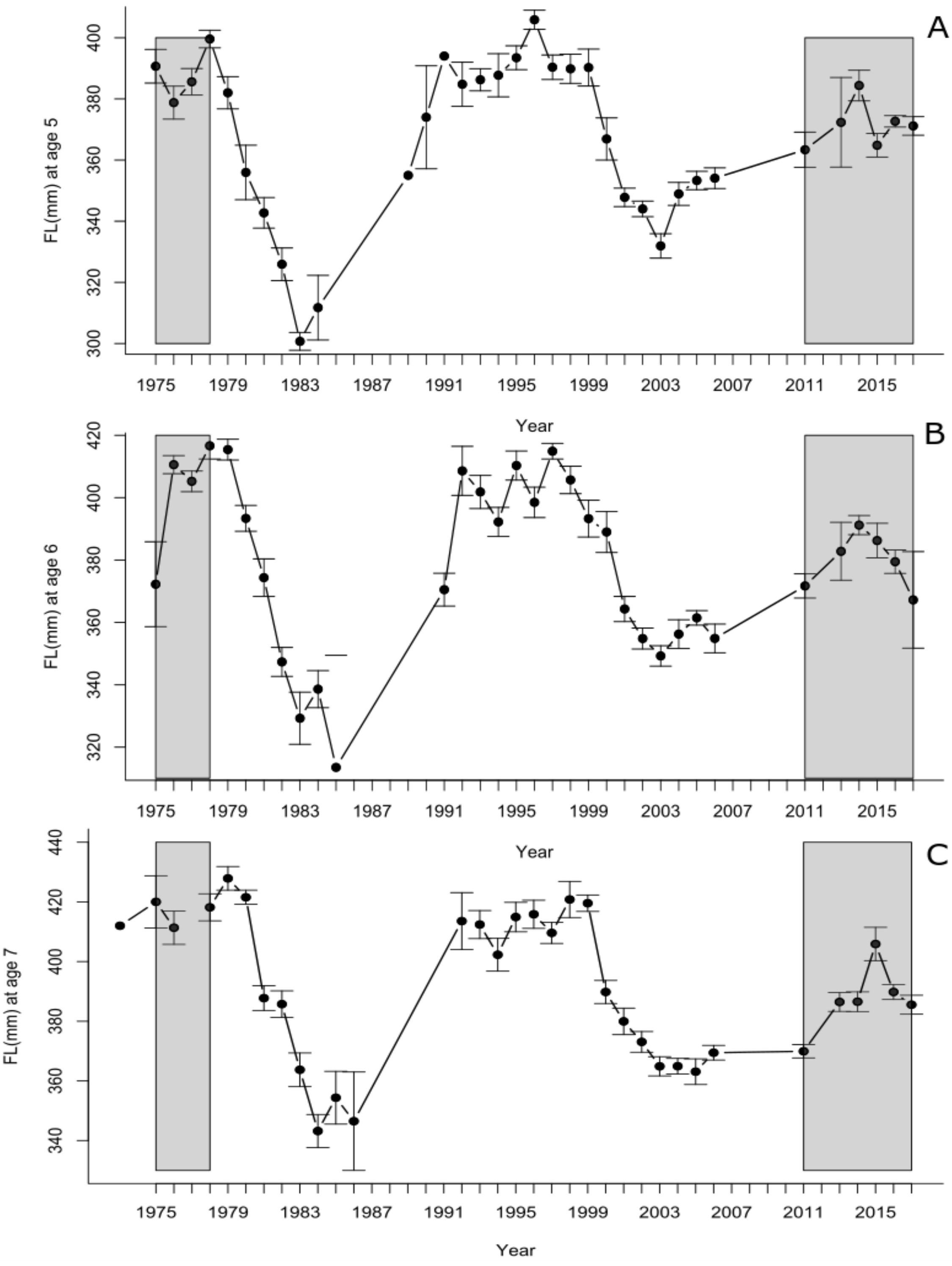

Figure 2.11. Lake 223 lake trout length-at-age for the three most common ages (5-7, A-C). Fork length in millimeters is on the y-axis. Grey bars indicate the time periods representing pre-*Mysis* extirpation (1975-1978), and the time period representing post-*Mysis* extirpation (2011-2017).

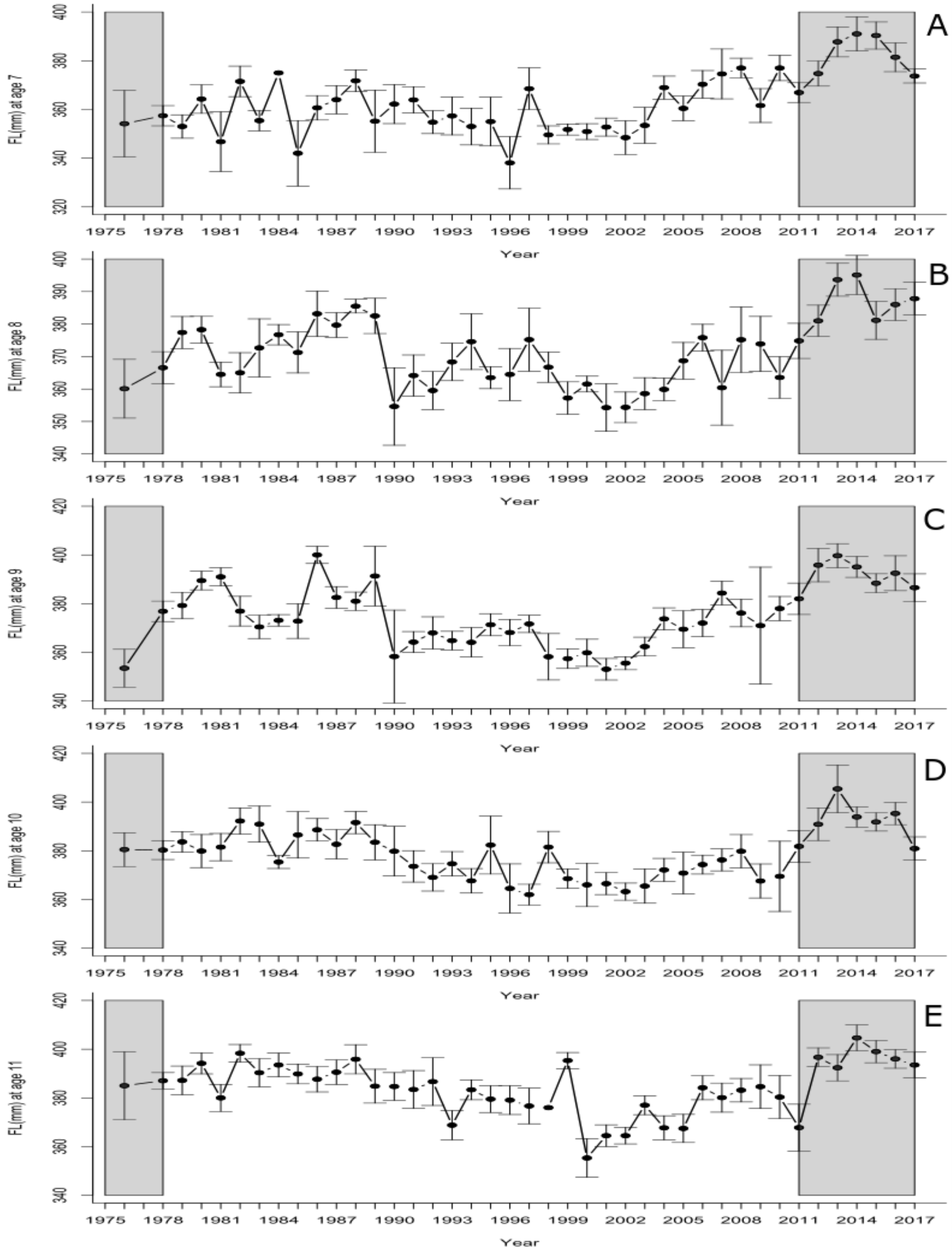

Figure 2.12. Lake 224 lake trout length-at-age for the five most common ages (7-11, A-E). Fork length in millimeters is on the y-axis. Grey bars indicate the time periods representing pre-*Mysis* extirpation (1975-1978), and the time period representing post-*Mysis* extirpation (2011-2017).

GENERAL CONCLUSION

The main objective of this thesis was to determine the ecosystem-level effects of the extirpation of *Mysis diluviana* from its native environment by comparing historical data collected before the acidification of Lake 223 (when *Mysis* were present), to the period of time after the lake was recovered from acidification but still missing *Mysis*. This analysis provides experimentally-derived evidence that *Mysis* likely play a significant role in structuring aquatic ecosystems in both introduced and native environments. Throughout the Lake 223 aquatic community, there have been substantial decreases in biomass of several key ecosystem compartments (lake trout, zooplankton, and slimy sculpin) correlated with the extirpation of *Mysis*. The zooplankton community has shifted to favour larger cladocerans, different than the shift in species composition observed in Lake 224 as revealed by multivariate analyses. Generally, larger species that would be prey to *Mysis* such as *Daphnia spp*. and *Holopedium glacialis* have increased in abundance in Lake 223 following the extirpation of *Mysis*. As *Mysis* occupy a central trophic position as a meso-predator in the pelagic habitat, combined with an omnivorous diet that is known to couple energy between benthic and pelagic habitats, the results of this work demonstrate the important structuring force *Mysis* have to all trophic levels within lake ecosystems.

The extirpation of *Mysis* has likely created a bottleneck in the flow of energy up the food-web, resulting in lower growth and abundance of the lake trout. As *Chaoborus* are now the largest predatory zooplankton species in Lake 223, and as numerous as *Mysis* once were prior to extirpation but with only a fraction of the total biomass, fish consuming *Chaoborus* are likely far less efficient relative to a diet of *Mysis*. Instead of increasing reliance on the littoral environment following the extirpation of *Mysis*, evidence from this study shows a decreased integration of littoral/pelagic energy pathways. During summer stratification, lake trout are thermally isolated from the littoral environment, and it is likely much less metabolically costly to forage on *Chaoborus* in the pelagic environment relative to making foraging trips into the littoral region. Lake trout have increased pelagic energy reliance (possibly from incorporating *Chaoborus* as an alternative to *Mysis*) correlated with lake trout growing 5.6% slower than before *Mysis* were extirpated. While nitrogen isotopic signatures provide evidence that both lake trout and white sucker have incorporated *Chaoborus* into their diet to some extent, future work should include stomach content analysis to corroborate SIA results.

Even though only the adult lake trout population was examined in this study, I speculate that the extirpation of *Mysis* has likely also affected the juvenile life stage of lake trout in Lake 223. Lake trout are planktivorous during the juvenile life stage and *Mysis* provide an intermediate-sized prey item during the switch to a piscivorous diet (Hudson et al. 1995, Gamble et al. 2011b). Without *Mysis*, juveniles may spend a longer time at a size where they are vulnerable to conspecific predation (consistent with elevated TP), resulting in adult lake trout reducing the abundance of the entire population via cannibalism. Although I have no evidence for increased juvenile mortality due to conspecific predation, stomach content analysis of adult lake trout could provide such evidence.

Based on this research, lakes that have been atmospherically acidified may see a dramatic change to the aquatic ecosystem if *Mysis* were to be re-established into habitats where they once were native. After decades of smelter emissions from mining operations, Ontario has some 19,000 lakes with a pH less than 6.0, with roughly a third of those in the Sudbury region (Neary et al. 1990, Yan et al. 1995). Since the reduction of sulfur emissions that have led to acid-rain, lakes in the Sudbury region are chemically recovering to their natural pH. Biological recovery follows the chemical recovery of these lakes, but land-locked lakes limit recolonization opportunities by aquatic species. Lake 223 is currently undergoing a *Mysis* re-introduction experiment, where *Mysis* from upstream Lake 224 are caught, transported, and released into Lake 223 during spring and fall iso-thermic conditions. Based on the findings in this study, once *Mysis* become re-established in Lake 223, I would expect a reversal of the effects outlined here from the extirpation of *Mysis*. Specifically, I expect *Mysis* to outcompete and prey on *Chaoborus* (Nordin et al. 2008) therefore reducing the abundance of *Chaoborus* larvae, resulting in greater energy transfer from lower to higher trophic levels.

Evidence from my thesis indicates that the extirpation of *Mysis* may have created a bottleneck of energy flowing from lower to higher trophic levels, resulting in the stunting of the growth and recruitment of the native lake trout population. Should *Mysis* re-establish in Lake 223, I also expect greater lake trout recruitment, and therefore greater total population abundance and biomass, as well as the growth rates of lake trout returning to those rates observed before acidification. As lake trout are only found in about 1% of Ontario lakes (OMNR 2006), this study provides evidence that re-introducing *Mysis* into lakes where they were previously extirpated due to human impacts may increase energy flow through the food web and restore these rare ecosystems.

The extirpation of *Mysis* has been shown here to be correlated with declines in the abundance, biomass, and growth rates of lake trout. A corollary of these finding is that the removal of *Mysis* from introduced waterbodies throughout western North America may help to reduce invasive lake trout. This unique opportunity to evaluate effects of a species extirpation shows clearly that *Mysis* are important for structuring aquatic ecosystems, and for increasing energy flow and biomass in aquatic ecosystems. This study provides further evidence that introducing *Mysis* into non-native ecosystems that are physically very similar to their native environment, would result in a restructuring of the aquatic community. In US states such as Montana and Idaho that have lake trout suppression strategies, this research suggests that reducing *Mysis* abundances in addition to lake trout may decrease growth, abundance, and recruitment of lake trout. Results from Lake 375 at the ELA has shown that eutrophication virtually eliminated *Mysis* from the lake (Paterson et al. 2011), and the acidification of Lake 223 demonstrated that *Mysis* are acid sensitive (Nero and Schindler 1983). Although it is unlikely that *Mysis* will ever become eradicated from introduced water bodies without large scale manipulations to the lake such as eutrophication or acidification, reducing non-native *Mysis* abundance may have large benefits to restoring natural ecosystems to which they have been introduced.

In conclusion, this unique opportunity to effectively remove one species from the ecosystem has allowed me to show the structuring role of *Mysis diluviana* in their native environment. This research suggests that *Mysis* are a potential keystone species in their native environment, whose extirpation has resulted in the decline in biomass of many species within Lake 223, with cascading effects to the lake trout population. The term “keystone alien” likely does not apply to *Mysis*, however it is difficult to explicitly define a keystone species (Power et al. 1996). Despite being known to restructure ecosystems in their non-native range, and to be an important part of the zooplankton community throughout their native range, this is the first study to describe the role they play in the structure and function of ecological communities based on their removal from an ecosystem. Restoration of acid-damaged lakes in lakes where *Mysis* were previously present should include consideration of the reintroduction of this species to fully restore the biological community to its natural state.

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APPENDIX

Zooplankton classification

D. catawba, D. pulex, and *D. schoedleri* are part of a complex that may or may not represent different taxa and consequently were lumped together. *D. galeata* and *D. dubia* are also very similar and were grouped together. Also, prior to 1995, early stage copepods were not identified to species, but were grouped together as simply cyclopoid or calanoid stage 1-3 or 4-5, regardless of species. After 1995 these early stage copepods were identified to species (M. Paterson, pers. coms.). To provide consistency throughout the dataset, all early stage copepods were summed together into the groups used prior to 1995. Rotifer species *Gastropus hyptopus*, *Gastropus sp.*, *Gastropus. stylifer*¸ *Kellicottia longspina, Keratella cochlearis and Polyarthra vulgaris,* were all grouped together into a general rotifer group after correlation plots revealed these species were highly (r>0.80) correlated.

Mark-recapture estimates

Occasionally, lake trout were caught in trap nets intended for white suckers during the spring. In order to satisfy the assumption of equal catchability across all time periods, lake trout caught in the spring were assumed to be adults in the population in the previous fall and included in the previous fall catch history. Lake trout caught in the spring incidentally cannot compare to lake trout caught in the fall in terms of catchability and would therefore violate the assumption of equal catchability between capture occasions.

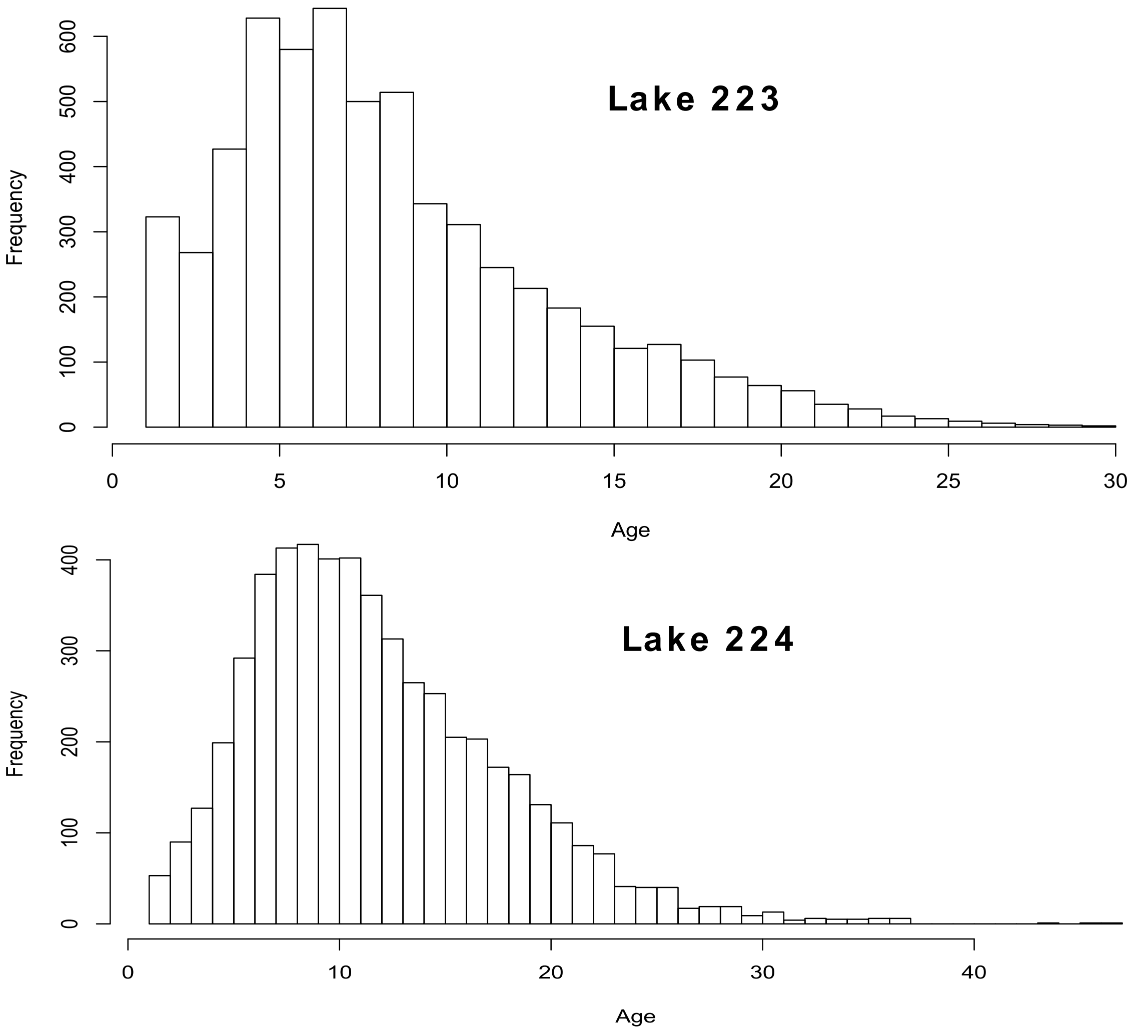

Figure A2.1. Lake 223 (top) and Lake 224 (bottom) lake trout age distributions from all years of sampling (1973-2017). Notice different scales on x-axis.