

**The Effects of Invasive Dreissenid Mussels on the Offshore Food
Web of Lake Simcoe, Ontario.**

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Abstract

Dreissenid mussels have successfully invaded North America, including the Great Lakes and their surrounding waterbodies. With their establishment, there have been many notable impacts including the rerouting of energy flow from offshore to nearshore energy sources. This study aimed to determine if such a shift had occurred in Lake Simcoe, Ontario following the establishment of zebra mussels (*Dreissena polymorpha*) and, more recently, quagga mussels (*Dreissena rostriformis bugensis*) with an emphasis on the fishes in the offshore region. With the relatively long time period of 13 years between establishment dates of zebra mussels and quagga mussels, Lake Simcoe provided a unique opportunity to differentiate the possible effects of zebra mussels independently from quagga mussels. All offshore fish investigated in this study appeared to decrease in trophic position (TP), closely following dreissenid invasion, except for lake trout, which increased. Interestingly, for smelt, herring, and lake trout this result may not be due directly to mussel establishment, but rather other factors impacting Lake Simcoe such as changes to the zooplankton community, as well as increased natural recruitment and ongoing stocking practices of lake trout and whitefish. Whitefish trophic position appeared to be consistent with increased dreissenid mussel abundance, as observations of whitefish stomachs in recent years revealed heavy reliance on dreissenids. In addition, and contrary to expectations, the percentage of littoral carbon incorporated in cold water fish decreased over time, especially in the most recent years. A shift towards nearshore resources was observed following zebra mussel invasion in 1996 among all offshore fish species, however, this quickly shifted towards more offshore resources in the following years. Therefore, it appears that the invasion of dreissenid mussels to Lake Simcoe has produced a

shift in energy pathways to favour more nearshore benthic-littoral energy production, in a similar manner to observed changes in the Great Lakes following dreissenid establishment. However, shifts in energy pathways in Lake Simcoe were observed to correct back to more offshore pelagic sources in years following initial dreissenid establishment. Ongoing monitoring of the Lake Simcoe offshore fish community is important to understand the effects of dreissenid mussels into the future.

Lay Summary

The biology mission statement is: “Faculty and students in the Department of Biology are bound together by a common interest in explaining the diversity of life, the fit between form and function, and the distribution and abundance of organisms.” The research conducted in this study highlights the theme of distribution and abundance of organisms as it looks at the effects of invasive dreissenid mussels on cold water fish communities. Dreissenid mussels have invaded much of North America including the Great Lakes and their surrounding waterways. This study aimed to determine what impacts these invasive mussels may have on the inland lake of Lake Simcoe. Unlike the Great Lakes, Lake Simcoe has a relatively long period of 13 years between the invasion of zebra mussels and the invasion of quagga mussels. This ecosystem provides a unique opportunity to observe the effects of zebra mussels without the presence of quagga mussels. In addition, Lake Simcoe is a good example of how dreissenid mussels may effect smaller inland lakes, rather than larger lakes such as the Great Lakes. It was found that offshore fishes in Lake Simcoe experienced a shift in energy source from pelagic to littoral energy following the invasion of zebra mussels. Interestingly, this shift seemed to correct itself fairly quickly, with energy sources becoming more pelagic in more recent years. Trophic position of all offshore fish was also found to change over time, with smelt, herring, and whitefish decreasing in trophic position, and lake trout increasing over time. These changes all occurred within a six-year window between 2000 and 2006. The decrease observed in trophic position of smelt and herring may be a result of changes in the diet of the fish from zooplankton predators to more herbivorous zooplankton. Whitefish trophic position may be directly influenced by mussel invasion, as whitefish stomachs in recent years show a heavy reliance on

dreissenid mussels. Lake trout trophic position may have increased as a result of increased cannibalism or whitefish consumption; young lake trout and whitefish were shown to have higher nitrogen values than either smelt or herring. With stocking practices introducing many small, young lake trout and whitefish to the lake, in addition to an increase in natural lake trout and whitefish recruitment, smaller lake trout and whitefish may be incorporated into larger lake trout diets more so now than in previous years. Regardless of the specific factors influencing the changes in energy source and trophic position, my study shows that the invasion of dreissenid mussels to Lake Simcoe has impacted cold water offshore fish in Lake Simcoe, either directly or indirectly.

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Table of Contents

Abstract.....	i
Lay Summary.....	iii
Acknowledgements.....	v
List of Figures	vii
List of Tables	xi
List of Appendices.....	xii
Introduction	1
Methods.....	11
Results.....	19
Discussion.....	27
References	37
Figures.....	46
Tables	57
Appendices.....	59

List of Figures

Figure 1: Sample sites for zooplankton, and benthic invertebrates collection from Lake Simcoe, ON. Zooplankton were collected at sites C6, E50, K42, K45, and M66. Benthic invertebrates were sampled at depths of 5, 10, 15, 20, 25, and 30 m along the transect from Sibbald Point to site K45. (Base graphic: Rennie and Evans (2012)). 46

Figure 2: Seasonal variation in isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for zooplankton collected from stations C6, E50, K42, K45, and M66 in Lake Simcoe, ON, during ice free period for the years 2009 and 2017. Isotopic values were corrected for Suess effect ($\delta^{13}\text{C}$) according to Verburg (2007) and anthropogenic N influences ($\delta^{15}\text{N}$) as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ values and mean $\delta^{15}\text{N}$ values for samples collected in all mesh sizes (63, 125, 250, 500, and 1000 μm). 47

Figure 3: Biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from zooplankton collected at site K45 (black Xs), profundal benthos ($\geq 15\text{m}$ depth) excluding dreissenids (red triangles), and profundal dreissenids ($\geq 15\text{m}$ depth, blue squares) for the years 2008, 2009, and 2017. Isotopic values were corrected for Suess effect ($\delta^{13}\text{C}$) according to Verburg (2007) and anthropogenic N influences ($\delta^{15}\text{N}$) as per Rennie et al. (2013). Points are individual measurements. Zooplankton samples sorted on mesh sizes 125, and 500 μm for 2009, and mesh sizes 125, 250, and 500 μm for 2017 were included. 48

Figure 4: Isotopic values of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) over time for zooplankton collected at stations K45 and M66 in Lake Simcoe, ON. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). $\delta^{15}\text{N}$ values are sediment-corrected as per

Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ and mean $\delta^{15}\text{N}$ values; line is regression line. Samples means were calculated from samples sorted on mesh sizes 125 and 500 μm for 2009, and mesh sizes 125, 250, and 500 μm for 2017. 49

Figure 5: Isotopic values of $\delta^{13}\text{C}$ over time for benthic invertebrates, excluding dreissenids, collected from Lake Simcoe, ON. Values of $\delta^{13}\text{C}$ are plotted by depth; A = 5m, B = 10m, C = 15m, D = 20m, E = 25m, F = 30m. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). Points are mean $\delta^{13}\text{C}$ values. 50

Figure 6: Isotopic values of $\delta^{15}\text{N}$ over time for benthic invertebrates, excluding dreissenids, collected from Lake Simcoe, ON. Values of $\delta^{15}\text{N}$ are plotted by depth; A = 5m, B = 10m, C = 15m, D = 20m, E = 25m, F = 30m. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{15}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{15}\text{N}$ values. 51

Figure 7: Isotopic values of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) over time measured from fish scale samples, and corrected to represent fish tissue isotopic values, collected from Lake Simcoe, ON. $\delta^{13}\text{C}$ values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). $\delta^{15}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ values and mean $\delta^{15}\text{N}$ values. 52

Figure 8: Breakpoints for isotopic values of $\delta^{13}\text{C}$ over time measured from fish scale samples, and corrected to represent fish tissue isotopic values, collected from Lake Simcoe, ON. Values of $\delta^{13}\text{C}$ are plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. $\delta^{13}\text{C}$ values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). Vertical dashed lines represent significant breakpoint years. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). Points are mean $\delta^{13}\text{C}$ values. Red line is zooplankton baseline; blue line is shallow benthos (5m) baseline. 53

Figure 9: Breakpoints for isotopic values of $\delta^{15}\text{N}$ over time measured from fish scale samples, and corrected to represent fish tissue isotopic values, collected from Lake Simcoe, ON. Values of $\delta^{15}\text{N}$ are plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. $\delta^{15}\text{N}$ values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). Vertical dashed lines represent significant breakpoint years. $\delta^{15}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{15}\text{N}$ values. Red lines are zooplankton baselines; blue line is deep benthos (15-30m) baseline; purple line is mean of smelt and lake herring baseline. 54

Figure 10: Percent contribution of littoral carbon calculated from tissue $\delta^{13}\text{C}$ (Table 1; Equation 1) values for fish collected from Lake Simcoe, ON. % littoral contribution is plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. Littoral contribution is shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). To account for the

accumulation of isotopes over the fish's lifetime adult diet, baseline values for zooplankton (pelagic) and nearshore benthos (littoral) were averaged over the previous years equal to the number of years the fish was feeding. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007)..... 55

Figure 11: Trophic position calculated from tissue $\delta^{15}\text{N}$ values for fish collected from Lake Simcoe, ON (Table 1; Equation 2). Trophic position is plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. Trophic position values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). To account for the accumulation of isotopes over the fish's lifetime adult diet, baseline values were averaged over the previous years equal to the number of years the fish was feeding. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{N}$ values were sediment-corrected as per Rennie et al. (2013)..... 56

List of Tables

Table 1: Difference between fish scale isotopic values relative to fish tissues for four species from Lake Simcoe, Canada. Positive values below indicate that scales are more enriched than tissues, negative values indicate scales are more depleted than tissue. To facilitate the use of fish tissues $\delta^{13}\text{C}$ in mixing models, tissue isotopic values were estimated from scale estimates by subtraction of correction values below. 57

Table 2: Significant breakpoint year classes for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (based on fish scale samples), % contribution of littoral carbon, and trophic position for each fish species considered in the study. 58

List of Appendices

Appendix 1: Comparison of the first reported sightings of zebra mussels and quagga mussels in each of the Great Lakes and Lake Simcoe. 59

Appendix 2: Sampling stations and dates for zooplankton collected in Lake Simcoe, ON in 2017. 60

Appendix 3: Latitude and longitude of zooplankton sampling locations for Lake Simcoe, ON. 61

Appendix 4: Zooplankton taxa across all size fractions (125, 250, and 500 µm) for samples collected from Lake Simcoe, ON in 2017. 62

Appendix 5: Latitude and longitude of benthic invertebrate sampling locations for Lake Simcoe, ON. Samples included are those that were used to obtain benthic invertebrates. Single hauls provided sufficient biomass for the three shallower sites (5-15m), whereas deeper sites (20-30m) required multiple hauls to obtain sufficient biomass for analysis. 63

Appendix 6: Benthic invertebrate taxa across all depths for samples collected from Lake Simcoe, ON in 2017. 64

Appendix 7: Isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ over time for benthic invertebrates, excluding dreissenids, collected from Lake Simcoe, ON, for depths of 15-30m. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). $\delta^{13}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ and mean $\delta^{13}\text{N}$ values; line is regression line. 65

Appendix 8: Benthic invertebrate taxa from 5m sites included in stable isotope analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for years 1982 to 2017. The presence of each taxonomic group is denoted by X.

..... 66

Appendix 9: Trophic position calculated from tissue $\delta^{15}\text{N}$ values for lake whitefish collected from Lake Simcoe, ON, over time (Table 1; Equation 2) estimated using both benthic invertebrates excluding dreissenid mussels (purple) and the same benthic invertebrates including dreissenid mussels (blue) for depths of 15-30m. Values of $\delta^{15}\text{N}$ are shown by year class to which the individual fish began feeding on an adult diet (age 3 for lake whitefish).

67

Appendix 10: Trophic position calculated from tissue $\delta^{15}\text{N}$ values for lake trout collected from Lake Simcoe, ON (Table 1; Equation 2). Trophic position is plotted based on estimates from baseline species; Panel A = smelt baseline, Panel B = herring baseline. Values of $\delta^{15}\text{N}$ are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish).....

68

Appendix 11: Comparison of $\delta^{15}\text{N}$ for smelt, herring, and lake trout (age 2). Only years with data available for all three species were included. Data presented represents the $\delta^{15}\text{N}$ values for scales of each fish species, sediment-corrected as per Rennie et al. (2013)..

73

Introduction

The impacts of the dreissenid mussel invasion in North America have been both rapid and devastating. Since their introduction to North America in the late 1980s, zebra mussels (*Dreissena polymorpha*) and quagga mussels (*Dreissena rostriformis bugensis*) have quickly spread throughout the Laurentian Great Lakes, as well as surrounding water bodies in both the U.S. and Canada (Hebert et al., 1989; Schloesser et al., 1996; Benson et al., 2019). Dreissenid mussels are thought to have been introduced to North America via the ballast waters of ships traveling from their native territories in Europe (Benson, 2019; Hebert et al., 1989; Smimova and Vinogradov, 1993). Although efforts have been made to slow the progression of dreissenid mussel invasion, their range continues to grow. This is primarily due to their ability to attach themselves to nearly any submerged surface, coupled with their ability to withstand long periods of desiccation (Ricciardi et al., 1995; Therriault et al., 2013). Large adult zebra mussels have been observed to have 73 percent survivorship over 10 days in good conditions (Ricciardi et al., 1995), and juvenile zebra mussels may survive ballast water transport for 11 to 15 days or longer (Therriault et al., 2013). This resistance to desiccation, as well as lengthy transport survival time, allows the mussels to establish on surfaces of boats, motors and other fishing equipment, or be sucked into bilges, live-wells and water lines and then be transported across large areas of land to another water body (Therriault et al., 2013). Increased shipping practices and movement of recreational watercraft throughout the Great Lakes and surrounding waterways has facilitated the dispersal of dreissenid mussels in North America at an alarming rate. While preventing the spread of dreissenid mussels to smaller inland lakes can typically be achieved by properly cleaning, draining, and drying all equipment that has been exposed to

waters where they are established, many fishermen and recreational boaters do not seem to perform this crucial step, as dreissenid mussels continue to spread throughout the continent.

Once zebra mussels have been introduced to a system, they can have several negative impacts. The sharp shells of mussels often diminish the quality of beaches and lake bottoms of popular swimming areas. In addition, their establishment has been linked to increases in algal blooms such as *Cladophora glomerata* (Higgins et al., 2008), which can lead to the growth of harmful bacteria such as *E. coli*, *Salmonella*, *Shigella*, or *Campylobacter* (Higgins et al., 2008; Therriault et al., 2013). Zebra mussel establishment can also cause many economic problems for industry as they establish in great numbers on submerged surfaces. This includes water intakes, hydroelectric dams, navigational buoys and scientific monitoring equipment (Evans et al., 2011; Therriault et al., 2013). Once established, these mussels can be extremely difficult to eradicate, with few successful eradications reported. Control methods are often costly, and may cause harm to other aquatic life in the ecosystem (DFO, 2014).

In addition to their effects on human activity, zebra mussel establishment can also have significant impacts on the ecosystems they invade. The mussel's ability to reproduce rapidly and in great numbers causes rapid population increases and quickly leads to a high density of mussels in invaded ecosystems (Therriault et al., 2013). The filter feeding activity of zebra mussels pulls phytoplankton out of the water column to the lake bottom, typically in the shallower areas of the lake (Hecky et al., 2004; Higgins and Zanden, 2010). Nutrients from these filtrates is excreted by the mussels and concentrated in these shallow areas (Hecky et al., 2004; Higgins and Zanden, 2010). Continuous filtration by such a large number of mussels causes an increase in water clarity that can lead to a number of effects on the structure of the ecosystem

including increased growth of nuisance plant species at deeper depths, and a deeper thermocline (Geisler et al., 2016). Their establishment has also been known to dramatically change community compositions of organisms within invaded ecosystems (Ricciardi et al., 1997; Winter et al., 2011). A reduction or possible elimination of fauna adapted to smooth-substrate may be observed following dreissenid mussel invasion, possibly as a result of their ability to change the habitat in which native benthic species live by altering bottom profiles and sediment composition or through competition for resources (Ricciardi et al., 1997). Ozersky et al. (2011a) observed increased abundance of non-dreissenid invertebrates in nearshore rocky habitats following dreissenid invasion, likely due to increased evenness of resource distribution provided by the deposition of dreissenid feces and pseudofeces, as well as a more homogeneous physical habitat structure provided by living mussels and empty shells. Conversely, Jimenez et al. (2011) observed decreased benthic abundance on soft sediments for many species in Lake Simcoe over most depths following dreissenid mussel invasion. A change in the benthic community in addition to decreased abundance of phytoplankton in the water column and increased water clarity are all factors that could induce changes to fish populations further up the food chain. For example, McNickle et al. (2006) observed a change benthic community structure toward a more homogeneous community dominated by mussels in shallow-water sites, and a significant decrease in *Diporeia* in the offshore community. Based on these changes to the benthic community and information on whitefish diets, they predicted that the diets of whitefish would only contain 57 to 84% of their previous energy content prior to dreissenid establishment. Although *Diporeia* are not present in Lake Simcoe, similar changes may still be expected as the benthic community reacts to the establishment of dreissenid

mussels. This initial research has been supported by subsequent studies indicating dreissenid establishment as a possible negative influence on cold water fish populations including lake whitefish (Hoyle et al., 2008; Rennie et al., 2009).

One of the main ecosystem impacts observed after the introduction of zebra mussels is a shift in energy flow and nutrients throughout the lake. Rush et al. (2012) found that lake trout in Lake Ontario became more reliant on nearshore energy resources following a decline in natural offshore forage fish populations, and an increase in invasive forage fish populations that occurred after the invasion of dreissenid mussels. Similarly, Rennie et al. (2009) also observed a shift in isotopic signatures, depth distribution and diets of offshore fish in South Bay, Ontario, that suggested greater reliance on nearshore energy sources after the establishment of dreissenid mussels. In a large scale temporal analysis examining lake whitefish populations across the Great Lakes, Fera et al. (2017) observed shifts in lake whitefish energy pathways from pelagic-profundal to benthic-littoral for several populations that had been invaded by dreissenid mussels, while observing no such change in uninvaded Lake Superior ecosystems. Such a shift may be due to the filtration of phytoplankton out of the water column which is then concentrated into shallower nearshore areas where the mussels are primarily established, through the excretion of feces and pseudofeces (Nogaro and Steinman, 2014), a phenomenon termed the nearshore phosphorus shunt (Hecky et al. 2004).

Shifts in energy pathways that have been associated with dreissenid establishment within the Great Lakes are well documented (Fera et al., 2017; Rennie et al., 2009; Rush et al., 2012). To determine if such a shift was apparent in the smaller inland lakes, a study by Rennie et al. (2013) was conducted in Lake Simcoe, Ontario after the establishment of zebra mussels.

This was accomplished by comparing data from both pre- and post-invasion time periods, 1983 and 2009 respectively. Evidence of a significant increase in the importance of benthic-derived primary production was found primarily in nearshore fish populations, however offshore populations appeared to be fairly stable between these years.

Since the time of that study, a new invader, the quagga mussel (*Dreissena rostriformis bugensis*), has become established in Lake Simcoe. Quagga mussels are similar to zebra mussels, with some important differences. Key among them is the quagga mussel's ability to inhabit colder, deeper waters and softer substrates (Dermott and Munawar, 1993; Lake Simcoe Region Conservation Authority, 2016; Therriault et al., 2013; Young and Jarjanzi, 2014). This allows these mussels to spread further into the lake, rather than colonizing only shallow, nearshore areas. They are also active at cooler temperatures, allowing them to have an increased growing season and inhabit areas that are unsuitable to support zebra mussels (Mills et al., 1993; Lake Simcoe Region Conservation Authority, 2016; Therriault et al., 2013). Quagga mussels were introduced to North America in 1988, and now inhabit all of the Great Lakes, as well as many surrounding inland lakes (Carlton, 2008; Leach, 1993; Mills et al., 1993). The more recent establishment of this species leaves much to be learned about their effects on ecosystems. The situation in Lake Simcoe is unique in comparison to other invaded waterbodies, as there was a relatively long period of time (13 years) between the first sighting of zebra mussels and that of quagga mussels (Appendix 1). Although quagga mussels have been present in the Great Lakes for several decades, little is known about their specific effects, as zebra mussel and quagga mussel invasion occurred in relatively fast succession on the Great

Lakes (typically less than a decade, see Appendix 1) relative to their observed impacts on other ecosystem components.

My study described changes in the offshore food web of Lake Simcoe, Ontario, associated with invasive dreissenid mussels. Specifically, my research evaluated the impact of recently established quagga mussels on the system versus those of zebra mussels. As such, an evaluation of this ecosystem provides a unique opportunity to describe what changes to offshore food webs may be expected after the invasion and establishment of both zebra and quagga mussels, and to potentially separate differential effects between the two given the lag in time between establishment.

The objectives of my study were: 1) to determine if the resource use of offshore fish in Lake Simcoe has changed over time using stable isotopes of carbon and nitrogen of fish, benthic invertebrates, and zooplankton and 2) to determine if these changes are consistent with the timing of both dreissenid mussel invasions.

I hypothesized that: 1) resource use of offshore fish would change over time, with offshore fish becoming more reliant on nearshore energy sources, similar to observations to date on the Laurentian Great Lakes and 2) changes in resource use towards more nearshore sources would be consistent with the timing of zebra mussel establishment, and then become more pronounced following the invasion of quagga mussels. In addition, I hypothesized that the large shift in isotopic values of nearshore benthic invertebrates observed by Rennie et al. (2013) would be continued in more recent years.

To test these hypotheses, I used archived scale samples of common offshore fish species, as well as zooplankton samples and benthic invertebrate samples collected from Lake

Simcoe, ON to conduct stable isotope analyses for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Historical data on invertebrates and zooplankton from Rennie et al. (2013) were used as well as previously unpublished zooplankton data from 2009, in combination with samples collected for all food web components during the 2017 sampling season. These results were used to interpret changes in food chain length and resource use by calculating both trophic position and percent carbon from benthic and pelagic origin, respectively.

Site description

Lake Simcoe is one of the largest inland lakes in Ontario outside of the Laurentian Great Lakes; due to its large year-round recreational fishery and close proximity to Toronto, Ontario (approx. 80 km North), it is a popular destination for tourists and commuters (Palmer et al., 2011). The lake has a surface area of 722 km² with a mean depth of 14-15 m and a maximum depth of 42 m. Zebra mussels (*Dreissena polymorpha*) were first observed on the hull of a boat in Lake Simcoe in 1991 (Palmer et al., 2011). The natural recruitment of this species was first detected in 1994 and by 1996 they were well established (Evans et al., 2011). This initial invasion was followed by the introduction of the quagga mussel in 2004 (Ozersky et al., 2011b), which was established by 2009 (Lake Simcoe Region Conservation Authority, 2016; Young and Jarjanzi, 2014). Prior to the establishment of dreissenid mussels, evidence suggests that Lake Simcoe was largely driven by offshore primary productivity, however it is likely that nearshore primary production has increased since the establishment of dreissenid mussels, resulting in a greater representation of benthic energy pathways (Rennie et al., 2013).

Shifts in the primary productivity and energy flow within a lake can be examined by analysing stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$; France, 1995; Peterson and Fry, 1987; Post, 2002). By observing the ratio of ^{13}C to ^{12}C , it is possible to determine the ultimate source of energy for an organism, as the base of nearshore littoral food webs are typically enriched with ^{13}C and have more positive $\delta^{13}\text{C}$ values. Pelagic areas, on the other hand, are typically dominated by the lighter isotope ^{12}C and have more negative $\delta^{13}\text{C}$ values (France, 1995; Peterson and Fry, 1987). Since carbon undergoes little fractionation between trophic levels (<1‰), sources of energy are preserved from one step in the food web to the next, thereby facilitating the use of mixing models to determine the ultimate source of carbon for an organism (France, 1995). The ratio of ^{15}N to ^{14}N present can provide information on the trophic position of an organism, as $\delta^{15}\text{N}$ signatures typically increase by 3-4‰ relative to diet (Peterson and Fry, 1987). Scale samples were chosen as a well-preserved and available structure to provide isotopic signatures of fish over time (Fera et al., 2017; Kelly et al., 2006; Rennie et al., 2009). Additionally, the use of scale samples also allows direct comparisons to be made to previous work by Rennie et al. in 2013 of stable isotope signatures for offshore fishes in Lake Simcoe.

The cold water fish species examined in this study include planktivorous rainbow smelt (*Osmerus mordax*) and lake herring (*Coregonus artedii*), benthivorous lake whitefish (*Coregonus clupeaformis*), and piscivorous lake trout (*Salvelinus namaycush*). Fish were chosen based on availability in provincial government archives, as well as representing a range of both diet (i.e. pelagic vs. benthic feeders), and trophic position (piscivorous, benthivorous, or planktivorous)

in the offshore ecosystem in Lake Simcoe. These species were also selected as they are all (at present or historically) important to the offshore recreational fishery of Lake Simcoe.

The once plentiful rainbow smelt has undergone a tremendous decline in population numbers in recent years. First introduced in the early 1960s, rainbow smelt populations took off in the 1970s with populations remaining high until the 1990s (North et al., 2013). Prior to 2012, rainbow smelt were still very common in Lake Simcoe, however in the years following their population numbers dropped dramatically. Currently, few rainbow smelt remain in the lake, to the extent that they are now rarely captured in surveys and assessments (Young and Jarjanzi, 2014).

In the past, lake herring provided a significant food source for many species of Lake Simcoe's fish community, as well as a large recreational fishery (Government of Ontario, 2014). This was until their populations began to decline in the 1980s as a result of low dissolved oxygen and loss of spawning habitat (Government of Ontario, 2014; Young and Jarjanzi, 2014). This led to the closure of the herring fishery in 2001 in an attempt to increase population numbers (North et al., 2013; Young and Jarjanzi, 2014). Today, the herring population has greatly increased and is maintaining stable levels; and as of 2015, the fishery has been re-opened (OFAH 2017).

Lake whitefish are another important species to the Lake Simcoe fishery. A collapse of the fishery caused by a lack of dissolved oxygen and eutrophic conditions occurred in the in the 1970s, and a significant stocking effort began in the 1980s (Young and Jarjanzi, 2014). Currently, approximately 150,000 fish are stocked annually, with stocking efforts remaining high as of 2015 (Lake Simcoe, Lake Whitefish stocking records). The whitefish population appears to be

recovering with catches of wild lake whitefish steadily increasing and exceeding catch rates of stocked fish since 2006 (Young and Jarjanzi, 2014).

Lake trout are another species where stocking efforts were historically required to maintain a healthy population. The collapse of the Lake Simcoe lake trout population began in the 1960s, again due to low dissolved oxygen levels and eutrophic conditions (Young and Jarjanzi, 2014). Stocking efforts began in 1966 (Borwick et al., 2009) by releasing approximately 250,000 stocked fish into the lake annually (Lake Simcoe, Lake Trout stocking records). Fish continued to be stocked at high numbers, however, there is increasing evidence of natural recruitment; in 2001, two wild young-of-the-year lake trout were caught (Young and Jarjanzi, 2014). Following this initial catch, wild lake trout have been caught every year since 2003 (Young and Jarjanzi, 2014). To encourage natural reproduction of wild lake trout, stocking efforts were cut in half beginning in 2010 (Borwick et al., 2009). Currently, approximately 50,000 fish are stocked annually (Lake Simcoe, Lake Trout stocking records).

Zooplankton are important for identifying what changes are occurring in pelagic regions near the base of the food web, and may vary across sampling locations. The analysis of stable isotopes of zooplankton from both before and after the establishment of quagga mussels were analyzed to provide a pelagic isotopic baseline to which stable isotope values of planktivorous lake herring and smelt could be compared, and to determine the degree of inter-annual variability in this baseline.

In addition to stable isotope analyses of fish and zooplankton samples, stable isotope analyses on benthic invertebrate samples were also measured. Isotopic signatures of benthic invertebrates over a similar timeframe as zooplankton provided a benthic isotopic baseline to

represent possible changes in the benthic food web. Together, these isotopic signatures permit an evaluation of benthic and pelagic energy sources for offshore fishes.

Overall, this study aimed to determine how the offshore food web has changed in Lake Simcoe since the establishment of zebra mussels, and more recently, quagga mussels. Through the analysis of stable isotopes of fish, zooplankton and benthic invertebrate samples, I hoped to provide a more complete picture of food web connectivity, and what energetic linkages currently exist. Additionally, I sought to determine whether shifts in fish isotopic signatures due to changes in diet, or wholesale energy shifts at the base of the food web within the Lake Simcoe ecosystem, have occurred following dreissenid mussel invasion.

Methods

Data acquisition and sampling

Zooplankton vertical hauls were conducted through the euphotic zone using MOE protocol and standard MOECC zooplankton nets; 12.2cm wide, 80cm long, with 80µm mesh (Mike Mueller; personal communication). Duplicate hauls were taken when possible to provide sufficient biomass for stable isotope analysis, and were concentrated into a single standard 500 mL polyethylene container (Appendix 2). Hauls were taken from four sampling stations; K42 in Kempenfelt Bay; K45 near the mouth of Kempenfelt Bay and representative of the centre of the lake; C6 in Cook's Bay and E50 near Georgina Island (Figure 1; Appendix 3). Samples were sorted on a series of metal sieves with mesh sizes of 500, 250, and 125 µm. The dominant taxonomy in each size fraction was noted, however, due to small sample size all taxa were included in isotopic analysis, with the exception of the spiny water flea (*Bythotrephes*

longimanus) which were removed from all samples due to their carnivorous diet (Appendix 4). Samples from 2009 followed similar protocols, with the exception that samples mid-lake were collected from station M66, near K45 (Appendix 3). Zooplankton samples collected in previous years by MOE followed similar procedures as in 2017, and are previously reported in Rennie et al. (2013). Historic zooplankton isotopic signatures from 1982-2008 were of Daphniidae taken at station K45 over all sampling events available in each given year.

Benthic invertebrates were collected from Lake Simcoe during the summer of 2017 at 6 depths (5, 10, 15, 20, 25, 30 m) along a transect from Sibbald Point to station K45 (Figure 1; Appendix 5), following Rennie and Evans (2012). A petite ponar was used to retrieve sediment samples at each site. Three locations were sampled at each depth, within roughly 10 m of one another. Each sample was immediately sorted on a 500 µm mesh and placed into a 500 mL container, with a small amount of lake water added and placed on ice. Samples were then returned to the lab and frozen at -20C. In the lab, benthic invertebrates were sorted to the lowest possible taxonomic level (Appendix 6). Common taxa included *Chironomidae*, *Amphipoda*, *Ephemeroptera*, *Gastropoda*, *Isopoda*, and *Dreissena*, which were further broken down into zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels. Stable isotopes were analysed for each taxonomic group at each depth based on sample availability. Data from previous years were from Rennie et al. (2013).

Fish scales were collected from archives at the Lake Simcoe Fisheries Assessment Unit. Targeted fish species included rainbow smelt, lake herring, lake whitefish, and lake trout. For fish collected from 1960 to 2017, a minimum sample size of six fish was targeted for each species and each year, a sample size shown to have statistical power to detect changes in

isotopic signatures between years of at least 2‰ (Rennie et al., 2009). Specific age classes were targeted for each species based on (a) sample availability to control for variability in mean fish size from year to year and (b) to maintain a reasonable assurance of accuracy in age determinations so as to eliminate size-based changes in fish over time; for instance, scale ages are most reliable at younger ages, and coregonids generally can be aged accurately via scales to age 5 (Rennie et al., 2009), and reasonable agreement between lake trout otoliths and scales is maintained until age 6-8 (Dubois and Lagueux, 1968). For this reason, I chose to target age 2 rainbow smelt, age 5 lake herring and lake whitefish, and age 8 lake trout for analysis.

Due to the small size of rainbow smelt scales, the entire scale was sampled, and often several scales from the same individual were needed to achieve the required weight for stable isotope analysis. Lake herring scales were sampled by cutting a pie-shaped piece of scale to the required weight to allow the entire lifetime diet of the fish to be incorporated in the analysis. Lake whitefish and lake trout scales were sampled to account for the ontogenic shifts in diet these species experience (e.g. planktivorous in early life to benthivorous or piscivorous later in life). This was accomplished by removing the centre of each scale with a 3mm biopsy punch for lake whitefish, or an 18.5-gauge hypodermic needle for lake trout. Approximately 2 years of scale material was removed in each case, and only the outer portion of these scales was analysed to represent adult isotopic signatures (3 years for whitefish, and 6 years for lake trout).

Scale samples of fish from 1960 to 2006 were collected from a variety of MNRF sampling programs including Fall Trap Netting, Winter Creel Survey, and Offshore Benthic Index Netting (OSBIN). Samples from 2007-2017 were collected only from OSBIN, which is a stratified mid-

summer benthic gill netting program. Multiple mesh size gillnets are set on the lake bottom overnight and removed the following day. Netting sites are randomly selected from several areas and depth strata. Sample sites are chosen based on a grid system. Grids consisting of depths greater than 20 m for more than 50% of the grid area were chosen. Shallow strata (15-20 m) were sampled for the years 2011 and 2012 but not sampled for any other years in the OSBIN program. An average of 35-40 sets were completed each year. Three strata are targeted for these sets (Main Basin 20-25 m, Main Basin >25 m, and Kempenfelt Bay > 25 m). Gill nets were made up of 9 panels of differing mesh size. Stretched mesh sizes, in order, were 51 mm, 114 mm, 38 mm, 76 mm, 127 mm, 89 mm, 25 mm, 102 mm, and 64 mm. Total length of set nets was 137.2 m with a panel height of 2.4 m (Langley, 2016). Fish collected using trap nets during 1964 to 2006 were captured in one of two ten-foot downhaul trap nets set at the northeast corner of Georgina Island and the southwest corner of Strawberry Island (Figure 1; Allen, 2005). Winter creel surveys conducted during 1976 to 2005 are whole-lake angler surveys where interviews and angler counts are performed, and scales are taken during angler interviews (LaRose and Langley, 2015). To determine if the use of multiple sampling programs influenced the stable isotope values of fish, an ANOVA was applied to fish from two different sampling programs (see results).

Stable isotope determination

Zooplankton and benthic samples were dried in a drying oven for approximately 48 hours at a temperature of 60°C. Dried samples were then ground using a spatula to create a uniform sample and weighed into tin cups. Fish scales were sampled to incorporate the diet

period of interest (i.e., adult feeding stage or entire lifetime diet as indicated previously). All samples were analysed for stable isotopes of ^{13}C and ^{15}N at Isotope Tracer Technologies Inc. in Waterloo, Ontario. Isotopic values were determined using a Finnigan MAT Delta^{Plus} Isotope Ration Mass Spectrometer (IRMS) (Germany) coupled with a CE instruments Elemental Analyzer EA 1110 CHN (Italy). Three international standards (IAEA-N1, IAEA-N2, IAEA-C6) and four calibrated internal standards, that bracket the samples, are used to correct and normalize data. Results are evaluated and corrected against standards, which are run at the beginning, middle, and end of every run, and reported against the international reference material. The standards used were IT²-13 / IT²-Beet Sugar / IAEA-C6 / Acetanilide (B2000) and IT²-601 / IAEA-N1 / IAEA-N2 / Acetanilide (B2000), for carbon and nitrogen, respectively. The isotope laboratory reported analytical error of C and N determinations at 0.15‰ and 0.3‰, respectively. Duplicate analysis was run for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of 129 randomly selected samples of fish scales to confirm analytical precision. A significant difference was observed for $\delta^{13}\text{C}$ (paired *t*-test; $t = 4.8314$, $df = 127$ *p*-value: < 0.0001 , with a mean of the differences as 0.2411‰, which is biologically a very small difference, well below trophic fractionation (typically on the order of 0.8-1‰) and only slightly greater than reported instrumentation precision (France, 1995). No significant difference was observed for duplicate analyses of $\delta^{15}\text{N}$ ($t = 0.19886$, $df = 127$ *p*-value = 0.8427).

Due to anthropogenic effects, isotopic values of atmospheric carbon (Verburg, 2007) and nitrogen (Holtgrieve et al., 2011) have changed over time. In addition, regions of anthropogenic impact are particularly vulnerable to changes in nitrogen deposited in lakes due to sewage and agriculture (e.g. Hiriart-Baer et al. 2011; Hodell and Schelske, 1998). For this reason, I applied corrections to isotopic values of carbon and nitrogen to all organisms under

investigation. To account for changes in atmospheric carbon and isolate isotopic changes in organisms due to energy source, a Suess correction was applied (Verburg, 2007) by standardizing $\delta^{13}\text{C}$ values of all material analysed to 1960-levels of atmospheric carbon. Similarly, down-core trends in $\delta^{15}\text{N}$ from dated sediments (Hiriart-Baer et al., 2011) were used to isolate isotopic changes in trophic position rather than anthropogenic inputs (as per Rennie et al., 2013). This function was applied to $\delta^{15}\text{N}$ values for the years 1960 to 2017 to correct $\delta^{15}\text{N}$ to 1960 values, calculated as the difference between sediment $\delta^{15}\text{N}$ for any given year and the $\delta^{15}\text{N}$ in 1960. Annual correction factors (e.g., increases in $\delta^{15}\text{N}$ due to anthropogenic activity) were then subtracted from the $\delta^{15}\text{N}$ of the organism, corresponding to collection year.

Trends in fish signatures

To facilitate percent carbon and trophic position estimates, paired estimates of scale and tissue isotopic signatures were required to convert scale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Scale $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were converted to tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using measured correction factors, where appropriate (Table 1). Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in Figure 7, Figure 8, and Figure 9 are also presented as isotopic tissue values for all species.

Trends in the annual means of fish $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for each species were evaluated over time using a segmented least squares linear regression (i.e. “breakpoint analysis”) with the R package *segmented* (version 0.5-0.3; Muggeo, 2008). Significance of breakpoints was conducted using likelihood ratio tests (implemented with ANOVA in R) to compare models with breakpoints to less complex models (e.g., either ones with fewer breakpoints or no breakpoints).

Trends in isotopic signatures of fish over time were analysed by year class rather than the year fish were collected to allow for comparisons to be made among fish of differing ages. Year class was determined to be the year the fish began feeding on an adult diet. For smelt and lake herring, this was assumed to be age 0 (year class = collection year – fish age). For whitefish and lake trout, adult diet was assumed to begin at age 3, since approximately two years of isotopic data was targeted for removal during the preparation of scales for isotope sampling to remove the effects of juvenile diets. Year class in these species was therefore determined to be the age of the fish minus the two juvenile feeding years that had been removed (year class = collection year – (fish age – 2)).

Estimating change of % pelagic resources in the Lake Simcoe offshore fish community

A two-source mixing model was used to determine the proportion of pelagic versus benthic energy present in fish. The $\delta^{13}\text{C}$ for fish and zooplankton were compared to a shallow benthic (5m) $\delta^{13}\text{C}$ end member using the following equation:

$$\% \text{ contribution of littoral} = (\delta^{13}\text{C}_c - \delta^{13}\text{C}_p) / (\delta^{13}\text{C}_b - \delta^{13}\text{C}_p) \quad \text{Eq. 1}$$

where $\delta^{13}\text{C}_c$ is the mean $\delta^{13}\text{C}$ of the consumer, $\delta^{13}\text{C}_b$ is the mean $\delta^{13}\text{C}$ of shallow benthic invertebrates (littoral), and $\delta^{13}\text{C}_p$ is the mean $\delta^{13}\text{C}$ of zooplankton (Vander Zanden and Vadeboncoeur, 2002). Percent carbon was estimated based on year class and therefore averages of $\delta^{13}\text{C}$ for zooplankton and shallow benthos end members were taken for years following the year the adult diet of that fish began. For lake trout and whitefish, $\delta^{13}\text{C}$ values of zooplankton and benthos were averaged over 3 years for age 5 whitefish, and 6 years for age 8 lake trout (for example, zooplankton and benthic invertebrate values were averaged over years

1990-1993 for a whitefish with the year class 1990). For prey items of herring and smelt, $\delta^{13}\text{C}$ values were simply averaged using the age of the fish (i.e., baselines were averaged over the year the fish was born to the year it was collected). Percent littoral carbon for all fish species was evaluated using breakpoint analysis as described for isotopic data.

Estimating change in trophic position in the Lake Simcoe offshore fish community

Trophic position of all fish was calculated using the equation:

$$\text{TP} = ((\delta^{15}\text{N}_c - \delta^{15}\text{N}_p)/3.4) + X, \quad \text{Eq. 2}$$

where $\delta^{15}\text{N}_c$ is mean $\delta^{15}\text{N}$ of the consumer, and $\delta^{15}\text{N}_p$ is mean $\delta^{15}\text{N}$ of the prey species, and X is the trophic position of the prey organism used to estimate $\delta^{15}\text{N}_p$ (Post, 2002). Prey $\delta^{15}\text{N}$ was determined to be the average $\delta^{15}\text{N}$ value for the prey species over the number of years equivalent to the lifetime adult diet of the fish. As with percent carbon, trophic position was estimated based on year class, integrating adult lifetime diet to sampling age, and therefore prey averages were taken for years following the year the adult diet of that fish began, similar to the process described above for $\delta^{13}\text{C}$. Trophic positions of rainbow smelt and lake herring were calculated using zooplankton as their prey species. Mean zooplankton $\delta^{15}\text{N}$ over appropriate years for a given age class of fish was determined by using predicted values for the years 1980 to 2015 based on a linear regression fit to corrected zooplankton $\delta^{15}\text{N}$ values with time (Figure 4). Trophic position of lake whitefish was calculated by using predicted mean $\delta^{15}\text{N}$ values of benthic invertebrates over time provided by a linear regression fit to the observed benthic $\delta^{15}\text{N}$ values integrated over depths of 15m to 30m (Figure 6, D-F; Appendix 7). For lake whitefish, dreissenid mussels were excluded from this regression model and only benthic

invertebrates collected at depths of 15 m or deeper were included (to reflect the profundal feeding habits of this species (Cossu and Wells, 2013; Young and Jarjanzi, 2014). Trophic position of lake trout was calculated using combined mean isotopic values of $\delta^{15}\text{N}$ for prey fish lake herring and rainbow smelt over appropriate years for the year class of fish being evaluated. Trophic position for all species was evaluated using breakpoint analysis.

Results

Trends in invertebrate isotopic signatures

Sample means of isotopic signatures of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for zooplankton collected from sites C6, E50, K42, and K45, during open water periods on Lake Simcoe for the years 2009 and 2017 revealed consistent seasonal trends across all sites, and size classes, over time. Combined averages of all sites and size classes were plotted over sampling months to identify possible trends in seasonal variability (Figure 2). Zooplankton isotopic values sampled from sites K45 and M66 over a number of years were averaged annually and these means were then plotted over time for the years 1986, 1990, 1991, 1997, 1999, 2005, 2008, 2009, and 2017. Sample means for years 1986-2008 consisted of only Cladocera, whereas means for 2009 and 2017 consisted of all taxa over all mesh sizes, with the exception of 250 μm mesh size for 2009 (see Appendix 4). Samples from 2009 in the 250 μm mesh size were removed as they were determined to be significantly different from patterns observed in the 125 and 500 μm mesh, and likely did not represent the targeted Cladoceran taxa present in previous samples. Samples from the 125 μm mesh size were included as they were not significantly different from the 500 μm mesh. Zooplankton isotopic signatures over time were evaluated using linear regression applied to

sample means for each year. This linear regression applied to the corrected zooplankton data over time revealed a significant increase in $\delta^{13}\text{C}$ over time (Figure 4; $F_{1,7} = 11.409$, $p = 0.0118$). No statistically significant trend was found for zooplankton $\delta^{15}\text{N}$ over time when a linear regression was applied (Figure 4; $F_{1,7} = 2.248$, $p = 0.1774$).

Sample means of isotopic values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of benthic invertebrates were plotted over time for each depth, and were also evaluated for trends over time using linear regression applied to sample means for each year. Separate linear regressions were applied for different depth categories; shallow (<15m) and deep ($\geq 15\text{m}$).

Dreissenids have been reported elsewhere to reflect pelagic resources more so than other benthic taxa (Rennie et al., 2009; Rennie et al., 2013). To determine if dreissenid mussels were isotopically different than other benthic taxa in the current study, carbon was plotted against nitrogen for deep-water benthos, dreissenids, and zooplankton (Figure 3). Dreissenids were found to have significantly lower $\delta^{15}\text{N}$ values compared to other benthic invertebrates, and therefore excluded from linear trends of combined benthic invertebrates over time at each depth zone ($t = 9.5$, $df = 10.0$, $p\text{-value} < 0.0001$). This was done to provide a more accurate representation of the benthic baseline, as dreissenid mussels have been shown to have a more pelagic signature than other benthic invertebrates.

Analyses of benthic invertebrate trends excluding *dreissenids* (given their signatures closer to pelagic resources) were used as an end member distinct from the pelagic resource (zooplankton) to generate predictive models for use in estimating trophic position and % littoral carbon contribution. All taxa were included in estimating annual means to build a general relationship for benthic end members (see Appendix 6).

Changes to the dominant taxa of littoral benthic communities have been observed in ecosystems following dreissenid mussel invasion (Jimenez et al., 2011; Ozersky et al., 2011a), and often result in a change in benthic isotopic signatures based on feeding strategy of predominant invertebrates present (i.e. more pelagic signatures with increased filter feeding species presence). However, taxa used for stable isotope analysis was fairly consistent over time, with the exception of samples taken in 2017, which were exclusively chironomids (Appendix 8). Standardized weighted samples for each benthic invertebrate taxon ensured that the same amount of organic material was analysed for each group of invertebrates. Therefore, changes in benthic invertebrate community composition were not likely to have a major influence on stable isotope trends. To test this, t-tests were applied to each group of invertebrate taxa to determine any significant differences between isotopic values of taxonomic groups for all years. No significant differences in carbon isotopic values, with the exception of a slightly significant difference found between *Ephemeroptera* and *Pelecypoda* ($t = 2.3011$, $df = 8.0285$, $p\text{-value} = 0.0503$), were found for any species when t-tests were applied. In addition, feces and pseudofeces may be capable of influencing the availability of nutrients, including nitrogen, to organisms in areas with heavy dreissenid establishment (Gergs et al., 2011; Izvekova and Lvova-Katchanova, 1972). Consumption of these feces and pseudofeces may influence organism $\delta^{15}\text{N}$ values. To control for this, down-core sediments samples were used to create a predictive model of sediment $\delta^{15}\text{N}$ values over time, with which corrections were applied to organism $\delta^{15}\text{N}$ values to account for possible increases in sediment nitrogen values over time (see methods). Furthermore, benthic invertebrate $\delta^{15}\text{N}$ signatures for shallow sites (5-10m) were observed to decrease over time, reflecting more littoral signatures than

those observed even before dreissenid mussel invasion (Figure 6). Therefore, it is unlikely that the incorporation of feces and pseudofeces into benthic invertebrate diets would produce a misleading shift towards pelagic resources regardless of the fact that feeding habits of offshore fish may have remained consistently littoral.

A linear regression of corrected benthic invertebrate isotopic signatures revealed a trend towards less negative $\delta^{13}\text{C}$ signatures over time for shallow sites (5 and 10 meter depths, combined; Figure 5 A, B; $F_{1,5} = 79.995$, $p = 0.0003$). Little change was observed for values of $\delta^{13}\text{C}$ for sites 15 meters and deeper (Figure 5 C-F), however a linear regression applied to sites 15 to 30 m deep (combined) revealed a significant increase in carbon isotopic values over time (Appendix 7; $F_{1,5} = 24.592$, $p = 0.0043$). All sites were observed to have trends of decreasing $\delta^{15}\text{N}$ values over time (5-10m: $F_{1,5} = 22.959$, $p = 0.0049$; $\geq 15\text{m}$: $F_{1,5} = 11.29$, $p = 0.0201$; Figure 6).

Benthic $\delta^{13}\text{C}$ from 5m sites was used for percent littoral calculations as it provided a representation of the benthic-littoral energy pathway. Isotopic signatures from 5m sites also provide an end member capable of encapsulating fish isotopic signatures between the benthic-littoral and pelagic end members. Mean $\delta^{15}\text{N}$ from sites 15-30m was used for trophic position calculations to provide a representation of benthos available to cold water benthivorous fish below the thermocline such as whitefish.

Trends in fish isotopic signatures

Fish diet is known to vary seasonally (Rennie et al., 2009). Though scale samples used in my isotopic analyses integrate information over years, the sources of scales span collections

over several sampling programs which target fish at different times of year (e.g., winter creel vs. fall trapnetting vs. summer OSBIN sampling). I therefore sought to determine if there were significant isotopic differences across methods of sampling. To do this, I evaluated differences between sampling methods that were most extremely different in their seasonal distribution (i.e., OSBIN and winter creel). The species most commonly sourced from multiple sampling programs were lake whitefish and lake trout. Therefore, an ANOVA was applied to data for these two species from both sampling programs for the years 2003, 2004, and 2005, examining differences among these three years and both sampling programs which were active during this time. Six fish of each species were selected for each year for both sampling programs. Age class selection was consistent with other fish samples for this study (i.e. age 5 for lake whitefish and age 8 for lake trout). No significant difference was found between fish from either sampling method for carbon ($F_{1,60} = 0.418, p = 0.520$) or nitrogen ($F_{1,60} = 0.43, p = 0.836$). Higher-order interaction terms in the ANOVAs were non-significant with the exception of a year-by-species interaction for $\delta^{15}\text{N}$, which was to be expected considering the difference in trophic position (and therefore isotopic signatures) between whitefish (benthivores) and lake trout (predators).

In addition, a stocking program for lake whitefish and lake trout (using gametes collected in the field during fall egg collection; Vandermeer, 2007), has been in place on Lake Simcoe since the 1980s for whitefish and since the 1960s for lake trout. Gametes are fertilized and then raised in a fish hatchery and released back into the lake as fingerlings. Until the mid-2000s, most fish in the lake were stocked. However, natural recruitment increased in the early 2000s (Young and Jarjanzi, 2014). Thus, changes in the proportion of stocked vs. wild fish might affect our analysis if these fish differ in terms of their feeding habits as adults. To determine if

the initial life history of a fish (stocked vs. wild) had an influence on their adult isotopic signature, an ANOVA was applied to data for lake trout and lake whitefish for the years 2008, 2009, and 2010. Six fish of each species were selected for each year and each of the two stocking statuses (stocked vs. wild) from the OSBIN sampling program. These years were chosen based on the availability of stocked and wild fish of both species for the appropriate age class. Again, age class selection was consistent with other samples for these species in this study. ANOVA results indicated no difference in foraging habits of adults between wild and stocked fish; no significant difference in carbon or nitrogen signatures was found between stocked and wild fish for either species ($F_{1,59} = 0.25, p = 0.87, F_{1,59} = 0.124, p = 0.726$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively). Higher-order interaction terms were non-significant (all $p \gg 0.05$).

Common patterns among trends in $\delta^{13}\text{C}$ among offshore fish species were apparent upon visual inspection (Figure 7A); nearly all species showed an increase in $\delta^{13}\text{C}$ towards increased benthic production during a year class that was coincident with *D. polymorpha* establishment (within 1-2 years of 1996; Figure 7A). All species then appeared to show trends towards more negative values for year classes from 2000 to 2009 (year of *D. bugensis* establishment) followed by another increase towards benthic production in coregonids (lake herring and lake whitefish, Figure 7A). Similarly, all species show a strikingly consistent decrease in $\delta^{15}\text{N}$ following the 2000 year class (Figure 7B).

Breakpoint analysis revealed significant breakpoints for year classes 1999 and 2001 in rainbow smelt $\delta^{13}\text{C}$ (Figure 8A; Table 2; $F_{22,26} = 10.813, p < 0.0001$). For year classes from 1960 to 1999, $\delta^{13}\text{C}$ was relatively stable, then became less negative sharply to 2001 and then became more negative to 2014. Year classes of lake herring $\delta^{13}\text{C}$ values appeared to become less

negative prior to 2000 but became more negative thereafter (Figure 8B; $F_{22,26}$: 3.351, p : 0.0251). Year classes of lake whitefish $\delta^{13}\text{C}$ increased over time with a significant increase after 2011 (Figure 8C; $F_{31,27}$: 11.607, p : <0.0001), and there were no significant breakpoints or temporal change observed in lake trout $\delta^{13}\text{C}$.

Rainbow smelt $\delta^{15}\text{N}$ year classes were found to have significant breakpoints, corrected for fish age, in 1961 and 2006, with a large decrease after 2006 (Figure 9A; Table 2; $F_{26,22}$: 28.561, p : <0.0001). Signatures of $\delta^{15}\text{N}$ for lake herring were found to significantly decrease after the 2001 year class, resulting in a significant breakpoint (Figure 9B; Table 2; $F_{29,27}$: 78.421, p : <0.0001). Lake whitefish were found to have decreasing $\delta^{15}\text{N}$ with significant breakpoints, corrected for age, observed in both the 1994 and 2007 year classes (Figure 9C; Table 2; $F_{31,27}$: 16.335, p : <0.0001). Lake trout $\delta^{15}\text{N}$ signatures also decreased over time with significant breakpoints for the year classes in 1990 and 2010 (Figure 9D; Table 2; $F_{28,24}$: 26.966, p : <0.0001).

Estimating change of % pelagic resources in the Lake Simcoe offshore fish community

A two-source mixing model revealed the proportion of littoral vs. pelagic energy for each fish species over time. For all species except lake whitefish, the percent of littoral energy consistently decreased over time with no significant breakpoints detected (Figure 10A,B,D; $F_{20,18}$: 1.5125, p : 0.2471, $F_{18,16}$: 2.864, p : 0.0846, $F_{14,12}$: 0.9827, p : 0.4025, respectively). A significant negative linear regression was found for all species (smelt: $F_{1,20}$: 69.44, p : < 0.0001, herring: $F_{1,18}$: 109.7, p : < 0.0001, whitefish: $F_{1,23}$: 21.47, p : 0.0001, lake trout: $F_{1,14}$: 56.5, p : < 0.0001). Lake whitefish had a significant breakpoint in the 2011 year class (two years after

quagga mussel establishment) followed by a sharp increase in the percent littoral energy consumed (Figure 10C; $F_{23,19}$: 7.0604, p : 0.0012).

Estimating change in trophic position in the Lake Simcoe offshore fish community

Trophic position estimates revealed differing trends over time for fish species analysed. A significant breakpoint was found in the 2006 year class for the trophic position of rainbow smelt ($F_{19,17}$: 50.953, p : <0.0001); rainbow smelt trophic position was consistent across year classes and then appeared to decrease from the 2006 to 2012 year classes (Figure 11A). Trophic position of rainbow smelt declined by 0.70 trophic positions between the 2006 and 2014 year classes.

Similar trends were observed for trophic position of herring over time. Trophic position of herring revealed a significant breakpoint in the 2000 year class ($F_{17,15}$: 76.684, p : <0.0001); the trophic position of lake herring increased slightly until the 2000 year class, and then decreased significantly until the 2011 year class (Figure 11B). Trophic position of herring was found to be depleted by more than one trophic position (1.4) between the 2000 and 2011 year classes.

A significant breakpoint for lake whitefish trophic position was found in the 2006 year class ($F_{22,20}$: 11.067, p : <0.0001). Trophic position of whitefish was observed to increase until the 2006 year class and then decreased until the 2013 year class (Figure 11C). Whitefish trophic position was found to decrease by 0.37 trophic positions between the 2006 and 2013 year classes. To determine if the exclusion of dreissenid mussels in the benthic invertebrate baseline used to calculate whitefish trophic position influenced trends in whitefish trophic position,

trophic position was plotted for whitefish using both benthic invertebrates excluding dreissenids and benthic invertebrates including dreissenid mussels. Trends in trophic position remained consistent over time, with the baseline including mussels producing a slightly lower trophic position value than that calculated excluding mussels (Appendix 9).

Trophic position for lake trout was calculated from the mean $\delta^{15}\text{N}$ values of prey fish herring and smelt (Figure 11D). For lake trout trophic position, a significant breakpoint was found in the 2004 year class ($F_{17,15}$: 6.0191, p : 0.0121). The trophic position of lake trout was found to increase by 0.34 trophic positions between the 2004 and 2006 year classes.

Discussion

Our observations provide compelling evidence of both long-term and punctuated changes in Lake Simcoe over the past 4 decades revealed by the isotopic patterns in the offshore fish species of the lake, relative to their putative energy sources, though not necessarily in the direction predicted. Punctuated changes were best represented by changes in $\delta^{15}\text{N}$ isotopes and estimates of trophic position. Values of $\delta^{15}\text{N}$ for fish appeared to all decline consistently in year classes associated with the early to mid-2000s for each of the four species analysed, and significant changes in trophic position were determined for year classes within a six-year window (2000 to 2006). Results for $\delta^{13}\text{C}$ indicated a consistent trend towards less negative, and therefore more nearshore, values following zebra mussel establishment, showing a maximum value for year classes of fish from the early to mid-2000s. This was followed by a trend towards more negative, and therefore more pelagic, values occurring prior to the establishment of quagga mussels in 2009. There was an apparent increase in $\delta^{13}\text{C}$ again for year

classes of herring and whitefish following quagga establishment, however it is unclear as to whether these changes towards more nearshore resources will be ongoing given the variation observed in this system over the past 3 decades reported here. Long-term changes observed in this study reflected in energy sourcing for offshore fishes demonstrated a consistent decline of % littoral energy contribution over time among species, although one punctuated change in lake whitefish towards more littoral reliance in most recent year classes was observed.

Rainbow smelt and lake herring trophic positions both decreased significantly over time. Smelt trophic position was shown to decrease by 0.70 trophic positions for year classes between 2006 and 2014, suggesting a greater reliance on herbivorous zooplankton rather than invertebrate predators relative to previous years. Herring was also observed to decrease even more than rainbow smelt (1.4 trophic positions) for year classes between 2000 and 2011, suggesting a greater reliance on herbivorous zooplankton rather than zooplankton predators such as *Mysis* and *Chaoborus spp.*. This is an interesting result considering the invasion of the zooplankton predator, *Bythotrephes longimanus* to Lake Simcoe in 1993 (Young and Jarjanzi, 2014). Although the abundance of *Bythotrephes* has been steadily decreasing since 1999 (Young and Jarjanzi, 2014) they were shown to be abundant in 2002 and 2005 (Kelly et al., 2013). However, this was not reflected in an increased trophic position for year classes of either rainbow smelt or lake herring occurring during those years (2000-2005). Lake herring have been known to feed on *Bythotrephes*, effectively increasing herring trophic position as a result (Coulas et al., 1998; Kelly et al., 2013; Rennie et al., 2011). Additionally, the impact of *Bythotrephes* on zooplankton communities has also been estimated to increase the overall trophic position of the zooplankton community as a consequence of community changes after

Bythotrephes invasion (Rennie et al. 2011). Despite this, trophic position of herring was observed to decrease over the time period when *Bythotrephes* was abundant in the lake. This decrease also occurred despite evidence of a change in the summer diets of lake herring from chironomids and cladocerans in 1983 to primarily *Bythotrephes* in 2009 (90% *Bythotrephes* contribution; Rennie and Evans unpubl.). One possible explanation for this decrease may be a reduced abundance of native zooplankton predators such as *Mysis* and *Chaoborus spp.*. Current and long-term abundance data regarding these native invertebrate predators are data deficient for Lake Simcoe.

The trophic position of whitefish varied over time, but was found to decrease by 0.37 trophic positions for year classes between 2006 and 2013, with a significant breakpoint found for the 2006 year class. Visual inspection of stomach contents of whitefish for the sampling years 2015 and 2016 indicated a heavy reliance on dreissenids (Langen, personal observation). Dreissenid mussels have been shown to have lower $\delta^{15}\text{N}$ values compared to other benthos (Figure 3), therefore a decrease in whitefish trophic position would be expected with an increase in dreissenid consumption. Rennie and Evans (unpubl.) observed the addition of both dreissenid mussels and *Bythotrephes* in lake whitefish diets in 2009 compared to 1983 diets. Though 2009 lake whitefish diets were still dominated by chironomid consumption, dreissenid mussels and *Bythotrephes* accounted for 11% and 14% of whitefish diets, respectively.

Lake trout, baselined to a combination of herring and smelt, were shown to have a significant change in trophic position in the 2004 year class. Trophic position remained fairly consistent until the 2004 year class, but then increased by 0.34 trophic positions with the 2006 year class. One possible explanation for the increase observed is the addition of herring as a

prey fish species found in lake trout diets. A detailed diet study (Adkinson, 2013) done for lake trout in Lake Simcoe between the years 1983 and 2008 indicated lake trout diets for years 1999 to 2002 were dominated by rainbow smelt, with species in the sculpin family as the secondary taxa observed. Between the years of 2003 and 2012, although still dominated by rainbow smelt, more reliance on yellow perch, whitefish subfamilies (*Coregonus spp.*) and minnow families was observed. The once depleted herring population also began to increase in abundance in 2006. The decrease in $\delta^{15}\text{N}$ of prey fish such as herring, smelt, and yellow perch might help to explain the increase in trophic position seen in lake trout (increasing separation between predator and prey), however, if this was the only explanation for the increase in lake trout trophic position, one would expect the $\delta^{15}\text{N}$ of lake trout to decrease at the same rate as their prey. That they did not, suggests the addition of a prey item to lake trout diets with higher a higher $\delta^{15}\text{N}$ signature. Interestingly, when lake trout trophic position was calculated based on an exclusively herring baseline, trophic position was observed to increase more dramatically than when combined with the baseline of rainbow smelt (Appendix 10). This is likely due to the rapid decrease in herring $\delta^{15}\text{N}$ observed following the 2000 year class; a period of time in which rainbow smelt with historically lower $\delta^{15}\text{N}$ has higher $\delta^{15}\text{N}$ values than herring. One possible prey item addition is whitefish. Whitefish were found to have higher $\delta^{15}\text{N}$ than herring or smelt, and with increased natural recruitment as well as heavy stocking inputs of young whitefish to the lake, may have provided an adequate food source to be incorporated into lake trout diets. This increase in whitefish consumption would also be consistent with increased observations of *Coregonus spp.* in lake trout stomachs by Adkinson, (2013). Similarly, smaller, younger lake trout may have also increased in adult lake trout diets. With increased natural recruitment

following 2003 and heavy stocking of young lake trout until 2010, the potential of young lake trout as a prey item of adult lake trout may have increased for year classes following 2004. Although there have been only a few reported occurrences of brook trout or lake trout in lake trout stomachs in 2007, there were many unidentified fish species in lake trout stomachs between 1983 and 2012 (Adkinson, 2013). Considering the high $\delta^{15}\text{N}$ of age 2 lake trout (Appendix 11) relative to rainbow smelt and lake herring (typically elevated by 2.5‰ compared with either rainbow smelt or herring), the introduction of young lake trout to adult lake trout diets could be a likely explanation for the recent increase in adult lake trout trophic position.

Though the introduction of round goby to Lake Simcoe may also be speculated to have had an effect on contemporary estimates of lake trout trophic position, an examination of the published literature on isotopic position of these fishes in Lake Simcoe suggests otherwise (Rennie et al., 2013). Round goby were introduced to Lake Simcoe in 2004, and were firmly established by 2010 (Young and Jarjanzi, 2014). Although round gobies were present in the lake, and have been known to become incorporated in diets of lake trout in other systems, such as Lake Ontario (Dietrich and Hoyle, 2006), evidence of their inclusion in lake trout diets was not apparent up to 2012 (Adkinson, 2013). However, in the 2016 sampling year, round gobies were observed in lake trout stomachs (Langen, personal observation). Round gobies were found to have lower $\delta^{15}\text{N}$ than yellow perch, herring, and rainbow smelt (Rennie et al., 2013) and would be expected to result in a decrease in lake trout trophic position if incorporated into lake trout diets in the future in significant numbers.

Interestingly, the increase in trophic position of lake trout occurs despite the invasion of *Bythotrephes* to Lake Simcoe in 1993. Cold water fish species including herring, whitefish, and

lake trout have been known to feed on *Bythotrephes*, and *Bythotrephes* have been found in the stomachs of each of these fish in Lake Simcoe (Amtstaetter, 2000; MacRae, 2001; Johanson and Amtstaetter, 2004). However, if lake trout have increased their consumption of *Bythotrephes* in place of fish in most recent years, it has not caused a decrease in trophic position thus far. Conversely, a reduction of predation on *Bythotrephes* in combination with prey fish to a primarily prey fish dominated diet between earlier year class years and the 2004 year class may help to explain the shift towards a higher trophic position for lake trout, as herring became more abundant and *Bythotrephes* populations began to decrease during this time period.

Contrary to my original hypothesis, the percent littoral derived carbon was observed to decrease over time for all species, though for whitefish, littoral-derived resources did become more important in the two most recent year classes. The high percentage of littoral energy historically, changing to pelagic derived carbon in most recent years was one of the most surprising results observed in this study. Historically there has been strong evidence of shifts away from pelagic production and towards more nearshore energy production in many other dreissenid-invaded ecosystems (Fera et al., 2017; Hecky et al., 2004; Rennie et al., 2013, 2009; Rush et al., 2012). Based on changes in $\delta^{13}\text{C}$, a shift from pelagic energy acquisition to more littoral resources can be observed for all species in year classes between 1996 and 2000, which is the expected response to zebra mussel invasion (1996), however, by the early to mid 2000s all species seem to have recovered from this initial invasion and begun to shift in the opposite direction.

Although the exact cause of the increase in pelagic energy consumption in recent years is not clear, there are several possible explanations for this shift. One possibility is a natural

correction of energy flow after the initial shock to the system that occurred immediately after mussel invasion. Over time, zebra mussel populations have decreased (Lake Simcoe Region Conservation Authority, 2016; Young and Jarjanzi, 2014), possibly allowing for decreased effects on energy flow through the nearshore as a result. It was reported that mussel abundance dropped from 32,529 mussels/m² in 1996 to 5,101 mussels/m² by 2005 (Young and Jarjanzi, 2014). With such a large decline in zebra mussel population prior to the invasion of quagga mussels in 2009, the driving forces of energy relocation would likely have been minimized, allowing for a natural shift back towards historical pelagic energy sources. Another hypothesis is the exclusion of cold water fish by warm water fish in the littoral zone. Vander Zanden et al. (1999) highlighted that it is possible for the nearshore community to effectively exclude cold water fish from taking advantage of nearshore resources. However, this may not be the case in Lake Simcoe. The Nearshore Community Index Netting (NSCIN) program completed by the Lake Simcoe Fisheries Assessment Unit has indicated that for most species in the nearshore community, aside from common carp (low abundance) and bluegill (high abundance), the average catch has not increased over time (Young and Jarjanzi, 2014). In addition, the average catch of certain species such as white sucker and smallmouth bass have actually decreased over time (Young and Jarjanzi, 2014). Although there has been an increase in yellow perch in the nearshore, it is not as great as the increase in yellow perch that has been observed in offshore sampling programs (Young and Jarjanzi, 2014).

It is important to note that the baseline $\delta^{13}\text{C}$ values for zooplankton and shallow benthos are historically very similar (difference of 3.9‰ in 1976 compared to a difference of 7.5‰ in 2015), which may explain the seemingly high percentage of littoral derived carbon

observed in the 1980s due to lack of differentiation between baselines. Greater uncertainty in earlier estimates of energy sources is expected as small shifts in the consumer relative to the end member during this time period could be seen as large differences of where carbon is being derived. Baseline $\delta^{13}\text{C}$ values for zooplankton and shallow benthos are becoming more differentiated over time, making it easier to distinguish between carbon sources.

Regardless of potential uncertainty in these early estimates, it is clear that carbon is becoming more pelagic-derived for cold water offshore fish species in Lake Simcoe over time. It does, however, appear that for herring and whitefish, the percent of littoral carbon has increased over the most recent year classes from 2009 onward. This is an interesting result as quagga mussels were known to become established in Lake Simcoe by 2009 (Young and Jarjanzi, 2014). Although detailed information is not available to determine the exact time that quagga mussels became established in Lake Simcoe, this result would be consistent with the expected response of a quagga mussel invader. However, quagga mussels have been observed to inhabit both shallow and very deep areas of the lake, including Kempenfelt Bay, and therefore may not cause a significant shift in energy allocation from offshore to nearshore, but rather from pelagic energy production to primarily benthic. Vanderploeg et al. (2010) suggested such a shift, termed the mid-depth sink, in Lake Michigan. Certain nearshore areas of Lake Michigan with sandy substrates and high wave action prevented dreissenid mussels from becoming established and therefore dreissenids began to inhabit mid-depth regions of the lake (30-50m) and appeared to be successfully intercepting C and P that would normally move from nearshore to offshore.

Considering the age of the fish used for this study and the fact that all data was expressed to represent the age class of when the fish began feeding on the prey of interest, it would be interesting to see if the observed trends highlighted in this study are continued in the future, particularly with regards to invasion of quagga mussels. Specifically, trends towards more littoral resources, or possibly benthic, for lake herring and lake whitefish may continue with respect to quagga mussel distribution in future years. In addition, more detailed diet studies for recent sampling years would be beneficial to determine what changes in diet may be driving the significant changes in trophic positions of offshore fish species.

In conclusion, I observed both punctuated and gradual changes of significance in the Lake Simcoe community. Overall, trophic position for all fish appears to change within a few years of mussel invasion. This result however may not be directly related to mussel invasion for all species, and for smelt, herring, and lake trout, is likely the result of other factors affecting Lake Simcoe. Such factors include changes to the zooplankton community, as well as fish stocking and increased natural recruitment of lake trout and whitefish, and increased abundance of the natural prey fish, lake herring. Trophic position of whitefish does appear to reflect increased dreissenid abundance, as observations of whitefish stomachs in recent years suggests heavy reliance on dreissenids. The most interesting result is the high percentage of pelagic derived carbon observed for all species in the most recent sampling years. Although a shift towards more nearshore energy acquisition is apparent following zebra mussel invasion in 1996, this shift is quickly corrected and followed by even more pelagic energy signatures. Lake herring and lake whitefish are observed to have an increase in percent littoral carbon following

2009, the year quagga mussels were abundant in Lake Simcoe, however it is unknown if this trend will continue into the future.

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Figures

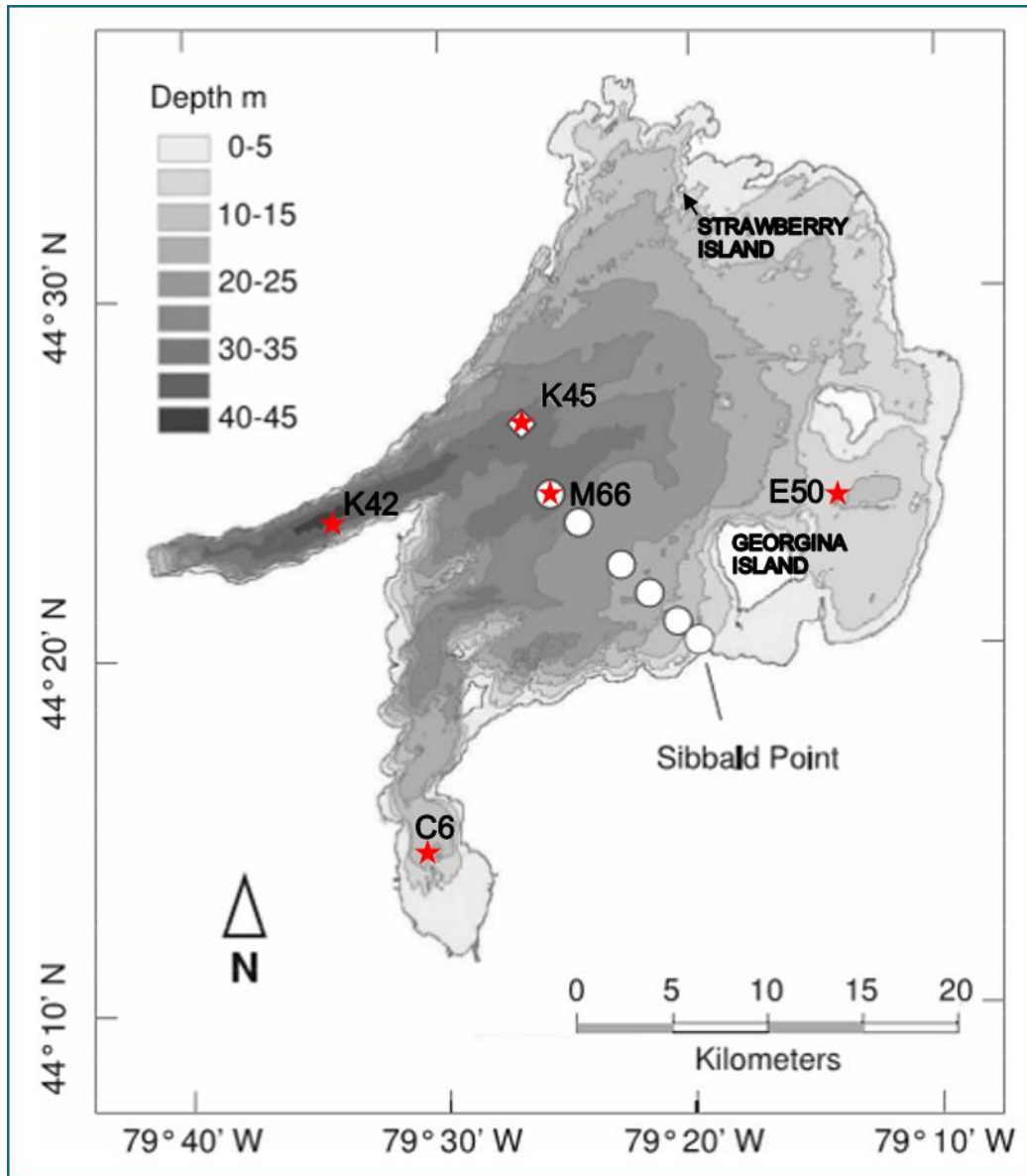


Figure 1: Sample sites for zooplankton, and benthic invertebrates collection from Lake Simcoe, ON. Zooplankton were collected at sites C6, E50, K42, K45, and M66. Benthic invertebrates were sampled at depths of 5, 10, 15, 20, 25, and 30 m along the transect from Sibbald Point to site K45. (Base graphic: Rennie and Evans (2012)).

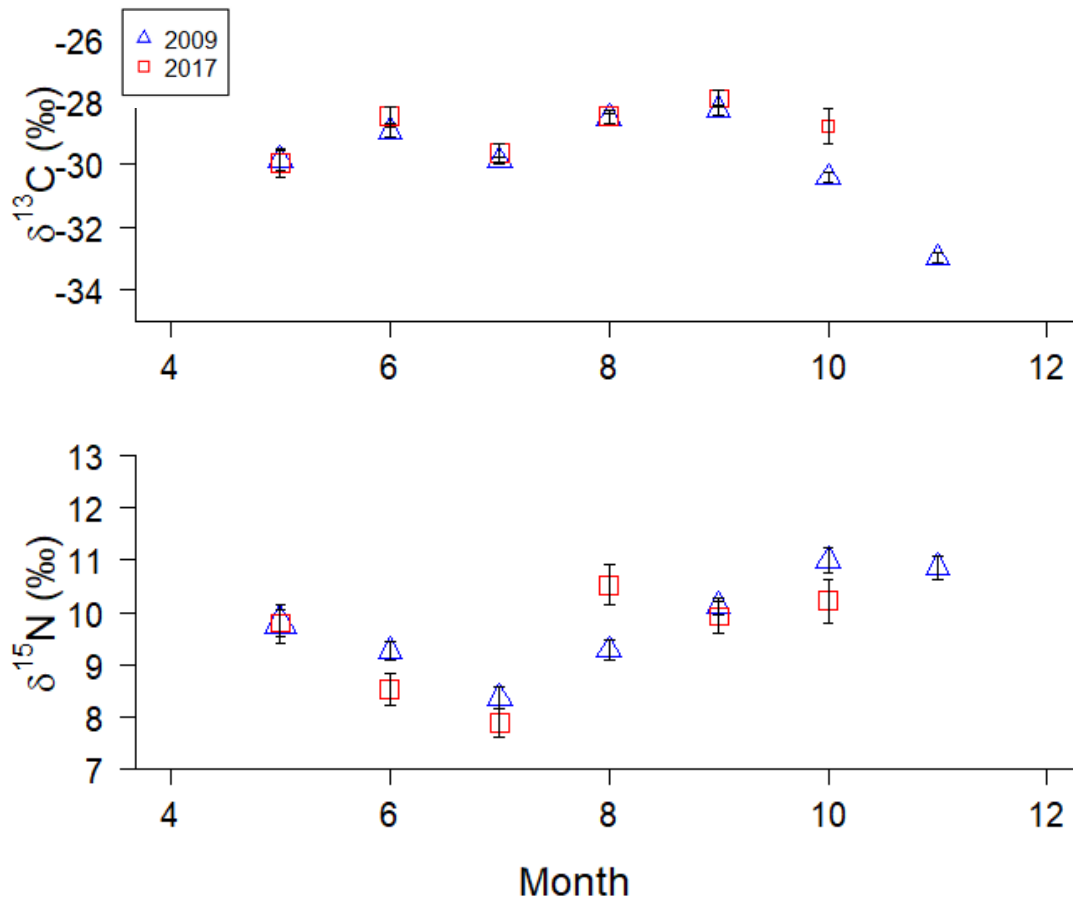


Figure 2: Seasonal variation in isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for zooplankton collected from stations C6, E50, K42, K45, and M66 in Lake Simcoe, ON, during ice free period for the years 2009 and 2017. Isotopic values were corrected for Suess effect ($\delta^{13}\text{C}$) according to Verburg (2007) and anthropogenic N influences ($\delta^{15}\text{N}$) as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ values and mean $\delta^{15}\text{N}$ values for samples collected in all mesh sizes (63, 125, 250, 500, and 1000 μm).

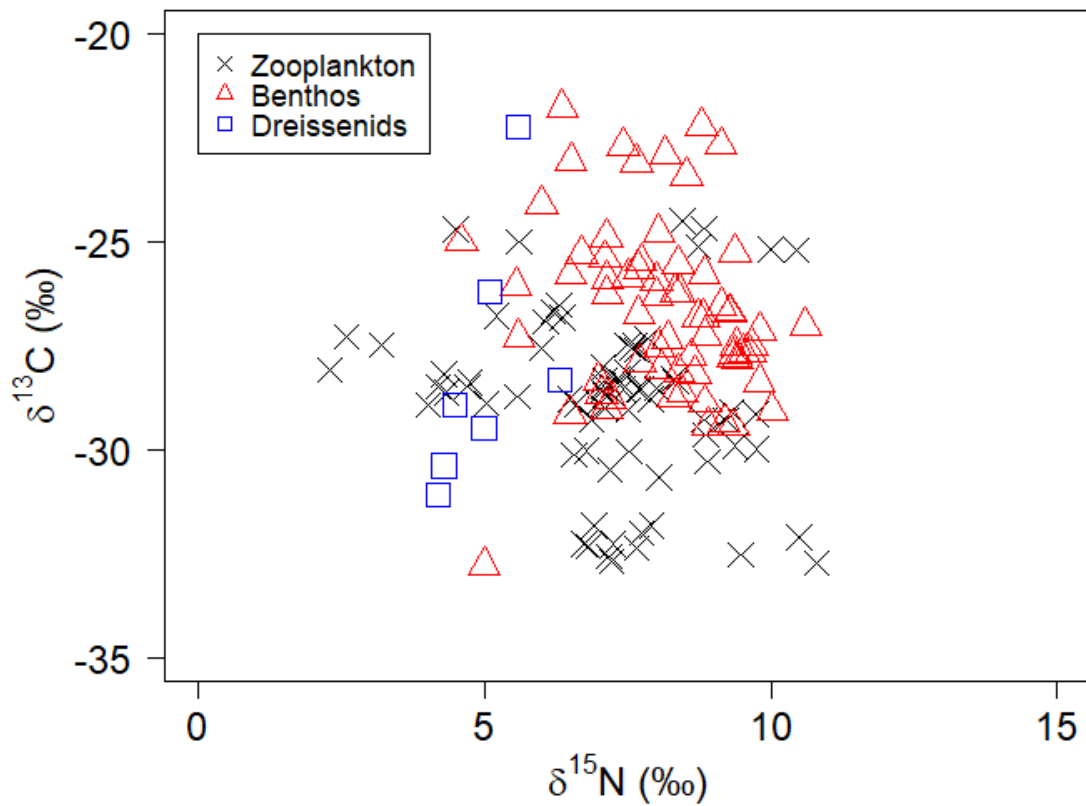


Figure 3: Biplot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from zooplankton collected at site K45 (black Xs), profundal benthos ($\geq 15\text{m}$ depth) excluding dreissenids (red triangles), and profundal dreissenids ($\geq 15\text{m}$ depth, blue squares) for the years 2008, 2009, and 2017. Isotopic values were corrected for Suess effect ($\delta^{13}\text{C}$) according to Verburg (2007) and anthropogenic N influences ($\delta^{15}\text{N}$) as per Rennie et al. (2013). Points are individual measurements. Zooplankton samples sorted on mesh sizes 125, and 500 μm for 2009, and mesh sizes 125, 250, and 500 μm for 2017 were included.

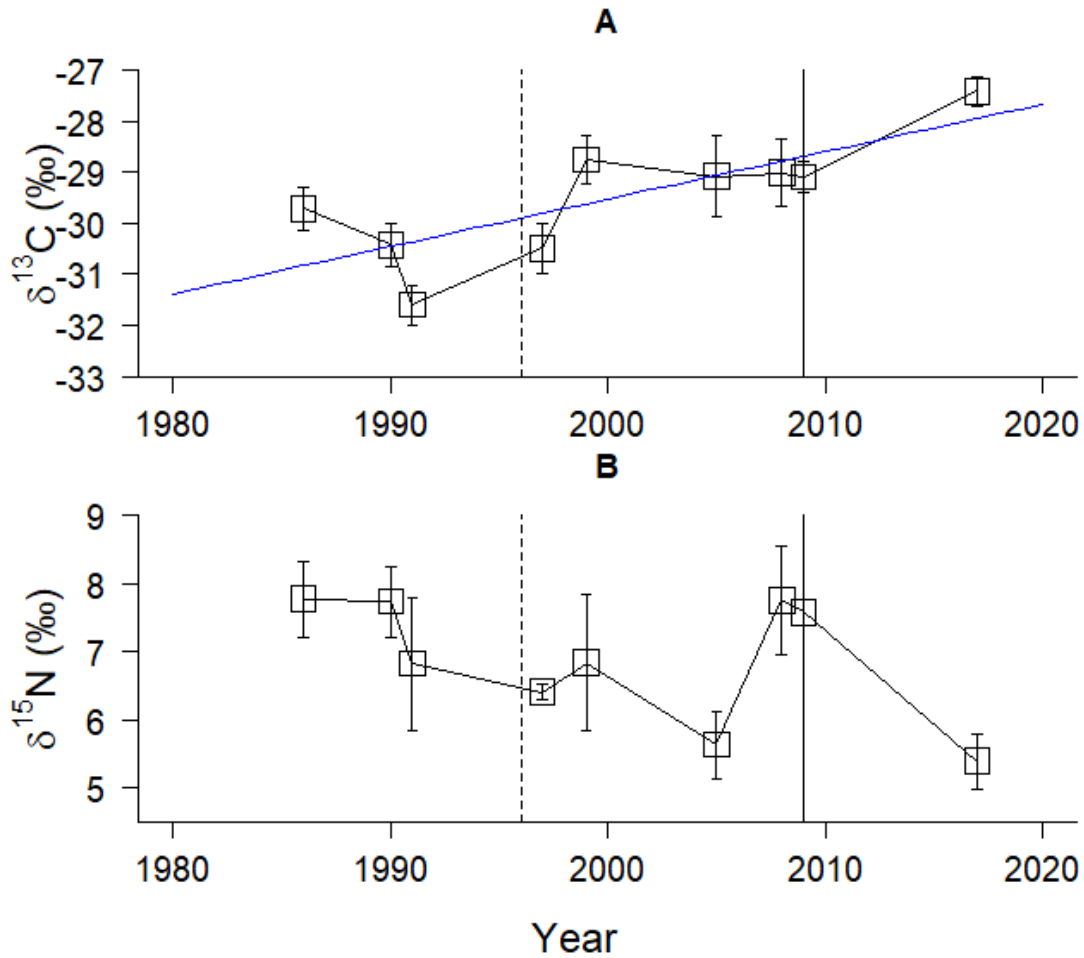


Figure 4: Isotopic values of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) over time for zooplankton collected at stations K45 and M66 in Lake Simcoe, ON. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). $\delta^{15}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ and mean $\delta^{15}\text{N}$ values; line is regression line. Samples means were calculated from samples sorted on mesh sizes 125 and 500 μm for 2009, and mesh sizes 125, 250, and 500 μm for 2017.

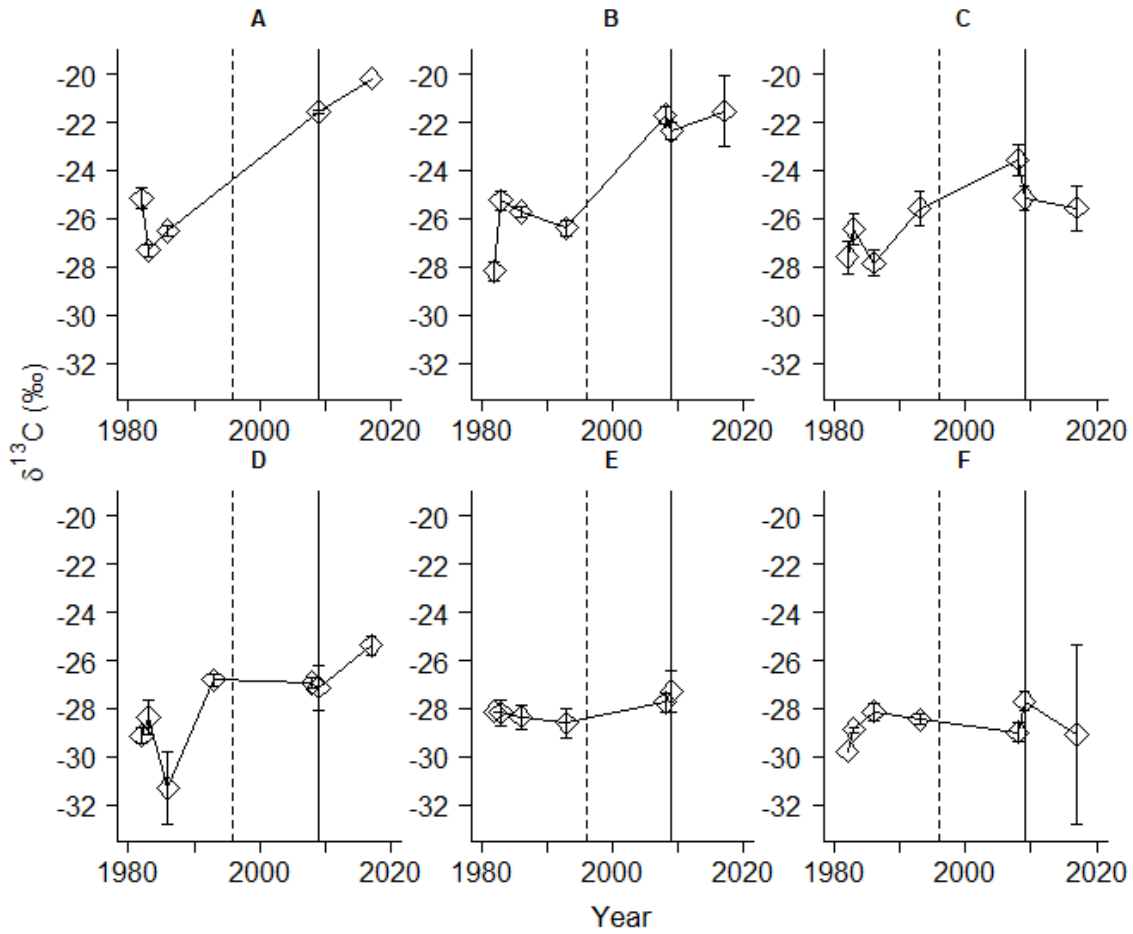


Figure 5: Isotopic values of $\delta^{13}\text{C}$ over time for benthic invertebrates, excluding dreissenids, collected from Lake Simcoe, ON. Values of $\delta^{13}\text{C}$ are plotted by depth; A = 5m, B = 10m, C = 15m, D = 20m, E = 25m, F = 30m. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). Points are mean $\delta^{13}\text{C}$ values.

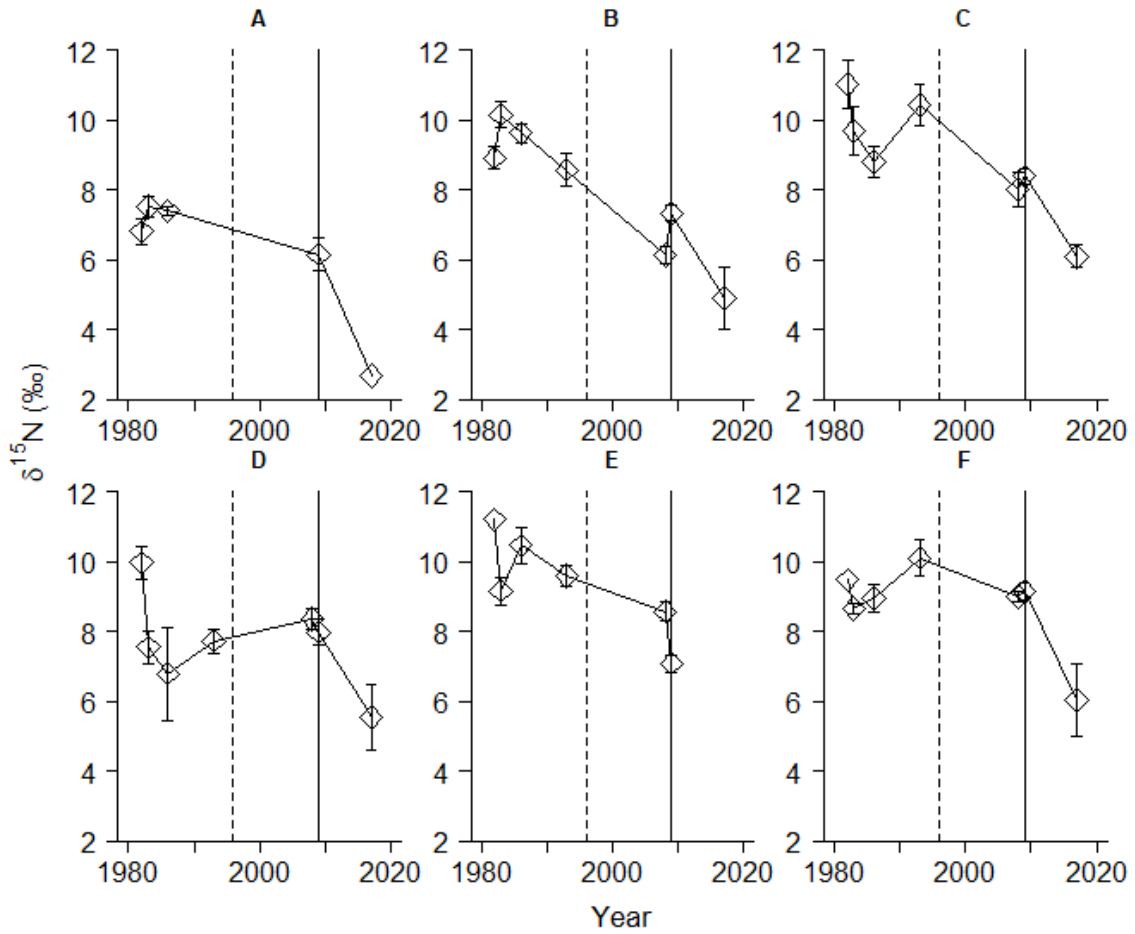


Figure 6: Isotopic values of $\delta^{15}\text{N}$ over time for benthic invertebrates, excluding dreissenids, collected from Lake Simcoe, ON. Values of $\delta^{15}\text{N}$ are plotted by depth; A = 5m, B = 10m, C = 15m, D = 20m, E = 25m, F = 30m. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{15}\text{N}$ values.

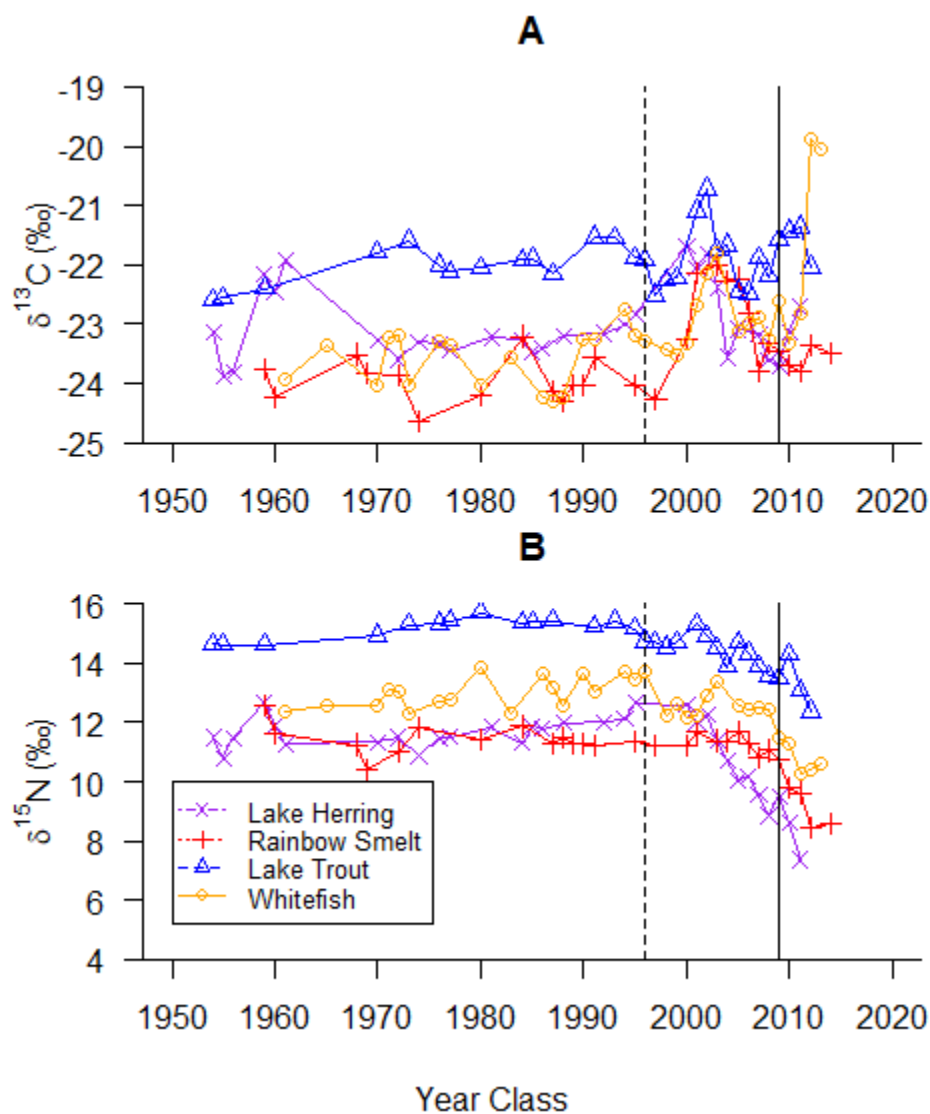


Figure 7: Isotopic values of $\delta^{13}\text{C}$ (A) and $\delta^{15}\text{N}$ (B) over time measured from fish scale samples, and corrected to represent fish tissue isotopic values, collected from Lake Simcoe, ON. $\delta^{13}\text{C}$ values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). $\delta^{15}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ values and mean $\delta^{15}\text{N}$ values.

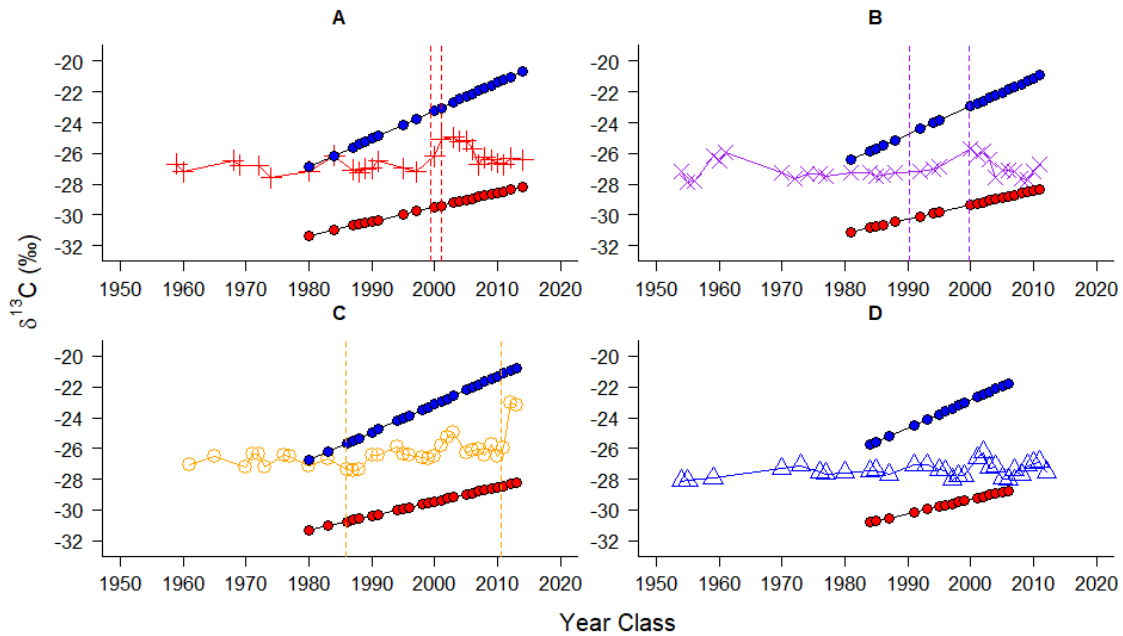


Figure 8: Breakpoints for isotopic values of $\delta^{13}\text{C}$ over time measured from fish scale samples, and corrected to represent fish tissue isotopic values, collected from Lake Simcoe, ON. Values of $\delta^{13}\text{C}$ are plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. $\delta^{13}\text{C}$ values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). Vertical dashed lines represent significant breakpoint years. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). Points are mean $\delta^{13}\text{C}$ values. Red line is zooplankton baseline; blue line is shallow benthos (5m) baseline.

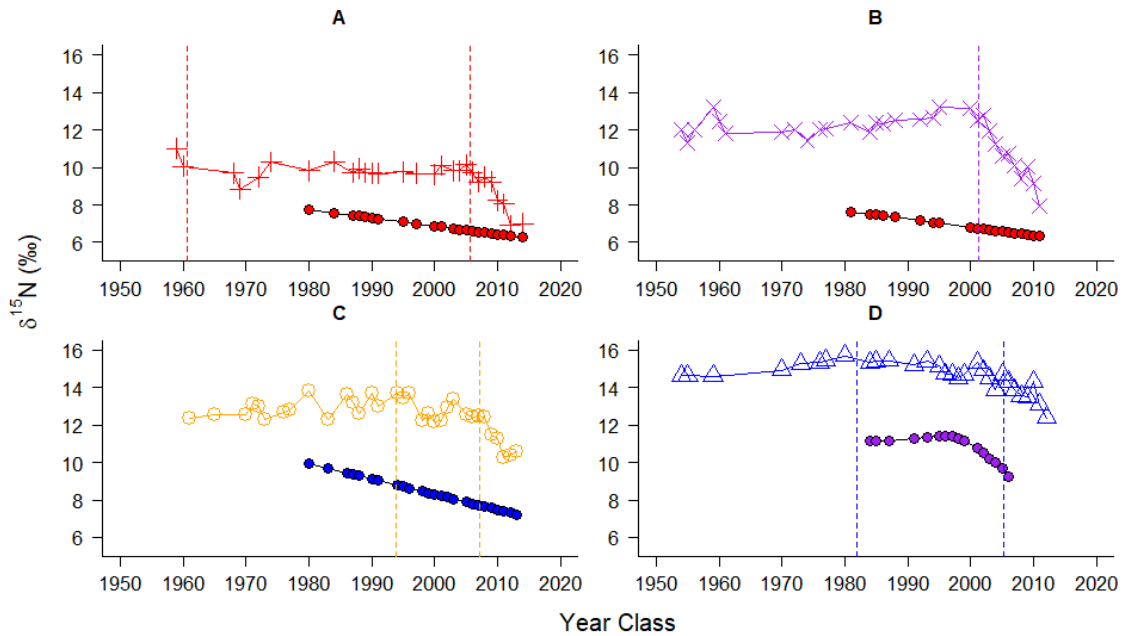


Figure 9: Breakpoints for isotopic values of $\delta^{15}\text{N}$ over time measured from fish scale samples, and corrected to represent fish tissue isotopic values, collected from Lake Simcoe, ON. Values of $\delta^{15}\text{N}$ are plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. $\delta^{15}\text{N}$ values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). Vertical dashed lines represent significant breakpoint years. $\delta^{13}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{15}\text{N}$ values. Red lines are zooplankton baselines; blue line is deep benthos (15-30m) baseline; purple line is mean of smelt and lake herring baseline.

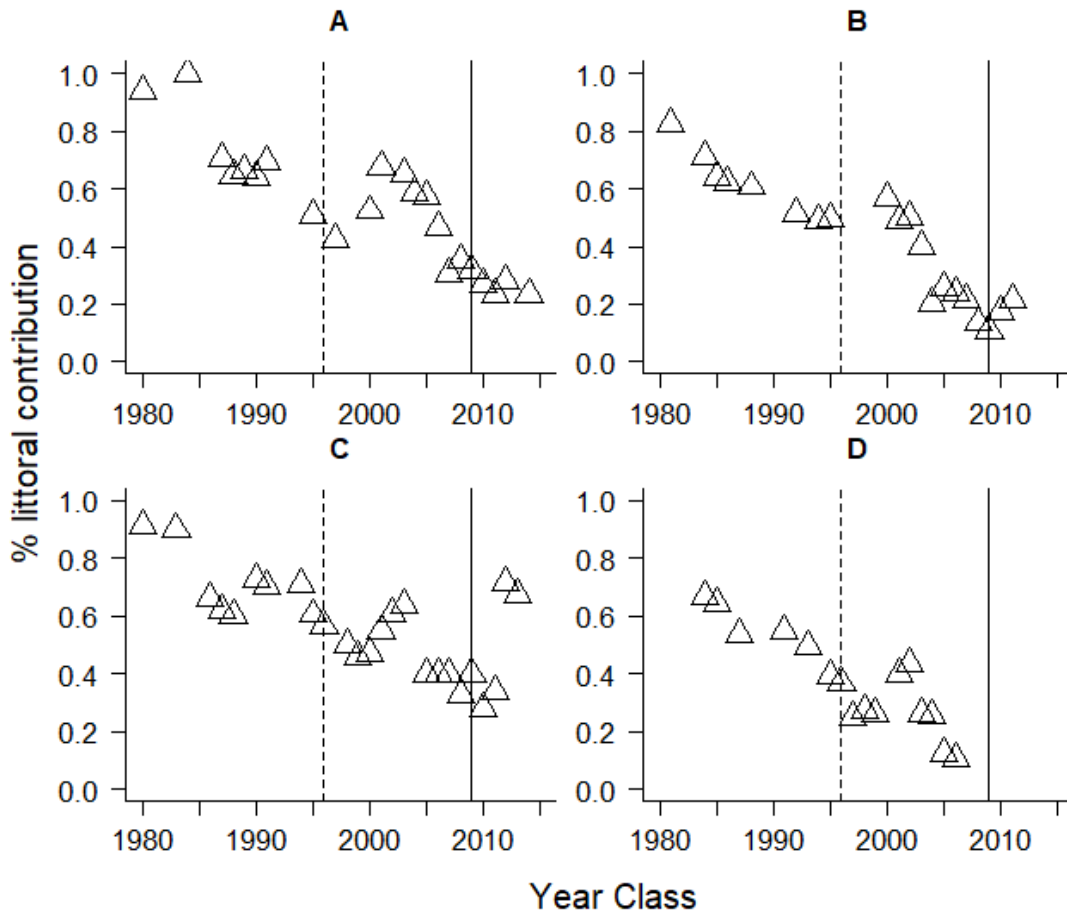


Figure 10: Percent contribution of littoral carbon calculated from tissue $\delta^{13}\text{C}$ (Table 1; Equation 1) values for fish collected from Lake Simcoe, ON. % littoral contribution is plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. Littoral contribution is shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). To account for the accumulation of isotopes over the fish's lifetime adult diet, baseline values for zooplankton (pelagic) and nearshore benthos (littoral) were averaged over the previous years equal to the number of years the fish was feeding. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007).

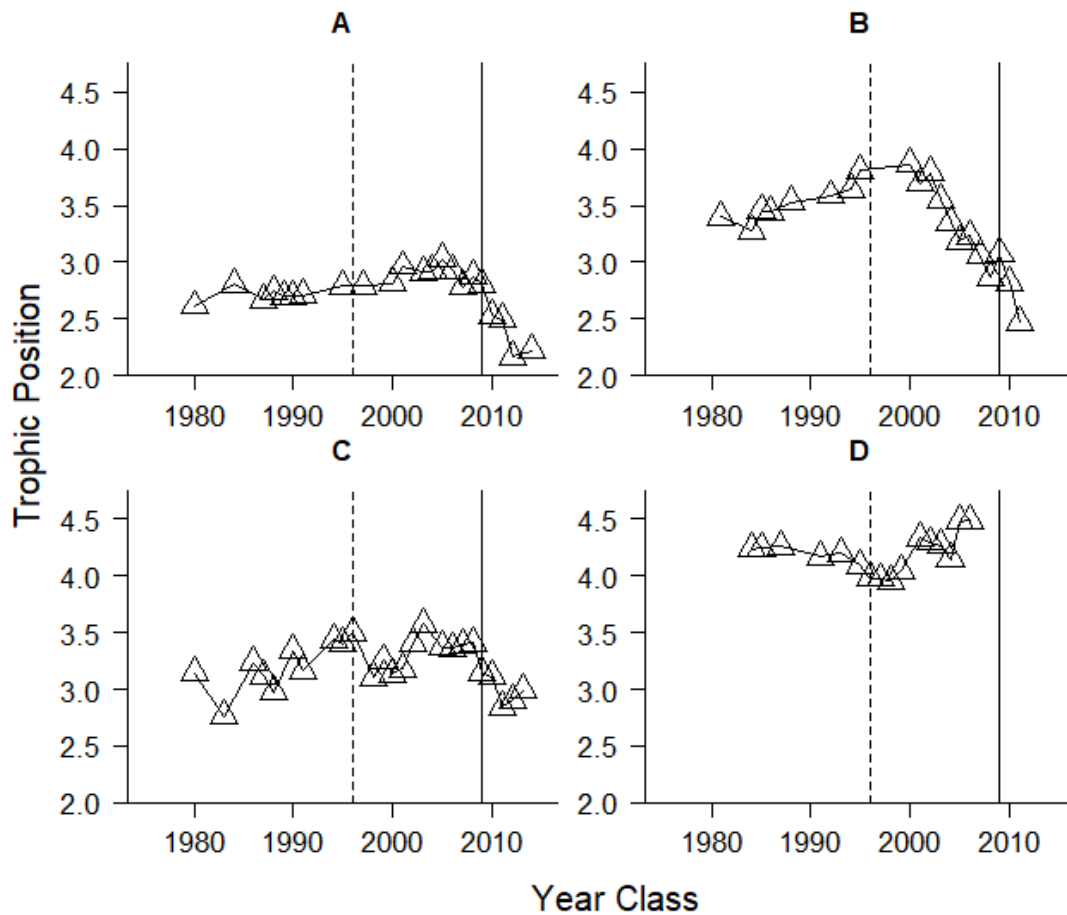


Figure 11: Trophic position calculated from tissue $\delta^{15}\text{N}$ values for fish collected from Lake Simcoe, ON (Table 1; Equation 2). Trophic position is plotted by species; A = smelt, B = herring, C = whitefish, D= lake trout. Trophic position values are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish). To account for the accumulation of isotopes over the fish's lifetime adult diet, baseline values were averaged over the previous years equal to the number of years the fish was feeding. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{N}$ values were sediment-corrected as per Rennie et al. (2013).

Tables

Table 1: Difference between fish scale isotopic values relative to fish tissues for four species from Lake Simcoe, Canada. Positive values below indicate that scales are more enriched than tissues, negative values indicate scales are more depleted than tissue. To facilitate the use of fish tissues $\delta^{13}\text{C}$ in mixing models, tissue isotopic values were estimated from scale estimates by subtraction of correction values below.

Species	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	dF	p ($\delta^{13}\text{C}$)	p ($\delta^{15}\text{N}$)
Smelt	+2.94	+1.56	29	< 0.0001	< 0.0001
Lake Herring	+4.02	-0.55	27	< 0.0001	< 0.0001
Lake Trout	+5.52	-0.11*	37	< 0.0001	0.33
Whitefish	+3.15	-0.13*	32	< 0.0001	0.24

**differences were not significant from zero, therefore scale tissues were considered to be equivalent to muscle tissues.*

Table 2: Significant breakpoint year classes for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (based on fish scale samples), % contribution of littoral carbon, and trophic position for each fish species considered in the study.

Fish Species	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		% littoral carbon	Trophic Position
Herring	1990	1999	NA	2001	NA	2000
Smelt	1999	2001	1961	2006	NA	2006
Lake Trout	NA	NA	1990	2010	NA	2004
Whitefish	NA	2011	1994	2007	2011	2006

Appendices

Appendix 1: Comparison of the first reported sightings of zebra mussels and quagga mussels in each of the Great Lakes and Lake Simcoe.

Lake	Zebra mussel	Quagga mussel	Years between sightings
Ontario	1989 ⁱ .	1990 ⁱⁱ .	1
Erie	1988 ⁱⁱⁱ .	1989 ^{iv} .	1
Huron	1990 ^v .	1997 ^x	~7
Michigan	1989 ^v .	1997 ^{vi} .	8
Superior ^{xi}	1989 ^v .	2005 ^{vii} .	16
Simcoe	1991 ^{viii} .	2004 ^{ix} .	13

i. (Griffiths et al., 1991), ii. (Marsden et al., 1992), iii. (Leach, 1993), iv. (Mills et al., 1993), v. (Benson, 2014) vi. (Nalepa et al., 2001), vii. (Grigorovich et al., 2008), viii. (Palmer et al., 2011), ix. (Ozersky et al., 2011b) x. Nalepa et al. 2009 reports Quaggas at low densities in 2000, but no surveys exist between 1972-2000 on Lake Huron. Given hydrological connections between Huron and Michigan, and low densities in 2000, it is reasonable to expect that Huron would be similar to Michigan. xi. Only localized regions of Lake Superior have either species of dreissenid mussel established, and as such the lake is relatively unaffected by their presence compared to the other lakes listed here.

Appendix 2: Sampling stations and dates for zooplankton collected in Lake Simcoe, ON in 2017.

Date	C6	E50	K42	K45
05/09/17	Single haul	Single haul	Single haul	Single haul
05/24/17	Single haul	Single haul	Single haul	Single haul
06/27/17	Double haul	Double haul	Double haul	Double haul
07/04/17	Double haul	Double haul	Double haul	Double haul
08/29-30/17	Double haul	Double haul	Double haul	Double haul
09/14/17	Double haul	Double haul	Double haul	Double haul
10/20/17	Double haul	Double haul	Double haul	Double haul

Appendix 3: Latitude and longitude of zooplankton sampling locations for Lake Simcoe, ON.

Station	Latitude	Longitude
C6	44.24305	-79.51222
E50	44.40833	-79.23750
K42	44.39916	-79.57055
K45	44.44305	-79.44472
M66	44.42139	-79.42556

Appendix 4: Zooplankton taxa across all size fractions (125, 250, and 500 µm) for samples collected from Lake Simcoe, ON in 2017.

	Date	Dominant taxa	Other taxa	Additional taxa	Additional taxa (removed)
C6	24/05/17	80% Cladocerans	20% Copepods		2 Chironomids, 1 Bythotrephes
E50	24/05/17	100% Copepods			
K42	24/05/17	70% Copepods	30% Cladocerans		
K45	24/05/17	80% Copepods	20% Cladocerans		
C6A	27/06/17	100% Cladocerans			
C6B	27/06/17	90% Cladocerans	10% Copepods		
C6B	04/07/17	80% Cladocerans	20% Copepods		
E50A	04/07/17	90% Copepods	10% Cladocerans		
E50B	04/07/17	80% Copepods	20% Cladocerans		
K42A	04/07/17	60% Cladocerans	40% Copepods		
K45A	04/07/17	90% Cladocerans	10% Copepods	2 Lepidoptera	
K45B	04/07/17	60% Cladocerans	40% Copepods		
C6	29/08/17	70% Copepods	30% Copepods		
E50	30/08/17	90% Copepods	10% Cladocerans	1 Lepidoptera	
K42	30/08/17	90% Cladocerans	10% Copepods	2 Lepidoptera	
K45	30/08/17	80% Cladocerans	20% Copepods	3 Lepidoptera	
C6	14/09/17	60% Copepods	40% Cladocerans		1 Bythotrephes
E50	14/09/17	60% Copepods	40% Cladocerans		
K42	14/09/17	95% Cladocerans	5% Copepods		3 Bythotrephes
K45	14/09/17	80% Copepods	20% Cladocerans		33 Bythotrephes
C6	20/10/17	80% Copepods	20% Cladocerans		3 Bythotrephes
E50	20/10/17	100% Copepods			2 Chironomids
K42	20/10/17	60% Cladocerans	40% Copepods	3 Lepidoptera	4 Bythotrephes
K45	20/10/17	90% Copepods	10% Cladocerans		8 Bythotrephes

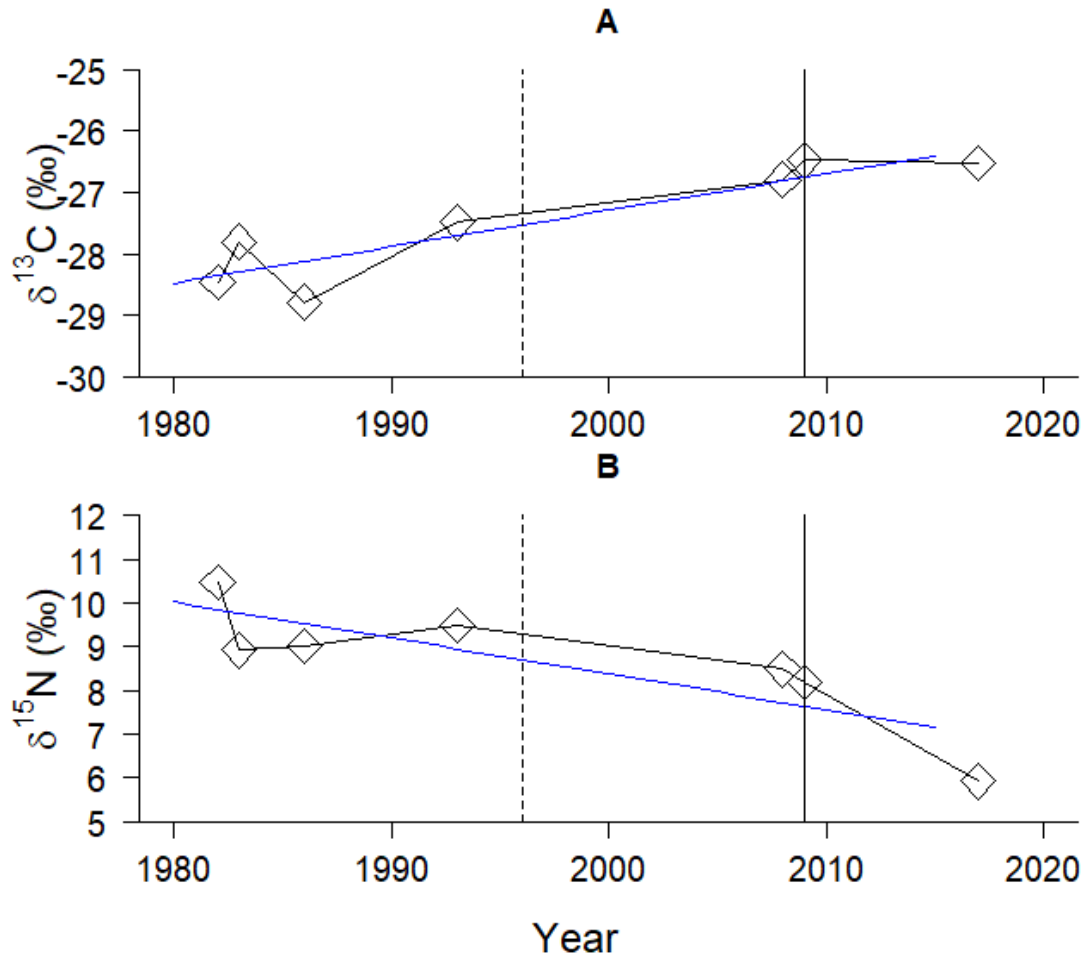
Appendix 5: Latitude and longitude of benthic invertebrate sampling locations for Lake Simcoe, ON. Samples included are those that were used to obtain benthic invertebrates. Single hauls provided sufficient biomass for the three shallower sites (5-15m), whereas deeper sites (20-30m) required multiple hauls to obtain sufficient biomass for analysis.

Depth	LAT	LON
5.5	44.34258	79.70000
10.3	44.34543	79.13333
15.4	44.81666	79.96666
19.5	44.63333	79.46666
19.7	44.30000	79.20000
19.7	44.35000	79.00000
24.9	44.10000	79.55000
25	44.71666	79.40000
25	44.65000	79.05000
30	44.71666	79.25000
30	44.73333	79.65000
30	44.33333	79.95000

Appendix 6: Benthic invertebrate taxa across all depths for samples collected from Lake Simcoe, ON in 2017.

Sam. #	Date	Depth	Quag.	Zeb	Gastro.	Chiron.	Amphi.	Isopod	Bivalve	Ephem.
4	10/07/17	5.5				16				
8	10/07/17	10.3	5	1		66				1
18	14/07/17	15.2	113	2	39	4	1	6		
13	12/07/17	19.5	1		8	5				
15	12/07/17	19.7	2		13					
14	12/07/17	19.7	97		10	1	3			
12	11/07/17	24.9	26				1			
11	11/07/17	25	70							
10	11/07/17	25	42						1	
3	10/07/17	30	3							
2	10/07/17	30				1			3	
1	10/07/17	30	2			1				

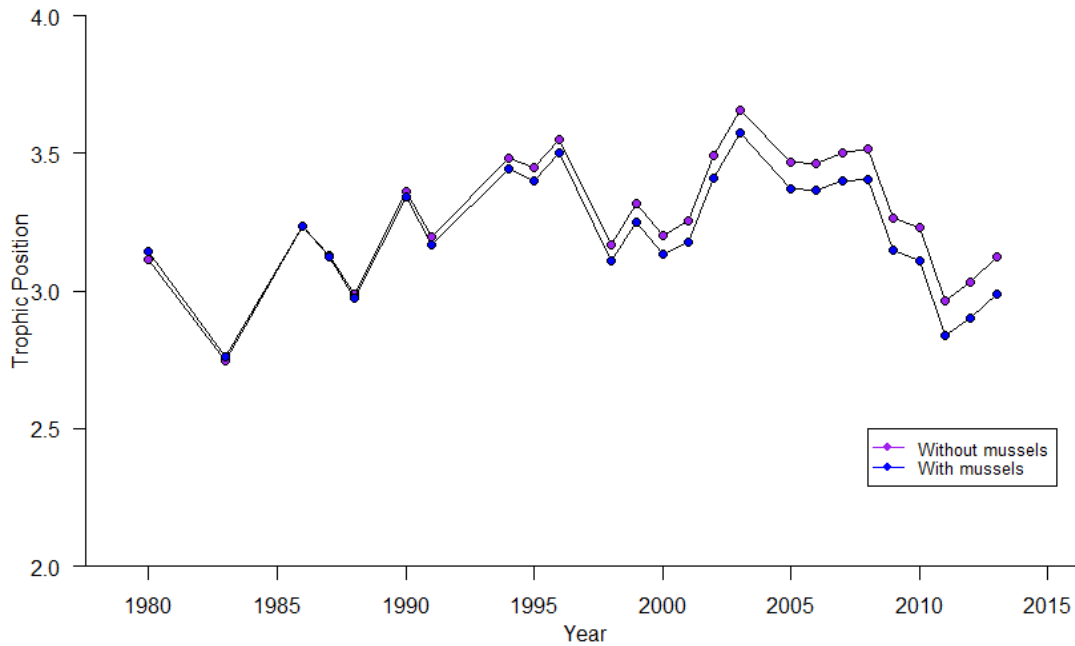
Appendix 7: Isotopic values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ over time for benthic invertebrates, excluding dreissenids, collected from Lake Simcoe, ON, for depths of 15-30m. Vertical dashed line and solid line depict the zebra mussel and quagga mussel establishment years, respectively. $\delta^{13}\text{C}$ values are Suess-corrected according to Verburg (2007). $\delta^{15}\text{N}$ values are sediment-corrected as per Rennie et al. (2013). Points are mean $\delta^{13}\text{C}$ and mean $\delta^{15}\text{N}$ values; line is regression line.



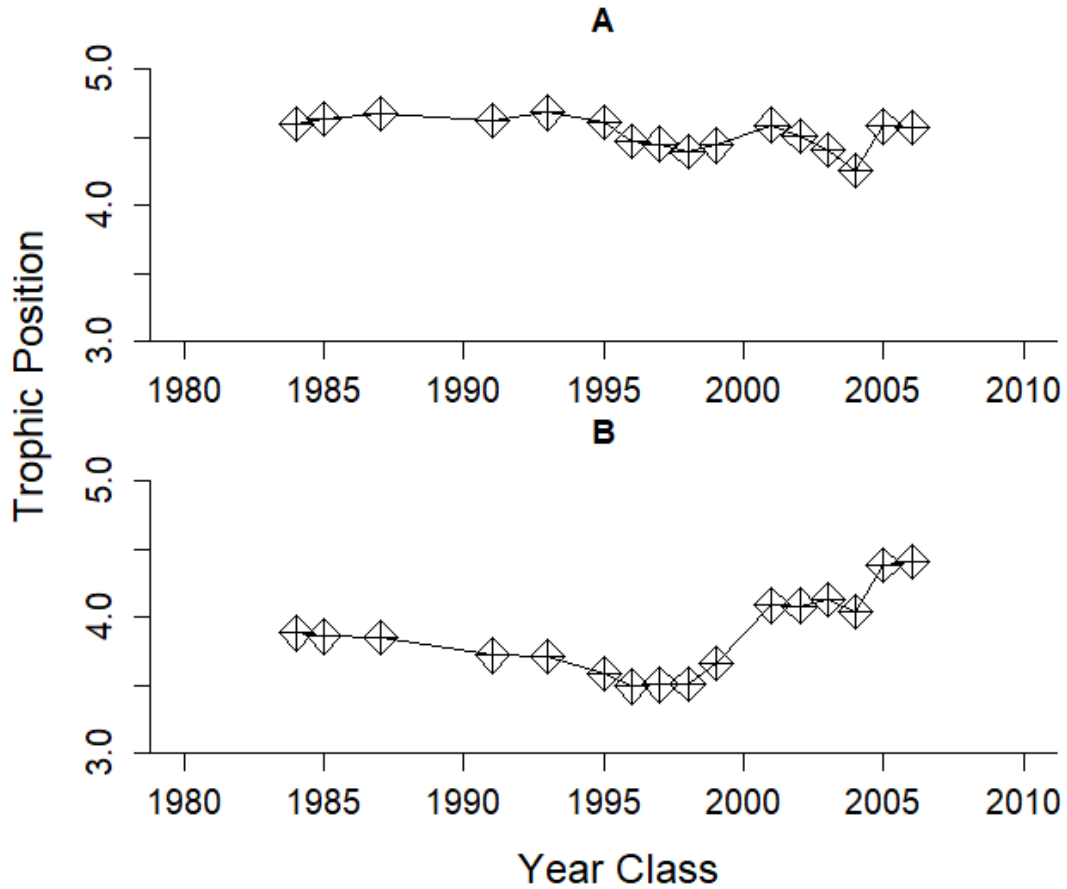
Appendix 8: Benthic invertebrate taxa from 5m sites included in stable isotope analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for years 1982 to 2017. The presence of each taxonomic group is denoted by X.

	1982	1983	1986	2009	2017
Amphipods	X	X	X		
Chironomids	X	X	X	X	X
Ephemeroptera	X	X	X	X	
Gastropoda	X	X	X		
Oligochaeta		X	X	X	
Isopoda		X			
Pelecypoda		X	X		
Copepods		X			

Appendix 9: Trophic position calculated from tissue $\delta^{15}\text{N}$ values for lake whitefish collected from Lake Simcoe, ON, over time (Table 1; Equation 2) estimated using both benthic invertebrates excluding dreissenid mussels (purple) and benthic invertebrates including dreissenid mussels (blue) for depths of 15-30m. Values of $\delta^{15}\text{N}$ are shown by year class to which the individual fish began feeding on an adult diet (age 3 for lake whitefish).



Appendix 10: Trophic position calculated from tissue $\delta^{15}\text{N}$ values for lake trout collected from Lake Simcoe, ON (Table 1; Equation 2). Trophic position is plotted based on estimates from baseline species; Panel A = smelt baseline, Panel B = herring baseline. Values of $\delta^{15}\text{N}$ are shown by year class to which the individual fish began feeding on an adult diet (age 0 for herring and smelt; age 3 for lake trout and lake whitefish).



Appendix 11: Comparison of $\delta^{15}\text{N}$ for smelt, herring, and lake trout (age 2). Only years with data available for all three species were included. Data presented represents the $\delta^{15}\text{N}$ values for scales of each fish species, sediment-corrected as per Rennie et al. (2013).

Year	$\delta^{15}\text{N}$ Smelt	$\delta^{15}\text{N}$ Herring	$\delta^{15}\text{N}$ Lake Trout (Age 2)
1995	11.3382	12.646778	13.4176
2004	11.3848	10.688144	13.6735
2005	11.7021	10.057712	14.4394
2006	11.3075	10.185179	14.3167
2008	11.053	8.8603	14.2392
2009	10.7756	9.501115	13.275
2010	9.78847	8.601489	13.3339
2011	9.62605	7.362568	11.7573