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Fatty Acid Profiles of Major Prey and Predator Fish in Lake Ontario: Use in Assessing Food Web Interactions

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Fatty Acid Profiles of Major Prey and Predator Fish in Lake Ontario: Use in Assessing Food Web Interactions

By

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Abstract

Fatty acid signatures (FAS) are currently used in food web studies to assess trophic interactions between predator and prey. In this study, three major prey fish (alewife, rainbow smelt, and round goby) were collected at three sites along the south shore of Lake Ontario (Olcott, Rochester, and Oswego) during the spring and fall of 2013. Major predator species (including lake trout, brown trout, Chinook salmon, coho salmon, chain pickerel, northern pike, yellow perch, and walleye) were collected along the south shore of Lake Ontario during the summer of 2013. Using multivariate statistics, FAS were compared among all predator and prey species as well as among location and between seasons for prey fish. Though notable seasonal differences were found in alewife FAS, differences in FAS among prey species were greater than any spatio-temporal differences detected within a single species. FAS among predator species were also significantly different though results were consistent with predator taxonomic family. Differentiating fatty acids were similar in among-species comparisons of prey and predator, respectively. Alewife and salmonids were differentiated by oleic acid (18:1n-9), round goby and percids by palmitoleic acid (16:1n-7), and rainbow smelt and esocids by DHA (22:6n-3). FAS suggested a prominent diet of alewife for salmonids and a round goby-rich diet for yellow perch while other species seemed to have a more balanced diet. Our results provide the first comprehensive FAS dataset for major prey and predator species in Lake Ontario. Though specific predator-prey FAS assimilation responses must first be investigated through controlled feeding experiments, the strong heterogeneity among FAS of Lake Ontario prey items suggests that the application of quantitative fatty acid signature analysis (QFASA) is a viable option for assessing predator feeding habits.

1. Introduction

1.1. Lake Ontario Food Web

Descriptions of Lake Ontario and its fish community prior to European settlement in the 1700's depicted an area lush with natural resources and great abundances of naturally reproducing fish (Smith 1995). The structure of the food web had a much different dynamic with a completely different array of species at each trophic level. Top predators of early fish assemblages that occupied offshore areas of the lake included Atlantic salmon (*Salmo salar*), lake trout (*Salvelinus namaycush*), and burbot (*Lota lota*). Additionally, three different species of coregonids (*Coregonus* spp.), bloater (*C. hoyi*), shortnose cisco (*C. reighardi*), and kiyi (*C. kiyi*) represented the greatest biomass in Lake Ontario (Nantel 1977). These planktivorous fish were prey to lake trout and burbot in the deep waters of the lake. Deepwater sculpins (*Myoxocephalus thompsoni*) and slimy sculpins (*Cottus cognatus*) were also prominent forage fish in deep water benthic habitats (Nantel 1977). Nearshore fish assemblages were dominated by perch (*Perca* spp.), sunfish (*Centrarchus* spp.), minnows (*Notropis* spp.), bass (*Micropterus* spp.), and pike (*Esox* spp.). Predominant forage species in these shallow, protected areas were the emerald shiner (*Notropis atherinoides*) and the spottail shiner (*Notropis hudsonius*) (Nantel 1977).

Since the 1700's, an array of direct and indirect anthropogenic forces have propagated drastic changes in the fish communities of Lake Ontario. Commercial overfishing, along with the introduction of the sea lamprey, were largely considered the driving force contributing to the severe degradation of native fish populations in Lake Ontario (Christie 1972). Overexploitation of several species including lake trout, Atlantic salmon, lake sturgeon (*Acipenser fulvescens*), and whitefish (*Coregonus* spp.) occurred throughout the 1800's and into the 1900's. As a result,

fish stocks drastically declined to irrecoverable levels toward the end of the 1800's. Even with reduced fishing effort brought on by the declines, populations were unable to rebound (Smith 1995). This considerable span of overfishing diminished a once abundant natural resource and began to destabilize the ecosystem in Lake Ontario.

During this time, invasive species also played a significant role in the changes to Lake Ontario's fish community. Nonnatives were able to thrive in an environment lacking mechanisms to control their populations, and establish themselves in Lake Ontario at the expense of native species. The sea lamprey (*Petromyzon marinus*), first documented in Lake Ontario in 1830, invaded the lake via the Erie Canal. As a parasite that attaches to, and often kills larger fish, the sea lamprey was detrimental to native top predator populations, namely lake trout (Larson *et al*. 2003). Another invasive species, alewife (*Alosa pseudoharengus*), was first detected in abundance in Lake Ontario in the spring of 1873 (Bean 1984). Originally, it was suggested that the mechanism of introduction was the accidental inclusion of alewife fry with released Atlantic shad (*Alosa sapidissima*) fry (Bean 1984). However, the current popular belief is that alewife reached Lake Ontario via the Erie Canal which connects the Hudson River system with the Oneida-Oswego River system, ultimately providing a passageway from the Atlantic Ocean to Lake Ontario (Smith 1970). By the 1960's, these planktivorous fish were thriving in Lake Ontario, benefitting from the degraded community of pelagic piscivores. In the absence of sufficient predation, thriving and expanding alewife populations are thought to have outcompeted coregonids, through competition for zooplankton (Crowder and Binkowski 1983). This displacement thrust alewife into an important role at the forage fish trophic level in Lake Ontario's food web. There is also evidence that alewife have some negative impacts on their predators through predation of fish larvae as well as elevated levels of thiaminase in alewife

which results in thiamine deficiencies and ultimately early mortality syndrome (EMS) in predators that consume alewife (Madenjian *et al.* 2008). Rainbow smelt (*Osmerus mordax*) were found in Lake Ontario in 1929 and likely entered via waterways leading from the Finger Lakes, New York, where they were previously introduced (Rooney and Paterson 2009). This introduction inserted yet another nonnative into a role in the food web. Rainbow smelt had a trophic role similar to that of alewife as both forage fish occupied habitats in the pelagic zone.

By the 1970's, alewife and rainbow smelt dominated the fish community in the offshore waters of Lake Ontario (Owens *et al*. 2003), and a mechanism to control populations of these fish, particularly alewife, became increasingly necessary. Alewife populations grew so rapidly that abundances exceeded the carrying capacity and massive die-offs that covered shorelines resulted. Stocking top predators in Lake Ontario provided an effective management strategy that would not only keep alewife populations in check and reestablish extirpated native predators, but also would create a sport fishery in Lake Ontario. Ultimately, the stocking of predator fish such as native lake trout and Atlantic salmon, brown trout (*Salmo trutta*), and Pacific salmon (Chinook salmon - *O. tshawytscha*, steelhead - *Oncorhynchus mykiss*, and coho salmon - *O. kisutch*) was implemented (Mills *et al*. 2003).

Lake Ontario is currently home to a mixture of native and non-native species that are part of an ecosystem that has remained generally resilient since the mid-1990s (Stewart *et al*. 2013). web in Lake Ontario reflects the changes brought about by centuries of anthropogenic influence. Alewife occupy a pivotal niche in Lake Ontario's food web as this planktivore not only influences zooplankton composition but is also the preferred food item of salmonids (Rand and Stewart 1998, Madenjian *et al*. 2002). Rainbow smelt are another planktitvorous invasive that currently occupy a similar trophic level as alewife. Like alewife, rainbow smelt have a diverse

diet that can range from zooplankton to small fish. Both of these forage fish are susceptible to sudden population fluctuations based on changes in the environment, predation, food availability or a combination of these factors (Rooney and Paterson 2009, Stewart *et al*. 2013). A more recent invader, round goby (*Neogobius melanostomus*), was first discovered in the Great Lakes region in the St. Clair River in 1990. This benthic fish, native to the Caspian Sea and Black Sea, expanded its range to the southwestern waters of Lake Ontario in 1998. Round goby have been expanding their range in the Great Lakes since their introduction (Chotkowski and Marsden 1999). Though round goby were not present in assessment surveys conducted by the U.S. Geological Survey – Lake Ontario Biological Station (USGS-LOBS) and the New York State Department of Environmental Conservation (NYSDEC) until 2002 (O'Gorman 2013), population abundance increased dramatically in the following years and has since begun to seemingly stabilize (Walsh *et al*. 2007).

The current state of the food The more recent introduction of the round goby is of particular interest to researchers as this benthic species has the potential to create new energy pathways within food webs across the Great Lakes. Round goby feed on a wide variety of organisms available to them in the benthic zones such as dreissenid mussels (zebra - *Dreissena polymorpha* and quagga - *Dreissena rostriformis bugensis*), invertebrates, small fish, and even fish eggs. As a main prey item of the round goby, dreissenid mussels are efficient filter feeders that consume large quantities of phytoplankton. While shifting this source of nearshore energy away from zooplankton and planktivores in the nearshore, vertical energy flow from dreissenid mussels to round goby may create a new energy pathway, reaching higher trophic levels in the lake (Mills *et al*. 2003).

Seasonal behaviors and feeding habits of prey assemblages are important to understand and consider in terms of prey availability and energy content for piscivores. For example, seasonal movements and diet shifts of alewife (Stewart *et al*. 2009) can have cascading implications for higher trophic levels as alewife diet compositions can affect their nutritional value. Further, offshore movements of round goby in late summer could alter the way in which energy is transferred through the food web (Pennuto *et al*. 2012). These temporal differences in forage fish assemblages can be easily altered by changes to the environment. Monitoring population dynamics of major prey fish is crucial for supporting fisheries as well as understanding the changing trophic interactions occurring in Lake Ontario.

It is important to understand the dynamics of a food web for the purpose of maintaining a diverse ecosystem that can sustain productivity and meet the needs of public use as well as those of other stakeholders. Salmonid species in Lake Ontario represent a large recreational fishery that contributes to local economies. Survival, growth, and the reproductive abilities of these species are directly influenced by the abundance, distribution, and dietary composition of their prey. Understanding the dynamics of predator-prey interactions in the transitional Lake Ontario food web can inform management decisions and the direction of further research. Adjusting stocking numbers in the lake is critical to maintaining a sustainable predator-prey balance. At the root of this relationship is the understanding of predator feeding habits and how predator species co-exist in Lake Ontario in terms of their utilization and sharing of resources.

1.2. Fatty Acids as Tracers

Fatty acids are constituents of lipids that play a major metabolic role in fish survival, growth, and reproduction (Tocher 2003). Fatty acids are comprised of a carboxylic acid with a

hydrocarbon chain. Fatty acids can be identified based on chain lengths, number of double bonds, and the position of those double bonds. The number of double bonds within the hydrocarbon chain is representative of the level of unsaturation. Saturated fatty acids contain no double bonds, while unsaturated fatty acids are comprised of at least one. Unsaturated fatty acids can be either monounsaturated (containing one double bond) or polyunsaturated (containing two or more double bonds). Nomenclature for fatty acids, defined by the International Union of Pure and Applied Chemistry (IUPAC), is based on carbon chain length, the number of double bonds, and the position of the first double bond with respect to the methyl end of the molecule. For example, linoleic acid (LA) is represented by 18:2n-6; "18" refers to the number of carbons in the chain, "2" refers to the number of double bonds, and "6" refers to the carbon position of the first double bond with respect to the methyl end. Certain fatty acids are required by freshwater fish but can only be obtained via diet. These are known as essential fatty acids. Linoleic and linolenic acid (ALA: 18:3n-3) represent two essential fatty acids which are necessary for the synthesis of long chain fatty acids and must be obtained through a fish's diet (Tocher 2003).

Fatty acids can be used as biochemical tracers in food web studies based on their conservative transfer from the prey item to the consumer (Lovern 1935). Dietary fatty acids, characterized by having a chain length greater than 14 carbons long, remain intact during digestion allowing researchers to analyze fatty acids strictly acquired via prey ingestion (Iverson 1993). These dietary fatty acids are transported into circulation and are stored in adipose tissues in predators (Iverson *et al*. 2004). Specifically, the major fat storage sites in salmonids are located in the fat of the belly (belly flap) and in the muscle (Tocher 2003). Thus, active fat storage deposits are typically representative of dietary fatty acids in predators (Iverson *et al*. 2004). The concentrations of different fatty acids comprise the fatty acid signature (FAS) of a

fish or fish species. Unlike diet analysis using stomach contents, FAS provide insight into the longer-term dietary habits of consumers. FAS are reflective of a fish's diet 4-12 weeks prior to sampling (Kinsch *et al.* 1998, Budge *et al*. 2006, Honeyfield *et al*. 2009) as fatty acids are released from lipids that have been ingested but are not degraded.

Fatty acid analysis has been reliably used in marine environments to characterize trophic interactions (Iverson *et al*. 1997, Smith *et al*. 1997) and study spatio-temporal variations (Budge *et al*. 2002, Iverson *et al*. 2002). Application of fatty acid analysis in freshwater ecosystems has revealed information regarding food web dynamics (Czesny *et al*. 2011, Happel *et al.* 2015[a], Happel *et al.* 2015[b]), though studies are limited. Essential to use of FAS, as a food web marker, is the ability to distinguish the FAS of prey species of interest. Such inter-species differences have been confirmed and utilized in marine environments (Budge *et al*. 2006) though the same cannot be said for prey assemblages in Lake Ontario.

Furthermore, analysis of FAS can be used to quantitatively estimate diet composition. This method, known as quantitative fatty acid signature analysis (QFASA), compares the fatty acid profiles of prey and predator species in an attempt to estimate proportions of prey species in predator diets (Iverson *et al*. 2004). This method has recently been used in a study conducted by Magnone *et al*. (2015) to quantify diets of flatfish (*Paralichthys orbignyanus*). The use of QFASA in this study was found to provide adequate approximations of this species natural diet. However, a clearer understanding of food web dynamics in Lake Ontario as well as the conducting of controlled feeding experiments to determine FAS assimilation rates are required before any quantitative diet estimations can take place in such an ecosystem.

Stable isotope analysis is another popular tracer method that allows assessment of energy flow through food webs via composition of elements such as carbon, nitrogen, and phosphorus. Certain stable isotope ratios serve as tracers during trophic energy transfer and can be used to attain qualitative information regarding the source of production at lower trophic levels. Similar inter- and intra-specific as well as spatio-temporal comparisons can be made using this method. The use of stable isotope analysis in Lake Ontario has shown variations in food web dynamics based on location and season (Zhang *et al*. 2012). Using both tracer methods (fatty acid and stable isotope analyses) simultaneously is becoming a popular method to identify trophic dynamics of aquatic ecosystems (Alfaro *et al*. 2006, Happel *et al*. 2015[a]). However, shortcomings of stable isotope analysis have been identified in the form of low assimilation rates of consumers to that of their diet (Dodds *et al*. 2014). Moreover, Dethier *et al*. (2013) found fatty acid analysis to be more consistent than stable isotope analysis in the ability to distinguish macrophyte taxa. Similarly, a study conducted on brown algae described clearer differentiation of producers as well as more accurate diet estimations of consumers using fatty acid analysis compared to stable isotope analysis (Crawley *et al*. 2009). Though both stable isotope and fatty acid analysis offer great potential for extracting long-term dietary information from predators, more needs to be known before reliable, quantifiable diet estimations can be made in food webs like that of Lake Ontario.

1.3. Objectives

A large scale, comprehensive study involving major prey and predator species has not yet been conducted for Lake Ontario's food web using fatty acid analysis. Our objectives were to 1) identify FAS of three prey species; alewife, rainbow smelt, and round goby and compare signatures spatio-temporally, 2) identify FAS of major predator species lake trout, brown trout,

Chinook salmon, coho salmon, steelhead trout, northern pike, chain pickerel, walleye, and yellow perch, and 3) use discriminating fatty acids to evaluate trophic food web interactions among prey and predators.

We hypothesized the presence of intra-species spatio-temporal variation in the FAS of prey fish. However, we expected inter-species differences to be more pronounced than any intra-species variation. Additionally, we hypothesized that predator FAS would reflect established prey FAS based on foraging habits.

2. Materials and Methods

2.1. Site Selection and Prey Sampling

During spring and fall 2013, three prey fishes (alewife, rainbow smelt, and round goby) were sampled during annual assessment surveys conducted by the USGS-LOBS aboard the R/V Kaho. Due to limitations of time and space on the R/V Kaho, samples were collected and preserved by the USGS-LOBS staff when I could not be on board. Nonetheless, I was able to participate as a collaborator on board for several cruise dates during the spring (4/23/2013, 4/26/2013, and 5/8/2013). Three transects were targeted for sampling along the south shore of Lake Ontario. Transects were Olcott, NY (Thirty Mile Point), Rochester, NY, and Oswego, NY (Nine Mile Point) (Figure 1a). Target sample size was 20 individuals of each species at each of the three locations and actual sample sizes ranged from 13 to 79 individuals (Table 1). Fish were collected using a 3-in-1 trawl net with an 18 m headrope and slotted, cambered V-doors. Bottom trawls were conducted at varying depths along each transect ranging from 15-185 m. Prey species were taken whole, bagged (Figure 2), stored on dry ice, and transferred to Dr. Rinchard's

lab at The College at Brockport, State University of New York where they were stored at -80˚C until biochemical analysis. Prior to lipid extraction, total length (mm) and weight (g) measurements were taken.

2.2. Predator Sampling

Major predator species (lake trout, brown trout, walleye, northern pike, chain pickerel, coho, Chinook, steelhead, smallmouth bass, yellow perch) were targeted at several areas along the south shore of Lake Ontario based on their availability through the cooperation of anglers and survey of agencies (USGS-LOBS) (Figure 1b, Table 2). I attended several fishing tournament events throughout the spring and summer months of 2013 at which I was able to obtain predator fish at weigh-in and cleaning stations. Working with members of the NYSDEC, who collected biological data from fish at these tournaments, I was able to interact with anglers who were willing to give up their fish once they had weighed them in. Some predator fish were also collected by use of vertical gillnets (The College at Brockport, State University of New York and USGS-LOBS). With the help of several students and faculty of the Department of Environmental Science and Biology at The College at Brockport, gillnets were set off the shore of Hamlin Beach, perpendicular to the shoreline using the R.V. Madtom. These gillnets had a soak time of approximately 24 h. They were 45.7 m multifilament of mesh sizes ranging from 25 to 102 mm with 12.5 mm increments. I was also on board the R.V. Kaho when vertical gillnets were set during lake trout surveys by the USGS-LOBS. These gillnets were made up of nine, 15.2 x 2.4 m panels of mesh sizes ranging from 51 to 151 mm with 12.5 mm increments. During the USGS survey, four of these gillnets were set off the shore of Hamlin Beach, perpendicular to the shoreline and had a soak time of approximately 24 h. Additionally, some predator fish were collected when I was on board the R.V. Kaho as bycatch during bottom trawls (USGS-LOBS).

Regardless of the collection method, predators were put on ice as whole fish and transported back to the Dr. Rinchard's research lab at The College at Brockport, State University of New York for processing. Total length (mm) and weight (g) measurements were taken, and a square section of the belly flap, approximately 4 x 4 cm, was removed for lipid and fatty acid analyses (Figure 3). Belly flap samples were immediately stored at -80°C until biochemical analysis.

2.3. Lipid Extraction

Both belly flap (predator) and whole fish (prey) were homogenized prior to analysis. Skin was removed from belly flap samples prior to homogenization. Homogenization was accomplished by placing whole samples (belly flap or prey) into a coffee grinder and grinding for 1-2 minutes or until a paste was developed. During lipid extraction, approximately one gram of homogenized sample was weighed precisely and placed into homogenization tubes. Twenty milliliters of 2:1 chloroform/methanol solvent containing 0.01% butylated hydroxytoluene used as antioxidant was added to these tubes for total lipid extraction (Folch *et al*. 1957). Tubes were then capped and placed on ice. A Fisher Scientific PowerGen homogenizer with a saw teeth stainless steel rotor stator probe (Fisher Scientific, Hampton, NH) was used to thoroughly homogenize each sample. Samples were homogenized for 1 minute at speed 4 and were kept on ice throughout the homogenization process. The homogenizer probe was rinsed and cleaned between samples using two deionized water solutions, followed by two solvent solutions. The samples were then filtered under vacuum pressure using 11 μm Whatman filters (Whatman International Ltd., Piscataway, NJ) and rinsed with solvent to collect all lipids. Lipids were transferred to larger test tubes and 4 milliliters of magnesium chloride (6 g $MgCl_26H_2O$:1000 ml deionized water) was added. These tubes were then filled with nitrogen gas, vortexed for 1 minute, refilled with nitrogen gas, sealed, and stored at room temperature for 24 hours. The

bottom solvent layer containing lipids was removed and transferred, using a Pasteur pipette, to a new homogenizing tube and evaporated under nitrogen gas in a 30-35°C water bath. This solvent layer was then transferred to a smaller, previously weighed tube once its volume had been reduced sufficiently and there was no water in the sample. This organic solvent was evaporated under nitrogen until it reached a stable weight for determination of total lipid content. Using the original weight of the tube and of the sample, total lipid content was measured gravimetrically and expressed as a percentage of the total sample weight, indicating percent lipids [(weight of lipid/weight of tissue)*100].

2.4. Fatty Acid Transmethylation and GC/MS Analysis

Transmethylation formed fatty acid methyl esters (FAMEs) by replacing the hydroxide group at the end of each fatty acid chain with a methyl group. This was accomplished by use of the methods described by Metcalfe and Schmitz (1961). Total lipids underwent saponification, wherein a hydroxide group was added to neutral lipids. This occurred through the addition of 1.5 milliliters of sodium hydroxide (NaOH 0.5 M in methanol) and incubation at 80°C for one hour. Samples were then left standing to cool at room temperature before 2 milliliters of borontrifluoride methanol was added. Borontrifluoride methanol functioned to replace the hydroxide group with a methyl group. Sample tubes were filled with nitrogen gas and incubated at 80°C for 30 minutes. The samples were once again allowed to cool at room temperature. One milliliter of hexane was then added to each sample, the sample tube was capped, and then vortexed for one minute. Then one milliliter of distilled water was added, the tube capped, and again vortexed for one minute. The hexane phase separated at the top and was transferred to tubes containing anhydrous sodium sulfate, which absorbed water residues. A double extraction occurred as an additional milliliter of hexane was added to each sample, tube capped, and

vortexed for one minute. This hexane phase was also added to the tube containing anhydrous sodium sulfate and the previous hexane phase to ensure the transfer of all FAMEs present in the sample. The tube containing the hexane phase was then vortexed for 20 seconds before the hexane phase was removed and transferred to 4 milliliter vials previously rinsed with hexane. The samples were then stored at -80°C until gas chromatography/mass spectrometry analysis.

Fatty acids profiles were determined using an Agilent Technologies 7890A GC system with Agilent Technologies 7693 Autosampler and Agilent Technologies 5975C inert XL EI/CI MSD with Triple-Axis detector (Agilent Technologies, Inc., Santa Clara, CA). The capillary column used was an Omegawax 250 Fused Silica Capillary Column with 30 m x 0.25 mm x 0.25 µm film thickness (Supelco, Bellefonte, PA). Helium was used as a carrier gas. The oven temperature was programmed from 175°C for 26 minutes to 205°C at 2°C per minute, and then held at 205°C for 24 minutes. The rate of helium carrier gas flow was 1.8 milliliters per minute. The source and analyzer temperature of the MS was set at 230°C. The individual fatty acid methyl esters (FAMEs) were identified by comparing the retention times of authentic standard mixtures (FAME mix 37 components, Supleco) with known spectrographic patterns of FAMEs. Spectrographic patterns for FAMEs were acquired from the National Institute of Standards and Technology Mass Spectral Library provided with the GC/MS and the Association Oil's Chemists' Society mass spectral library provided online at http://lipidlibrary.aocs.org/index.html. Nonadocanoic acid (19:0) was used as the internal standard and was added to each sample based on the amount of total lipids present (8 mg/50 mg of lipids). Individual FAMEs were identified by their retention times and their peak area was quantified in proportion to that of the internal standard. The composition of each was then calculated and reported as a percentage of the total FAMEs.

2.5. Quality Control Procedures

During GC/MS analysis, all samples were injected similarly through use of an auto injector. The auto injector removes human error which eliminates consistency issues associated with variable sample volumes and contamination. Blanks were run through the GC/MS every 10 samples to adjust baselines and account for any background noise that may be occurring. Peaks representing individual FAMEs were identified based on their retentions times, the retention times of authentic standard mixtures, and known spectrographic patterns. A log was kept to track changes in retention times and compounds were calibrated accordingly. Each peak was also manually evaluated to ensure that the software ChemStation was correctly identifying and integrating FAMEs. All data resulting from peak areas were thoroughly reviewed to check for integration errors.

2.6. Statistical Analysis

Univariate statistical analyses were conducted on lipid data from predator and prey samples for within-species and among-species comparisons using IBM SPSS 22.0 (SPSS Inc., Chicago, Illinois). Lipid data were checked for both normality and homogeneity of variance using the Shapiro-Wilk test and Barlett's test, respectively. Prey and predator lipid content data failed both the Shapiro-Wilk test and Barlett's test. Consequently, lipid data were analyzed with non-parametric methods using the Kruskal-Wallis test. Statistical differences were analyzed further using ANOVA's *post hoc* Tamhanes's test as equal variance is not assumed with this test. Prey and predator lipid data were analyzed separately. Prey lipid data were aggregated over season and sample site for species comparison and over sample site for seasonal comparison.

Multivariate statistical analyses were conducted on fatty acid data. Non-parametric methods were performed using PRIMER v.6 (Primer-E, Plymouth, U.K.) with fatty acid data expressed as relative concentrations of 29 total detected fatty acids. Data were untransformed as they were expressed as percentages. Since non-parametric methods were used, there were no stringent sample size requirements to abide by and all data were included in analyses. Fatty acid data were analyzed using these methods to assess intra-specific and inter-specific variability in FAS among prey and predator fish as well as variability between season and among sample site for prey species.

Initially, average and standard deviation values were calculated for all predator species as well as prey species which were separated by season and location. A Bray-Curtis similarity matrix was computed separately for all prey and then all predator samples. Prey samples were also separated by species while predator samples were separated by family and Bray-Curtis similarity matrices were computed for each. Cluster analysis (CA) was used to analyze intra-specific similarity levels. CA was run for datasets of all prey samples, all predator samples, as well as salmonid species. Nonmetric multidimensional scaling (nMDS) plots were constructed to provide a two-dimensional representation of groupings based on FAS. The relative level of distortion of the data in the nMDS plots is represented by a stress value. This value is a measure of distortion of data on the plot such that stress values < 0.1 are indicative of accurate representation of data while stress values > 0.2 should be interpreted with caution as data may be distorted to the point of misrepresentation. Contours on the nMDS plots enclose data points based on defined levels of similarity between 0 and 100%. These contours were a result of CA and were used to further support intra-specific similarities in prey as well as inter-specific similarities in predators.

From the individual fatty acid data (represented by percent weight of total fatty acids as before) similarity percentage routine (SIMPER) was conducted to evaluate the levels of dissimilarity among prey species, predator family groups, and between alewife season groups as well as levels of similarity among prey species and predator family groups. Additionally, SIMPER was used summarize the contribution of fatty acids most responsible for similarities within and differences among these groups.

Analysis of similarity (ANOSIM) was conducted to determine differences between groupings formed based on prey and predator species, prey location and season, and predator family. It was also used to compare make comparisons between each predator and prey species, individually. This test allows for the entire FAS to be considered for group comparisons and yields an R-statistic value representing the level of similarity between two groups. R-statistics can range from 0.0 to 1.0. An R-statistic of 0 would indicate that the FAS of samples within a group are no more similar to one another than they are to the FAS of samples from the group to which they are being compared. Conversely, an R-statistic of 1.0 would indicate that FAS of all samples within a group are more similar to one another than to those of the group to which they are being compared. This particular analysis tested the null hypothesis that there are no differences in FAS between species or between sample sites within each species.

Principal component analysis (PCA) was conducted to reduce the dimensionality of fatty acid data and provide a descriptive means for comparison of prey and predator ordinations. Prey and predator samples were analyzed separately and plotted on two principal component axes that explain the majority of variance in the respective dataset. Vectors represent the fatty acids that primarily contribute to each principal component and those with correlations of at least 0.4 were selected and displayed.

3. Results

3.1. Fish Collection and Morphology

A total of 812 prey fish were collected from Lake Ontario during the spring and fall of 2013 and were analyzed for lipid content and fatty acid signature. Fish size, length, and weight displayed in Table 1 were separated by species, season, and location. Sample sizes for each group were variable as they were dependent on availability, though all groups had a sample size of at least $n = 13$. Additionally, fish lengths and weights were highly variable as a wide range of sizes were targeted for each group.

A total of 240 belly flap samples for lipid and fatty acid analyses were collected from predator species in Lake Ontario during the summer and fall of 2013. Fish location, length, and weight were displayed for each predator species in Table 2. Some weight data were unavailable as it was not recorded by the collection agency. Mean fish lengths were similar among location for each predator species.

3.2. Total Lipids in Prey and Predator

Total lipid data for prey aggregated over season and location yielded significant differences among species (Kruskal-Wallis, Chi-square = 300.08, df = 2, $p < 0.05$) (Figure 4). Alewife were found to have significantly higher total lipid content than rainbow smelt or round goby (Tamhane's *post hoc*, $p < 0.001$). Lipid content comparisons between seasons within species revealed significant differences for alewife (Kruskal-Wallis, Chi-square = 107.04, df = 1, $p < 0.05$) and round goby (Kruskal-Wallis, Chi-square = 58.05, df = 1, $p < 0.05$) between the spring and fall. Alewife collected in the fall were found to have significantly greater lipid content than those collected in the spring. Conversely, round goby from the spring were significantly

higher in lipid content than those collected during the fall. Seasonal differences were not observed in the total lipid content of rainbow smelt (Kruskal-Wallis, Chi-square = 0.17, df = 1, $p = 0.683$).

Alewife collected in the spring showed significant differences in total lipid content based on location (Kruskal-Wallis, Chi-square = 21.81, df = 2, $p < 0.05$). In the spring, alewife from Rochester had significantly lower total lipid content than alewife from Nine Mile Point and Thirty Mile Point (Tamhane's *post hoc*, $p < 0.001$). Round goby collected in the spring also had significant differences in total lipid content by location (Kruskal-Wallis, Chi-square = 20.28, df = 2, $p < 0.05$). Round goby from Nine Mile Point that were collected in the spring had significantly lower lipid content than spring round goby from Rochester or Thirty Mile Point (Tamhane's *post hoc*, $p < 0.001$).

Statistical differences were observed in total lipids of belly flap samples among predator species (Kruskal-Wallis, Chi-square = 175.32, df = 8, $p < 0.05$). Highest concentrations of total lipids were found in lake trout and brown trout while the lowest concentrations of total lipids were found in northern pike, chain pickerel, and yellow perch (Figure 5). Chinook salmon, coho salmon, steelhead, and walleye were found to have medial lipid content levels and showed no significant differences from one another.

3.3. Comparison of Prey Fatty Acid Signatures

For all prey samples, 29 different fatty acids were detected consistently and were used for analysis of FAS (Tables 3, 4, 5). In all three prey species, the most abundant SAFAs (saturated fatty acids) were palmitic acid (16:0) followed by stearic acid (18:0). MUFAs (monounsaturated fatty acids) oleic acid (18:1n-9) and palmitoleic acid (16:1n-7) were most abundant in alewife

and round goby while rainbow smelt MUFAs were dominated by oleic acid and 18:1n-7. All prey species' PUFAs (polyunsaturated fatty acids) were dominated by docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3).

High degrees of similarity were determined when examining intra-species FAS of prey regardless of location or season (Table 8). Alewife FAS were found to be 85.55% similar to one another with the fatty acids most responsible for this similarity being 16:0 at 21.74% contribution and 18:1n-9 contributing to 18.26% of similarity among all alewife samples. Both of these fatty acids were found at relatively high concentrations in alewife with average abundances of 19.89% and 17.86%, respectively. Rainbow smelt FAS, across all locations and seasons, were 83.73% similar each other. Contributing to this similarity were fatty acids 16:0 at 21.43% contribution and 22:6n-3 at 16.64% contribution. Average abundances detected for each were 19.11% and 17.42%, respectively. Finally, round goby FAS aggregated over space and time were 86.62% similar to each other. Similar to alewife, the two top contributing fatty acids for this similarity were 16:0 at 19.55% contribution and 18:1n-9 at 12.67% contribution. Average abundances for each were 18.14% and 12.08%, respectively. Intra-species similarities among all prey species were highlighted by 16:0 as the top contributing fatty acid (Table 8).

If the FAS of all prey species regardless of season or location was considered, alewife, rainbow smelt, and round goby were all significantly different (ANOSIM: Table 7). The strongest differences in FAS existed between alewife and round goby ($R = 0.74$, $P < 0.001$) followed by rainbow smelt and round goby ($R = 0.67$, $p < 0.001$) and alewife and rainbow smelt ($R = 0.45$, $p < 0.001$). Fatty acids primarily responsible for the differences in alewife and round goby FAS included 16:1n-7 and 18:1n-9 (Table 9). In round goby, 16:1n-7 is found at higher levels on average than in alewife (3.91% and 11.46% respectively), while alewife have higher

levels of 18:1n-9 than round goby (17.86% and 12.08% respectively). Fatty acids 16:1n-7 and 18:1n-9 contributed to 17.17% and 14.69 % of the difference between alewife and round goby FAS, respectively. Discriminating fatty acids responsible for the differences between rainbow smelt and round goby included 22:6n-3 (19.42% contribution) and 16:1n-7 (14.22% contribution). Higher levels of 22:6n-3 were detected in rainbow smelt while round goby again had higher levels of 16:1n-7. On average 22:6n-3 was detected at 17.42% of total fatty acids in rainbow smelt and at 9.79% in round goby. Less prevalent than in round goby, 16:1n-7 was detected at 6.10% in rainbow smelt. Finally, the fatty acids that contributed most to differences found between alewife and rainbow smelt were 22:6n-3 and 18:1n-9. Rainbow smelt had higher concentrations of 22:6n-3 and alewife had higher concentrations of 18:1n-9. The less abundant 22:6n-3 was detected at 9.77% in alewife while the less abundant 18:1n-9 was detected at 13.20% in rainbow smelt.

Inter-species differences and intra-species similarities among these three prey species are further represented in Figure 6 where prey species are plotted in a nMDS plot. The stress level of the plot was 0.16. Prey fish samples, which are plotted in space based on the degree of similarity of their FAS with respect to all other prey samples, showed some distinct groupings based on species. Moreover, cluster analysis, overlaid on Figure 6 as contours showing similarity groups, further supports intra-species similarities and inter-species differences among the three species. Three large clusters were formed at 80% similarity, and enclosed groups representative of alewife, rainbow smelt, and round goby. Round goby were more distinctly separated from alewife and rainbow smelt. While alewife and rainbow smelt groups were slightly overlapped, there were still identifiable clusters separating most samples of each species.

3.4. Spatio-Temporal Comparison of Prey Fatty Acid Signature

Strong differences in prey species FAS were not detected based on location (Table 10, Figures 7a, 8a, 9a). Though R-values showed some significant spatial differences, the values were generally low ($R < 0.29$: Table 10). Additionally, nMDS plots did not display distinct groupings that would indicate strong spatial differences among locations for any of the three prey species (Figures 7a, 8a, 9a).

Alewife collected in the spring of 2013 had significantly different FAS than those collected in the fall ($R = 0.60$, $p < 0.001$). This temporal difference found in alewife was much stronger than temporal differences in rainbow smelt or round goby (Table 10, Figure 7b). The fatty acids primarily contributing to this difference were 22:6n-3 and 18:1n-9 at 21.07% and 13.08%, respectively (Table 11). Alewife collected in the spring had higher levels of 22:6n-3 (12.88%) than those that were collected in the fall (5.76%). Also, on average, alewife collected in the fall were found to have slightly higher concentrations of 18:1n-9 (18.26%) than alewife collected during the spring (17.55%). Seasonal differences in FAS of rainbow smelt and round goby were weaker (ANOSIM: Table 10) and not evident by strong groupings in nMDS plots (Figures 8b and 9b).

3.5. Comparison of Predator Fatty Acid Signature

For all predator belly flap samples, 29 different fatty acids were routinely detected and used to create FAS. These 29 fatty acids were consistent with those detected and used for prey FAS. Predator SAFAs were dominated by 16:0 for all species while the most abundant MUFA for all species was 18:1n-9. Unlike other predator species the PUFA with the highest average concentration for Chinook salmon was linoleic acid (18:2n-6). The dominant PUFA for all other

predator species was 22:6n-3 (Table 6). All predator species were found to be significantly different from each other (ANOSIM: Table 12). However, some species comparisons revealed stronger differences than others. Walleye showed the strongest difference in FAS from salmonid species steelhead, coho, and Chinook salmon ($R = 1.00$, $p < 0.001$). Additionally, chain pickerel and northern pike showed strong differences in FAS from Chinook salmon ($R = 0.99$, $p < 0.001$). Lower R-values were detected between salmonid species such as brown trout and steelhead ($R = 0.31$, $p < 0.001$) and brown trout and coho salmon ($R = 0.34$, $p < 0.001$). Similarly, esocid species such as chain pickerel and northern pike displayed weak differences in FAS between one another ($R = 0.34$, $p < 0.001$).

Predator species showed similarities in FAS based on family (Figure 10). The stress level of this nMDS plot was 0.10. At 80% similarity, clusters formed groups separating salmonid species from esocids and percids and at 85% similarity, three large clusters formed separating the three families individually. However, walleye FAS were grouped with salmonid species rather than the percid species yellow perch (Figure 10). High degrees of similarity were determined when predator species were grouped by family (Table 14). On average salmonid FAS were 89.92% similar, esocids were 87.80% similar and percids were 85.70% similar to one another. Contributing to 26.16% of the similarity in salmonid species was 18:1n-9 with an average abundance of 24.48% while 16:0 contributed to 15.74% of similarity and had an average abundance of 14.99%. In esocids, 22:6n-3 was most important for similarity with 18.67% contribution and an average abundance of 18.84% followed by 16:0 at 17.86% contribution with an average abundance of 16.53%. Finally, similarity in percid FAS was facilitated by 16:0 and 18:1n-9 with contributions to similarity of 20.08% and 17.62%, respectively. Average abundance of 16:0 in percid species was 19.08% while 18:1n-9 was found on average at 18.14%.

Predator FAS also displayed differences among families (Table 13). The strongest differences were found between salmonid and percid species ($R = 0.87$, $p < 0.001$) and salmonid and esocid species ($R = 0.86$, $p < 0.001$) (Table 13). There was also a significant difference between esocids and percids, though differences were weaker ($R = 0.43$, $p < 0.001$). Differences between salmonids and esocids as well as salmonids and percids were explained primarily by 22:6n-3 and 18:1n-9. Salmonids species showed much higher concentrations of 18:1n-9 (24.48%) than esocids (15.57%) or percids (18.14%). However, 22:6n-3 was found at much lower levels in salmonids (8.34%) than in esocids (18.84%) or percids (14.45%). Differences in the FAS of esocids and percids were chiefly due to differences in concentrations of 16:1n-7 and 22:6n-3. On average percids had higher concentrations of 16:1n-7 than esocids while the opposite was true for 22:6n-3 (Table 15).

Salmonid fatty acid signatures were 80% similar to each other (Cluster analysis: Figure 11). However, at 90% similarity a new cluster is formed separating Chinook salmon FAS from all other salmonids. The stress value for this nMDS plot was 0.14. When compared to the rest of the salmonid species, Chinook salmon had notably higher concentrations of 16:0, 18:2n-6, and 18:3n-3 (Table 6).

3.6. Comparison of Prey and Predator Fatty Acid Signatures Using Principal Component Analysis and Analysis of Similarity

In both predator and prey, PCA reduced 29 routinely detected fatty acids into five principal components. For prey species the first two principal components explained 73.8% of the variance in the fatty acid data (Figure 12). The variance in the first principal component (PC1)

was explained positively by 22:6n-3 and was contributed to negatively by 18:1n-9. The second principal component (PC2) was explained positively by 16:1n-7 and was also contributed to negatively by 18:1n-9. For predator species, the first two principal components explained 83.6% of the variance in the data. Variance in PC1 was explained positively by 22:6n-3 and negatively by 18:1n-9. The variance in PC2 was explained positively by 16:1n-7 and negatively by 18:3n-3 (Figure 13).

Fatty acids 16:1n-7, 18:1n-9, and 22:6n-3 were all important for explaining variance in the FAS of prey species (Figure 12) as well as predator species (Figure 13). Fatty acids associated with the three different prey species are consistent with those associated with predator species based on family groupings. Fatty acid 18:1n-9 was found in high concentrations in both alewife and salmonid species (Tables 8 and15). Figures 12 and 13 support this common association as salmonid and alewife groupings are differentiated by 18:1n-9. A similar comparison was drawn between round goby and yellow perch. Both species were differentiated by high concentrations of 16:1n-7 (Tables 9 and 15) which is supported by common positioning in Figures 11 and 12 with 16:1n-7. Finally, rainbow smelt and esocid species were commonly differentiated by high concentrations of 22:6n-3 (Tables 9 and 15). PC1 was positively explained by 22:6n-3 representing groups formed for rainbow smelt as well as esocids (Figures 12 and 13).

FAS of all salmonid species along with walleye were most similar to the FAS of alewife than any other prey species (ANOSIM: Table 16). Furthermore, esocid species had the most in common with rainbow smelt and yellow perch showed the greatest similarities with round goby (ANOSIM: Table 16).

4. Discussion

4.1. Lipid Content in Prey and Predator

Our study provided a comprehensive evaluation of lipid content in prey and predator species of Lake Ontario. Lipid levels in alewife were significantly higher than in round goby or rainbow smelt regardless of season or location. Reported lipid content by Honeyfield *et al*. (2012) of Lake Ontario prey fish also showed much higher lipid content in alewife than in that of round goby, rainbow smelt, sculpin, or stickleback. High lipid content in alewife is likely a reason for the nutritional importance of the species as the main prey item for top predators across Lake Ontario.

Alewife had higher lipid content in the fall than in the spring. Similar seasonal trends were reported by Madenjian *et al*. (2000) for Lake Michigan population where alewife had average lipid contents of 7.4% in the spring and 12.2% in the fall. Increases in lipid content are potentially due to increased feeding rates, suggesting an increase in food availability or the emergence of a more lipid-rich diet source for alewife during the fall. Stewart and Binkowski (1986) estimated that nearly 50% of an individual alewife's annual feeding in Lake Michigan occurred during the fall months of September and October. Similarly, Rand *et al*. (1994) described strong seasonal changes in alewife of both Lake Ontario and Lake Michigan with regard to energy density. Energy density for Lake Ontario alewife in the fall more than doubled the densities in the spring (Rand *et al.* 1994). Alewife in Lake Ontario, spawn from late spring into early summer and it is common for many fish species to reduce their caloric intake prior to and during their spawning season. These seasonal differences could also be related to the importance of lipid reserves as an energy source for overwintering in fish (Toneys and Coble

1980). For example, the significant increase in lipid content of alewife in the fall compared to the spring could be a result of the need to build up lipid reserves in preparation for winter months when food availability is scarce. It is also important to note that alewife collected in the spring (April-early May) were collected from the bottom of Lake Ontario were conditions were still "winter-like". Thus, spring conditions may be more reflective of overwintering behaviors.

Though less significant, round goby also displayed seasonal changes in lipid content while rainbow smelt did not. Seasonal movements of prey could play a role in lipid content variation as nearshore to offshore movements have the potential to expose fish to different prey items, ultimately altering their diet. In the case of the round goby, documented offshore movement in the fall as nearshore waters cool (Walsh *et al*. 2007) could alter the diet and consequently the amount of essential nutrients consumed, resulting in the observed decrease in total lipids. Lake Ontario round goby have been found to have significant seasonal differences in feeding habits. Brush *et al*. (2012) reported gut contents of round goby in Lake Ontario that suggest increased reliance on dreissenids as well as, in one case, fish eggs during the spring compared to a more diverse diet including various invertebrates during the fall. Studies by Madenjian *et al*. (2000) and Rand *et al*. (1994) support our findings of little seasonal change in rainbow smelt lipid content. Furthermore, Rand *et al*. (1994) found energy density of rainbow smelt in Lake Ontario to vary less than those of Lake Michigan. These trends suggest that rainbow smelt may consume a lipid-deficient diet or simply maintain smaller lipid stores relative to alewife despite any seasonal shift in feeding habits,

In determining lipid content of predators, a section of the belly flap was processed for lipid and fatty acid analyses. This tissue type was determined my Budge *et al*. (2011) to better reflect the diet of Atlantic salmon in a controlled feeding experiment. This portion of tissue is

also very concentrated in lipids, resulting in high percent lipid values (Table 6 and Figure 5). Salmonid species along with walleye demonstrated significantly higher lipid concentrations than esocids or yellow perch. A great deal of trophic niche overlap among salmonid species determined by Yuille *et al*. (2015) is speculated to be due to these species utilizing alewife as their primary prey item in Lake Ontario. This commonality in feeding habits offers a potential explanation for higher lipid content in these species. Over a timespan from the 1970s-2008 lipid content in brown trout, Chinook salmon, coho salmon, and yellow perch in Lake Ontario has been trending in significant downward trajectory (Neff *et al*. 2012). This trend is important to monitor as it can provide indications of food web shifts as well as suggest changes in contaminant levels present in commonly consumed fish, emphasizing the need for further spatial and temporal comparisons of lipid content.

4.2. Prey FAS

Differences among alewife, rainbow smelt, and round goby FAS aggregated over space and time were likely influenced by distribution, feeding habits, and species-specific internal synthesis of fatty acids. Alewife, differentiated from the other two prey species by higher levels of 18:1n-9, are known to feed primarily on *Mysis relicta* in Lake Ontario (Stewart *et al*. 2009). Alewife were found to grow larger when fed diets rich in 20:5n-3 and 22:6n-3 highlighting the importance of *Mysis* as a food source as it pertains to the entire food web in Lake Ontario from a bottom-up perspective (Snyder *et al*. 2011).

Rainbow smelt occupy a similar ecological niche as alewife as they are both pelagic, open-water feeders. A study on Lake Champlain conducted by Simonin *et al*. (2012) showed extensive overlap of alewife and rainbow smelt distribution that was age, light, and temperature

dependent. Despite this perceived overlap, Lake Ontario alewife and rainbow smelt had significantly different FAS. Paterson *e al.* (2014) suggest increased importance of nearshore energy for rainbow smelt and alewife in Lake Ontario and report greater PCA contributions from the benthic-associated 16:1n-7 for rainbow smelt than alewife. In the present study, we also found higher concentrations of 16:1n-7 in rainbow smelt compared to alewife, suggesting that rainbow smelt in Lake Ontario may occupy a niche that is more reliant on benthic energy than alewife. This difference could also be the result of species-specific biosynthesis. In this case, even if alewife and rainbow smelt consumed a similar diet, their metabolic differences in fatty acid synthesis could account for differences in how the diet is reflected in the FAS.

Round goby had the most distinguishable FAS from the other two prey species. This is the expected result as round goby are benthic fish which implies a completely different feeding environment than alewife or rainbow smelt. Round goby feed in an environment where invertebrates and dreissenid mussels are present. Additionally, the benthic-oriented niche they occupy is characterized by higher rates of decomposition and oxidation that may contribute to FAS differences from pelagic environments at the lowest trophic level (Frederickson *et al*. 1986). In the present study, round goby could be differentiated from alewife and rainbow smelt as having higher concentrations of 16:1n-7 and lower levels of 22:6n-3. Similar concentration differences were reported by Czesny *et al*. (2011) in Lake Michigan. Thus, 16:1n-7 is associated with benthic than pelagic environments while 22:6n-3 is associated more with pelagic habitats in Lake Ontario.

Fatty acid profiles of major prey fish in Lake Ontario have yet to be extensively reported with spatial and temporal comparisons. Though significant inter-specific differences among prey species FAS regardless of season or location were established in the present study, intra-specific

differences were found in a seasonal comparison of alewife FAS. An organism's dietary composition changes as the prey items it encounters changes and the FAS of the consumer assimilate accordingly. Thus, FAS have the power to provide time integrated insights into the feeding habits of consumers (Budge *et al*. 2011). In this case, the temporal variation found in alewife FAS, similar to the same variation in lipids previously discussed, is likely due to shifts in diet leading to consumption of different prey items with different FAS. Stewart *et al*. (2009) reported dietary shifts in sub-adult alewife in Lake Ontario from *Mysis* dominated diets in the spring to zooplankton dominated diets in the fall. Furthermore, this study reported an increase in biomass of prey consumed by alewife from spring to fall. These trends offer a potential explanation for the differences in FAS observed in the present study. However, our reported seasonal differences in the FAS of alewife were due chiefly to a dramatic decrease in the presence of the fatty acid 22:6n-3 from spring to fall. In terms of fatty acid composition, *Mysis* has been characterized as having high levels of 18:1n-9 as well as seasonally fluctuating levels of 22:6n-3 (Hinderer *et al*. 2012). This fluctuation in 22:6n-3 is thought to be caused by starvation of *Mysis* (Schlechtriem 2008) as 22:6n-3 is a major component of membrane lipids which remain stable, unlike depleting storage lipids, during starvation. Therefore, it is possible that alewife fed primarily on starved *Mysis* during the spring of 2013, accounting for the high levels of 22:6n-3. However, this is not the only potential explanation. O'Gorman *et al*. (2011) describe an influx of alewife in Lake Ontario in nearshore waters that coincides with a decrease in zooplankton length. This illustrates the potential dietary shifts that could result not only from the seasonal movements of alewife exposing them to alternative food sources, but also from their consumption altering the dynamic of their own prey base. Seasonal zooplankton composition changes are also a likely contributor to the differences in alewife FAS. For example, O'Gorman *et al*. (2011) suggest

zooplankton composition becomes dominated by *Bosmina* spp. as the water in Lake Ontario begins to warm. Though there are numerous factors that obscure the ability to identify the exact contributors to this seasonal change, the reported fatty acid data illustrating the seasonal FAS difference in alewife is important to consider as a potential confounding factor when attempting to assess predator feeding habits.

4.3. Predator FAS and Implication for Predator-Prey Interaction

Predator species were grouped into taxonomic families based on FAS similarity. Differences among predator families may stem from inherent similarities in internal synthesis of fatty acids. Niche overlap among species of the same family is also a possible mechanism for levels of within-family similarity. However, each predator species was significantly different from one another regardless of family as family groupings clustered at only 85% similarity. Again, these differences may be attributed to smaller-scale biosynthesis differences among species within taxonomic family groupings. Alternatively, closely related species may be utilizing alternative food sources in an effort to supplement dietary requirements.

Similar to alewife distinction from rainbow smelt and round goby, salmonid species were differentiated from esocids and percids by higher levels of 18:1n-9. Salmonid species Chinook salmon, coho salmon, steelhead, lake trout and brown trout in Lake Michigan and Lake Superior have also been found to have similar levels of 18:1n-9 (Williams *et al*. 2014). The trophic niche overlap of salmonid species in Lake Ontario described by Yuille *et al*. (2015) supports attribution of this similarity in FAS to a common dietary composition among salmonid species

that heavily features alewife. A study conducted by Legard *et al*. (2014) which examined gut content of major predators in Lake Ontario coincided with sampling conducted for the present study. This study reflected the implications of the FAS data as gut contents revealed that alewife were the most abundant prey item for all salmonid species in Lake Ontario in 2013. Legard *et al.* (2014) also reported rainbow smelt as the second most consumed prey item by salmonids in Lake Ontario. Though alewife may be the prominent prey item for salmonids in Lake Ontario, presence of prey diversity is important as it enables species to supplement their diets in an ecosystem that is ever changing. Specifically, the current research on contributions of alewife to EMS in top predators may emphasize a need to switch from alewife to alternate prey items.

Williams *et al*. (2014) suggest that fatty acid compositions are better predicted by evaluating the dietary composition of a predator than by that predator's taxonomic family. This notion may relate to walleye showing more similarities in FAS to salmonid species than to the other percid species, yellow perch. This may indicate that walleye are consuming an alewife-rich diet like other salmonid species. However, strong differences between walleye and salmonid species Chinook salmon, coho salmon, and steelhead based on R-values are contradictory (Table 12). Walleye FAS seem to have a higher degree of similarity to lake trout and brown trout FAS (Table 12). Thus, it is suggested that there are some dietary similarities among these three species. The establishment of round goby as a viable prey item has been documented via their introduction into the diets of lake trout in Lake Michigan, Lake Huron (Jacobs *et al.* 2010, McKenna *et al*. unpublished data) and Lake Ontario (Dietrich *et al*. 2006). Furthermore, Legard *et al*. (2014) found round goby to comprise 44% of brown trout diets and 15% of lake trout diets in Lake Ontario in 2013. In the present study, walleye, lake trout, and brown trout had the highest concentrations of the benthic associated 16:1n-7 of any predator, aside from yellow perch.

Therefore, it is suggested that walleye may have feeding tendencies that utilize benthic round goby as well as pelagic alewife at a ratio similar to that of brown trout and lake trout.

Yellow perch were differentiated from all other predators as the discriminating fatty acid 16:1n-7 was found at the highest concentration of any predator species. The same was true for round goby when compared with alewife and rainbow smelt. While our results suggest that walleye may consume a combination of round goby and alewife, FAS of yellow perch support a more round goby-dominated diet. Stomach contents from yellow perch in Lake Ontario in 2013 were dominated by round goby, supporting FAS data (Legard *et al*. 2014).

Docosahexaenoic acid (22:6n-3) was important for differentiating esocid species northern pike and chain pickerel from other predators as well as rainbow smelt from other prey fish. High concentrations of 22:6n-3 accompanied by low concentrations of 18:1n-9 for esocids as a whole suggest a diet that may be comprised of rainbow smelt more so than any of the other predator species sampled in this study. Though there is limited research on the dietary habits of these species in Lake Ontario, rainbow smelt or other prey items with comparable FAS may be more readily available to esocids based on competition for alewife with salmonids species or simply due to optimal foraging within their preferred habitat.

4.4. Conclusions and Further Research

FAS in prey and predator species in Lake Ontario not only revealed distinguishable profiles among species, but suggested general feeding habits of top predators and highlighted the potential use of FAS to assess and monitor changing feeding habits in top predators which comprise a world class sport fishery in Lake Ontario. Our results provide the first comprehensive FAS dataset for major prey and predator species in Lake Ontario. Similar to work done in Lake

Michigan by Czesny *et al*. (2011), this type of data provides a foundation for lake-wide food web assessment using FAS. Seasonal differences in FAS of alewife signify seasonal changes in composition at lower trophic levels. These seasonal changes are important to consider from a management perspective when assessing the nutritional value available for top predators at certain times of the year. At the forage fish trophic level, FAS were indicative of pelagic or benthic environments, supporting use of FAS to assess the utilization of different food sources by top predators. Specific to the present study, general trends in FAS identify walleye, lake trout, and brown trout as species that may be utilizing round goby as a food source as this invasive species expands its habitat range to deeper waters in Lake Ontario (Dietrich *et al*. 2006). With the changes to the nearshore environment in Lake Ontario brought about by invasions of round goby and dreissenid mussels, this shift in diet composition of top predators may provide a pathway for energy, that would have otherwise been trapped in the nearshore, to reach offshore environments and a higher trophic level.

Our results depicted general trends in FAS of prey and predator species from which we were able to draw broad conclusions regarding predator feeding habits. Fatty acid data supported findings of gut contents and modeling in similar studies (Legard *et al.* 2014, Czesny *et al*. 2011). FAS suggested a prominent diet of alewife for salmonids and a round goby-rich diet for yellow perch. We were also able to use FAS data to speculate the composition of predator diets that may have been more balanced. However, in order to quantitatively predict predator diet composition, there is a need for controlled feeding experiments. As previously discussed, FAS are reflective of both the diet of an organism as well as its metabolic synthesis of fatty acids. During controlled feeding experiments, fish FAS will assimilate to the FAS of their diet only to a certain degree. The differences that remain between the FAS of the consumer and its diet can be attributed to the

metabolic synthesis of fatty acids which is typically species-specific. Therefore, controlled feeding experiments can produce species-specific calibration coefficients that can be implemented in QFASA to adjust for such variability (Iverson *et al*. 2004). Calibration coefficients have been used in modeling to successfully estimate diets of freshwater fish in a controlled environment (Happel *et al*. unpublished data). Further knowledge of specific predator-prey metabolic responses should be investigated before FAS data, like the dataset reported in the present study, can be manipulated to provided quantitative information on diet estimations.

Vital to the implementation of a quantitative assessment of predator feeding habits is the ability to distinguish FAS of potential prey items. Prey items that are indiscernible from one another have the potential to confound findings of a quantitative and even qualitative assessment of predator dietary composition based on FAS. Strong heterogeneity among major prey species FAS relative to spatio-temporal variation was illustrated in this study. Moreover, two of these prey species (alewife and rainbow smelt) have similar feeding habits and ecological niches. Thus, it is suggested that the application of QFASA is a viable option for assessing predator feeding habits in Lake Ontario.

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Table 1. Prey sample summary for spring and fall of 2013 including sample site, species, sample size (*n*), and length and weight (mean ± standard deviation). Collection agency for all samples was the U.S. Geological Survey.

		Sample site								
		Thirty Mile Point			Rochester			Nine Mile Point		
		Alewife	Rainbow Smelt	Round Goby	Alewife	Rainbow Smelt	Round Goby	Alewife	Rainbow Smelt	Round Goby
Spring	*n*	66	65	20	71	43	70	42	37	13
	Length (mm)	123.5 ± 43.1	101.7 ± 24.6	93.6 ± 18.9	108.8 ± 37.7	99.3 ± 31.7	90.6 ± 25.6	128.1 ± 40.3	91.8 ± 23.2	86.8 ± 24.9
	Weight (g)	19.6 ± 16.7	6.6 ± 4.6	14.7 ± 9.2	12.3 ± 12.3	8.0 ± 10.5	15.1 ± 12.1	21.5 ± 18.3	5.1 ± 3.5	4.5 ± 3.4
Fall	*n*	57	32	79	45	20	37	36	22	57
	Length (mm)	149.5 ± 27.9	78.2 ± 29.7	71.2 ± 25.6	103.8 ± 43.1	103.8 ± 6.7	72.5 ± 21.4	100.7 ± 37.9	71.9 ± 21.8	73.8 ± 27.8
	Weight (g)	29.2 ± 12.8	4.4 ± 5.3	7.4 ± 8.3	13.9 ± 13.7	6.5 ± 1.0	7.5 ± 9.0	12.3 ± 12.5	2.8 ± 3.9	8.5 ± 11.4

Table 2. Predator sample summary for spring-summer of 2013 including sample site, species, sample size (*n*), collection agency and method, and length (mm) and weight (g); mean ± standard deviation (- indicates absence of data).

		Sample site					
		Wilson	Olcott	Oak Orchard	Rochester	Oswego	Henderson
Lake trout	*n*	–	3[a]	13[a]	23[b] + 21[c] + 8[d]	–	7[a]
	Length (mm)	–	744.2 ± 82.6	665.1 ± 71.2	516.7 ± 300.2	–	696.0 ± 76.7
	Weight (g)	–	6350.3 ± 4571.2	3449.6 ± 1416.9	4281.7 ± 2149.8	–	–
Brown trout	*n*	–	–	9[a]	6[b] + 6[c]	–	3[a]
	Length (mm)	–	–	578.9 ± 76.1	609.3 ± 115.6	–	629.3 ± 23.5
	Weight (g)	–	–	3392.8 ± 1158.0	–	–	–
Chinook salmon	*n*	15[a]	5[a]	–	1[b] + 1[c]	10[a]	–
	Length (mm)	774.9 ± 87.4	819.2 ± 38.6	–	614.0 ± 319.6	960.3 ± 49.3	–
	Weight (g)	5402.8 ± 1825.0	5815.3 ± 1107.2	–	–		–
Coho salmon	*n*	–	17[a]	–	1[b]	–	–
	Length (mm)	–	574.7 ± 40.2	–	620.0 ± –	–	–
	Weight (g)	–	1919.6 ± 279.5	–	–	–	–
Steelhead	*n*	–	–	15[a]	–	–	–
	Length (mm)	–	–	680.4 ± 66.3	–	–	–
	Weight (g)	–	–	3068.6 ± 882.5	–	–	–
Chain pickerel	*n*	–	–	–	–	–	19[a]
	Length (mm)	–	–	–	–	–	601.1 ± 35.0
	Weight (g)	–	–	–	–	–	
Northern pike	*n*	–	–	–	–	–	15[a]
	Length (mm)	–	–	–	–	–	731.8 ± 79.1
	Weight (g)	–	–	–	–	–	–
Walleye	*n*	–	–	–	5[b]	–	3[a]
	Length (mm)	–	–	–	627.8 ± 52.1	–	708.3 ± 69.0
	Weight (g)	–	–	–	–	–	–
Yellow perch	*n*	–	–	–	29[c] + 5[b]	–	–
	Length (mm)	–	–	–	234.9 ± 40.5	–	–
	Weight (g)	–	–	–	–	–	–

Collection agencies and methods: a: The College at Brockport Department of Environmental Science and Biology at fishing tournaments, donated by anglers, b: The College at Brockport Department of Environmental Science and Biology by use of vertical gillnets, c: U.S. Geological Survey by use of vertical gillnets, d: U.S. Geological Survey as bycatch during bottom trawling.

Table 3. Lipid concentration (% wet mass) and fatty acid composition (% detected) in alewife collected from Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP) during the spring and fall of 2013. Data expressed as mean ± standard deviation.

	Spring			Fall		
	TMP	ROCH	NMP	TMP	ROCH	NMP
Lipid (%)	6.6 ± 3.4	4.2 ± 2.4	4.2 ± 2.4	11.3 ± 3.7	10.0 ± 4.1	9.6 ± 3.3
Fatty acids (%)						
12:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
14:0	4.0 ± 0.9	3.4 ± 1.0	4.8 ± 1.0	6.5 ± 0.5	5.6 ± 1.1	5.0 ± 0.7
16:0	19.8 ± 2.1	18.8 ± 2.4	20.6 ± 2.4	20.2 ± 1.6	19.5 ± 1.8	19.7 ± 1.6
17:0	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.8 ± 0.1
18:0	4.0 ± 0.9	4.6 ± 0.8	3.9 ± 0.8	3.2 ± 0.4	3.6 ± 0.6	3.8 ± 0.5
20:0	0.3 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
22:0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:1n-9	0.9 ± 0.2	0.8 ± 0.3	1.0 ± 0.2	1.2 ± 0.1	1.2 ± 0.2	1.2 ± 0.1
16:1n-7	3.6 ± 0.7	2.8 ± 0.9	4.1 ± 1.0	4.9 ± 0.4	4.4 ± 0.8	4.2 ± 0.8
17:1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.2
18:1n-9	18.6 ± 3.7	15.9 ± 5.9	18.7 ± 4.1	18.0 ± 2.4	17.8 ± 2.7	19.3 ± 3.1
18:1n-7	3.4 ± 0.5	3.0 ± 0.3	3.4 ± 0.4	3.6 ± 0.3	3.8 ± 0.4	3.6 ± 0.3
20:1n-9	1.9 ± 0.6	1.8 ± 0.8	1.8 ± 0.7	1.2 ± 0.3	1.2 ± 0.4	1.6 ± 0.6
22:1n-11	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1
22:1n-9	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:2n-6	3.9 ± 0.8	3.6 ± 1.0	4.0 ± 0.8	5.3 ± 0.5	4.8 ± 0.5	4.6 ± 0.5
20:2n-6	0.9 ± 0.3	0.7 ± 0.3	0.8 ± 0.3	0.4 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
20:3n-6	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
20:4n-6	5.3 ± 1.3	6.0 ± 1.6	5.2 ± 1.1	4.3 ± 0.4	4.7 ± 0.8	4.8 ± 0.5
22:4n-6	0.4 ± 0.1	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
22:5n-6	1.9 ± 0.6	2.2 ± 0.6	1.6 ± 0.5	0.7 ± 0.1	0.8 ± 0.2	0.9 ± 0.2
18:3n-3	3.8 ± 1.6	3.1 ± 1.1	3.7 ± 0.9	6.0 ± 0.5	6.1 ± 0.6	6.1 ± 0.6
18:4n-3	1.4 ± 0.6	1.5 ± 0.6	1.6 ± 0.4	4.3 ± 0.7	4.4 ± 0.6	4.0 ± 0.9
20:3n-3	1.2 ± 0.4	0.9 ± 0.4	1.0 ± 0.4	0.5 ± 0.3	0.4 ± 0.1	0.4 ± 0.3
20:4n-3	1.8 ± 0.4	1.7 ± 0.4	1.7 ± 0.4	1.5 ± 0.2	1.4 ± 0.2	1.3 ± 0.2
20:5n-3	7.4 ± 0.8	7.9 ± 1.1	7.4 ± 0.9	9.0 ± 0.8	9.5 ± 1.1	9.1 ± 1.2
21:5n-3	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0
22:5n-3	2.1 ± 0.3	2.3 ± 0.4	1.9 ± 0.3	1.4 ± 0.3	1.7 ± 0.5	1.7 ± 0.2
22:6n-3	11.3 ± 3.9	16.0 ± 6.6	10.1 ± 4.2	5.6 ± 1.1	6.0 ± 1.9	5.7 ± 1.0

Table 4. Lipid concentration (% wet mass) and fatty acid composition (% detected) in rainbow smelt collected from Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP) during the spring and fall of 2013. Data expressed as mean ± standard deviation.

	Spring			Fall		
	TMP	ROCH	NMP	TMP	ROCH	NMP
Lipid (%)	2.0 ± 0.8	3.1 ± 0.7	3.2 ± 1.1	3.0 ± 1.9	3.2 ± 1.2	2.5 ± 1.2
Fatty acids (%)						
12:0	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
14:0	3.0 ± 1.2	4.0 ± 0.8	4.3 ± 1.2	3.1 ± 2.0	4.4 ± 1.0	2.8 ± 1.1
16:0	19.6 ± 2.1	17.5 ± 2.2	19.1 ± 1.8	19.4 ± 1.9	19.1 ± 1.4	19.6 ± 1.5
17:0	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1
18:0	3.2 ± 0.6	3.2 ± 0.7	2.9 ± 0.7	5.0 ± 1.9	3.8 ± 0.5	5.4 ± 1.1
20:0	0.1 ± 0.1	0.2 ± 0.3	0.1 ± 0.0	0.3 ± 0.2	0.2 ± 0.0	0.3 ± 0.1
22:0	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
16:1n-9	0.7 ± 0.2	0.6 ± 0.3	0.8 ± 0.2	0.6 ± 0.1	1.0 ± 0.1	0.8 ± 0.1
16:1n-7	6.8 ± 2.7	6.0 ± 2.7	8.2 ± 2.7	4.4 ± 3.0	4.9 ± 1.0	3.6 ± 2.6
17:1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
18:1n-9	13.2 ± 3.6	12.1 ± 4.9	14.3 ± 3.7	13.4 ± 3.8	14.1 ± 1.4	12.9 ± 2.2
18:1n-7	3.1 ± 0.3	2.8 ± 0.6	3.1 ± 0.2	2.7 ± 0.5	3.4 ± 0.3	3.2 ± 0.2
20:1n-9	0.6 ± 0.4	1.0 ± 0.3	1.0 ± 0.2	0.8 ± 0.3	1.0 ± 0.3	0.6 ± 0.2
22:1n-11	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
22:1n-9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
18:2n-6	2.7 ± 1.2	4.0 ± 0.9	3.6 ± 1.1	3.9 ± 1.3	5.3 ± 1.1	4.7 ± 0.7
20:2n-6	0.5 ± 0.3	0.6 ± 0.2	0.7 ± 0.1	0.4 ± 0.1	0.6 ± 0.2	0.4 ± 0.2
20:3n-6	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
20:4n-6	5.8 ± 1.2	4.7 ± 1.6	4.6 ± 0.9	5.3 ± 1.1	5.6 ± 1.0	7.3 ± 1.5
22:4n-6	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
22:5n-6	1.2 ± 0.3	1.5 ± 0.3	1.3 ± 0.4	2.0 ± 1.1	1.2 ± 0.2	1.5 ± 0.4
18:3n-3	2.3 ± 1.0	4.1 ± 1.2	3.3 ± 1.2	3.8 ± 1.0	6.0 ± 1.4	5.4 ± 1.2
18:4n-3	0.7 ± 0.6	2.6 ± 1.6	1.4 ± 0.5	2.0 ± 1.1	2.7 ± 0.7	2.2 ± 0.7
20:3n-3	0.6 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	0.5 ± 0.2	0.9 ± 0.3	0.4 ± 0.3
20:4n-3	0.7 ± 0.3	1.2 ± 0.5	0.9 ± 0.3	0.8 ± 0.2	1.1 ± 0.3	0.7 ± 0.2
20:5n-3	11.8 ± 2.2	11.1 ± 1.6	11.0 ± 1.6	11.3 ± 1.2	10.9 ± 1.5	12.5 ± 1.6
21:5n-3	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
22:5n-3	0.9 ± 0.4	1.4 ± 0.4	1.2 ± 0.3	1.0 ± 0.4	1.4 ± 0.3	1.0 ± 0.3
22:6n-3	21.0 ± 6.0	18.6 ± 3.7	15.6 ± 5.4	17.3 ± 6.8	10.6 ± 3.1	12.8 ± 2.8

Table 5. Lipid concentration (% wet mass) and fatty acid composition (% detected) in round goby collected from Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP) during the spring and fall of 2013. Data expressed as mean ± standard deviation.

	Spring			Fall		
	TMP	ROCH	NMP	TMP	ROCH	NMP
Lipid (%)	4.6 ± 1.2	3.9 ± 1.6	2.4 ± 0.8	2.7 ± 0.9	2.9 ± 0.9	2.2 ± 0.6
Fatty acids (%)						
12:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
14:0	3.4 ± 0.6	2.8 ± 0.5	3.2 ± 1.1	2.2 ± 0.7	2.5 ± 0.8	2.2 ± 0.8
16:0	17.6 ± 1.6	17.3 ± 1.4	20.4 ± 2.2	17.5 ± 2.0	18.8 ± 2.2	19.8 ± 2.3
17:0	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.0	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.2
18:0	2.9 ± 0.5	4.0 ± 1.3	3.2 ± 0.5	4.9 ± 1.1	4.2 ± 0.8	5.6 ± 1.5
20:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
22:0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
16:1n-9	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	1.0 ± 0.2	0.8 ± 0.1	1.0 ± 0.2
16:1n-7	16.8 ± 3.2	12.6 ± 3.7	7.2 ± 3.2	10.5 ± 3.5	11.4 ± 2.7	9.5 ± 3.8
17:1	0.7 ± 0.1	0.7 ± 0.2	0.3 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.2
18:1n-9	13.6 ± 1.4	12.7 ± 2.2	12.1 ± 2.3	11.6 ± 2.1	12.0 ± 1.2	11.5 ± 1.6
18:1n-7	5.5 ± 0.5	5.6 ± 1.0	3.2 ± 0.3	5.8 ± 0.9	4.8 ± 0.8	5.1 ± 1.0
20:1n-9	1.9 ± 0.4	2.2 ± 0.9	0.8 ± 0.2	2.2 ± 0.8	2.5 ± 0.7	1.8 ± 0.7
22:1n-11	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
22:1n-9	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
18:2n-6	2.6 ± 0.4	2.9 ± 0.6	2.8 ± 1.2	3.6 ± 0.8	2.6 ± 0.7	3.5 ± 1.0
20:2n-6	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2
20:3n-6	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
20:4n-6	5.5 ± 1.1	6.3 ± 1.3	4.9 ± 1.3	6.5 ± 1.4	6.8 ± 1.1	7.4 ± 1.6
22:4n-6	0.8 ± 0.3	0.9 ± 0.4	0.1 ± 0.1	0.7 ± 0.3	1.0 ± 0.3	0.7 ± 0.3
22:5n-6	2.1 ± 0.7	2.8 ± 1.1	1.3 ± 0.3	2.1 ± 0.7	3.2 ± 1.0	2.0 ± 0.8
18:3n-3	2.3 ± 0.5	2.8 ± 0.8	2.6 ± 1.4	2.9 ± 1.2	2.4 ± 0.8	2.4 ± 1.0
18:4n-3	1.6 ± 0.3	1.9 ± 0.8	1.2 ± 0.8	1.4 ± 0.6	1.3 ± 0.5	1.1 ± 0.5
20:3n-3	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:4n-3	0.4 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
20:5n-3	8.4 ± 1.0	9.0 ± 2.2	12.9 ± 2.0	10.1 ± 1.5	8.0 ± 1.8	9.8 ± 2.1
21:5n-3	0.0 ± 0.0	0.0 ± 0.1	0.0 ± 0.0	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.2
22:5n-3	2.9 ± 0.8	3.2 ± 0.7	1.0 ± 0.3	3.3 ± 1.0	3.2 ± 0.5	2.9 ± 0.7
22:6n-3	8.4 ± 1.8	9.3 ± 1.7	19.4 ± 5.2	10.0 ± 2.6	10.7 ± 2.1	10.1 ± 2.7

Table 6. Lipid concentration (% wet mass) and fatty acid composition (% detected) in belly flap tissue of predator fish collected from Lake Ontario during the summer and fall of 2013.

	Lake trout	Brown trout	Chinook salmon	Coho salmon	Steelhead	Northern pike	Chain pickerel	Walleye	Yellow perch
Lipid (%)	34.8 ± 9.1	38.7 ± 11.9	25.4 ± 7.2	18.0 ± 6.8	23.5 ± 15.8	3.0 ± 1.7	1.5 ± 0.5	20.3 ± 15.3	4.2 ± 3.5
Fatty acids (%)									
12:0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
14:0	3.3 ± 0.4	3.3 ± 0.5	4.4 ± 0.4	3.3 ± 0.4	3.8 ± 0.5	2.6 ± 0.8	1.8 ± 0.6	3.4 ± 0.3	2.6 ± 0.4
16:0	14.1 ± 1.1	15.8 ± 1.5	16.5 ± 1.0	15.1 ± 1.7	15.5 ± 1.5	15.6 ± 1.6	17.4 ± 1.0	12.5 ± 1.1	20.6 ± 1.8
17:0	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
18:0	2.9 ± 0.4	4.1 ± 0.7	4.8 ± 1.0	3.9 ± 0.6	4.0 ± 0.5	3.9 ± 0.7	4.7 ± 0.8	1.1 ± 0.6	3.2 ± 0.7
20:0	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
22:0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
16:1n-9	0.6 ± 0.2	0.7 ± 0.2	1.2 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.2	0.8 ± 0.2	1.4 ± 0.5	1.1 ± 0.2
16:1n-7	8.9 ± 1.9	8.1 ± 2.2	4.9 ± 0.5	5.2 ± 0.7	4.8 ± 0.9	7.6 ± 2.7	5.3 ± 1.5	8.8 ± 1.1	12.5 ± 3.4
17:1	0.6 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
18:1n-9	24.6 ± 1.8	25.1 ± 2.4	25.3 ± 1.5	23.5 ± 1.6	22.7 ± 1.3	17.3 ± 1.8	14.7 ± 4.0	28.9 ± 1.1	15.7 ± 2.8
18:1n-7	4.2 ± 0.5	4.5 ± 0.6	3.2 ± 0.3	3.4 ± 0.5	3.6 ± 0.2	3.2 ± 0.7	2.6 ± 0.4	2.9 ± 0.3	3.7 ± 0.4
20:1n-9	2.0 ± 0.3	2.5 ± 0.3	2.4 ± 0.4	2.2 ± 0.4	2.1 ± 0.4	1.4 ± 0.3	1.0 ± 0.3	1.1 ± 0.2	1.1 ± 0.3
22:1n-11	0.3 ± 0.1	0.4 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	0.4 ± 0.3	0.1 ± 0.0	0.1 ± 0.2	0.1 ± 0.1	0.7 ± 0.9
22:1n-9	0.1 ± 0.0	0.1 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.1 ± 0.1
18:2n-6	3.8 ± 0.7	3.1 ± 0.8	5.6 ± 0.4	4.3 ± 0.4	4.9 ± 0.5	4.5 ± 0.6	4.1 ± 0.8	4.5 ± 0.3	2.1 ± 0.5
20:2n-6	0.8 ± 0.2	0.7 ± 0.3	0.9 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.6 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.4 ± 0.1
20:3n-6	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.5	0.4 ± 0.2	0.2 ± 0.1
20:4n-6	3.6 ± 0.3	3.5 ± 0.3	3.1 ± 0.2	4.0 ± 0.3	4.1 ± 0.3	5.9 ± 1.2	8.2 ± 2.3	5.7 ± 0.4	5.1 ± 1.1
22:4n-6	1.0 ± 0.2	1.1 ± 0.3	0.5 ± 0.1	0.9 ± 0.2	1.2 ± 0.5	0.6 ± 0.2	0.5 ± 0.1	0.6 ± 0.1	0.9 ± 0.2
22:5n-6	1.7 ± 0.3	1.7 ± 0.4	0.8 ± 0.2	1.7 ± 0.4	1.7 ± 0.4	2.0 ± 0.6	1.7 ± 0.5	2.4 ± 0.2	2.4 ± 0.6
18:3n-3	3.4 ± 0.7	2.9 ± 1.0	5.5 ± 0.4	4.3 ± 0.5	3.9 ± 0.6	2.7 ± 0.6	2.1 ± 0.5	3.1 ± 0.6	1.5 ± 0.5
18:4n-3	1.4 ± 0.3	0.9 ± 0.3	1.9 ± 0.2	1.4 ± 0.2	1.0 ± 0.3	1.3 ± 0.4	0.8 ± 0.3	0.7 ± 0.2	0.8 ± 0.2
20:3n-3	1.0 ± 0.3	1.0 ± 0.7	1.0 ± 0.2	0.9 ± 0.1	1.4 ± 0.4	0.5 ± 0.2	0.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
20:4n-3	2.2 ± 0.6	2.1 ± 0.6	2.8 ± 0.3	2.2 ± 0.4	2.7 ± 0.4	1.4 ± 0.4	1.2 ± 0.4	1.3 ± 0.2	0.6 ± 0.3
20:5n-3	5.2 ± 0.7	4.4 ± 1.0	3.9 ± 0.6	5.4 ± 0.7	5.1 ± 1.0	5.5 ± 0.5	6.6 ± 1.1	4.6 ± 0.7	5.0 ± 0.9
21:5n-3	0.3 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1
22:5n-3	3.5 ± 0.5	3.7 ± 0.5	2.5 ± 0.5	3.6 ± 0.7	4.1 ± 0.7	3.3 ± 0.6	3.3 ± 0.4	2.6 ± 0.2	2.5 ± 0.3
22:6n-3	9.0 ± 1.1	8.2 ± 1.1	5.2 ± 1.3	10.4 ± 1.9	8.7 ± 1.0	16.6 ± 4.1	19.9 ± 5.2	11.2 ± 1.0	15.2 ± 3.4

Table 7. Lake Ontario prey fish: 1-Way ANOSIM pairwise tests for each species. Samples within each species group are aggregated spatio-temporally. Comparisons sorted by R statistic (lowest to highest).

Species comparisons	R-statistic
Rainbow smelt, Alewife	0.45
Rainbow smelt, Round goby	0.67
Alewife, Round goby	0.74

Table 8. Prey fish from Lake Ontario: SIMPER routine for similarity within species groups. Fatty acids (% detected) that contribute to ≥ 70% of cumulative similarity are reported.

Species	Average similarity (%)	Average abundance (%)	Contribution (%)
Alewife	85.55		
16:0		19.69	21.74
18:1n-9		17.86	18.26
20:5n-3		8.30	8.88
22:6n-3		9.77	8.02
20:4n-6		5.12	5.27
14:0		4.78	4.65
18:2n-6		4.33	4.44
Rainbow smelt	83.73		
16:0		19.11	21.43
22:6n-3		17.42	16.64
18:1n-9		13.20	13.33
20:5n-3		11.53	12.54
20:4n-6		5.41	5.48
16:1n-7		6.10	5.29
Round goby	86.62		
16:0		18.14	19.55
18:1n-9		12.08	12.67
16:1n-7		11.46	10.64
22:6n-3		9.79	9.79
20:5n-3		9.33	9.47
20:4n-6		6.59	6.67
18:1n-7		5.43	5.65

Table 9. Prey fish from Lake Ontario: SIMPER routine for dissimilarity among species groups. Fatty acids (% detected) that contribute to ≥ 70% of cumulative dissimilarity are reported.

Group comparison	Average dissimilarity (%)	Average abundance		Contribution
Rainbow smelt, Alewife	20.26	Rainbow smelt	Alewife	
22:6n-3		17.42	9.77	23.51
18:1n-9		13.20	17.86	15.22
20:5n-3		11.53	8.30	8.35
16:1n-7		6.10	3.91	7.20
16:0		19.11	19.69	5.92
18:3n-3		3.62	4.62	5.12
14:0		3.53	4.78	4.71
18:4n-3		1.71	2.73	4.28
Rainbow smelt, Round goby	21.36	Rainbow smelt	Round goby	
22:6n-3		17.42	9.79	19.42
16:1n-7		6.10	11.46	14.22
18:1n-9		13.20	12.08	7.78
20:5n-3		11.53	9.33	6.62
16:0		19.11	18.14	6.00
18:1n-7		3.01	5.43	5.67
22:5n-3		1.10	3.17	4.85
20:4n-6		5.41	6.59	4.41
18:3n-3		3.62	2.63	4.03
Alewife, Round goby	22.13	Alewife	Round goby	
16:1n-7		3.91	11.46	17.17
18:1n-9		17.86	12.08	14.69
22:6n-3		9.77	9.79	10.57
16:0		19.69	18.14	6.16
14:0		4.78	2.52	5.37
18:3n-3		4.62	2.63	5.29
20:5n-3		8.30	9.33	4.70
18:1n-7		3.42	5.43	4.59
20:4n-6		5.12	6.59	4.39

Table 10. Lake Ontario prey fish: 2-Way ANOSIM for location and season represented as R-statistics. Locations are Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP). Location and season comparisons were analyzed separately.

	R-statistic		
Comparison	Alewife	Rainbow smelt	Round goby
TMP, ROCH	0.14	0.28	0.06
ROCH, NMP	0.13	0.18	0.15
TMP, NMP	0.19	0.14	0.12
Spring, Fall	0.60	0.31	0.18

Table 11. Alewife from spring and fall of 2013 in Lake Ontario: SIMPER routine for dissimilarity between seasons. Samples within species-season group are aggregated spatially. Fatty acids (% detected) that contribute to ≥ 70% of cumulative dissimilarity are reported.

Fatty acid	Average abundance (%)		Contribution (%)
	Spring	Fall	
22:6n-3	12.88	5.76	21.07
18:1n-9	17.55	18.26	13.08
18:4n-3	1.53	4.27	8.03
18:3n-3	3.49	6.08	7.84
16:0	19.59	19.84	6.75
14:0	3.94	5.85	5.95
20:5n-3	7.60	9.19	5.21
16:1n-7	3.40	4.57	4.03

Table 12. Predator fish from Lake Ontario: 1-Way ANOSIM comparisons for species groups. Comparisons sorted by R-statistic (lowest to highest).

Species comparison	R-statistic	Species comparison	R-statistic
Brown trout, Steelhead trout	0.31	Brown trout, Yellow perch	0.91
Brown trout, Coho salmon	0.34	Chain pickerel, Coho salmon	0.91
Chain pickerel, Northern pike	0.34	Walleye, Yellow perch	0.91
Coho salmon, Steelhead trout	0.35	Lake trout, Northern pike	0.93
Brown trout, Lake trout	0.38	Steelhead trout, Yellow perch	0.93
Coho salmon, Lake trout	0.45	Chain pickerel, Steelhead trout	0.94
Northern pike, Yellow perch	0.60	Chain pickerel, Walleye	0.94
Lake trout, Steelhead trout	0.61	Chinook salmon, Coho salmon	0.94
Chain pickerel, Yellow perch	0.72	Chinook salmon, Lake trout	0.95
Coho salmon, Northern pike	0.76	Brown trout, Chain pickerel	0.95
Brown trout, Northern pike	0.82	Lake trout, Yellow perch	0.97
Brown trout, Walleye	0.82	Chinook salmon, Northern pike	0.99
Northern pike, Steelhead trout	0.83	Chinook salmon, Yellow perch	0.99
Northern pike, Walleye	0.83	Chain pickerel, Lake trout	0.99
Brown trout, Chinook salmon	0.83	Chain pickerel, Chinook salmon	0.99
Chinook salmon, Steelhead trout	0.87	Coho salmon, Walleye	1.00
Lake trout, Walleye	0.88	Chinook salmon, Walleye	1.00
Coho salmon, Yellow perch	0.90	Steelhead trout, Walleye	1.00

Table 13. Lake Ontario predator fish: 1-Way ANOSIM pairwise tests for predator fish grouped by family. Comparisons sorted by R statistic (lowest to highest).

Family comparison	R-statistic
Esocidae, Percidae	0.43
Salmonidae, Esocidae	0.86
Salmonidae, Percidae	0.87

Table 14. Predator fish from Lake Ontario: SIMPER routine for similarity within family groups. Fatty acids (% detected) that contribute to ≥ 70% of cumulative similarity are reported.

Family	Average similarity (%)	Average abundance (%)	Contribution (%)
Salmonidae	89.92		
18:1n-9		24.48	26.16
16:0		14.99	15.75
22:6n-3		8.34	8.09
16:1n-7		7.27	6.56
20:5n-3		4.87	4.85
18:2n-6		4.16	4.01
18:1n-7		3.93	3.97
20:4n-6		3.58	3.75
Esocidae	87.80		
22:6n-3		18.84	18.67
16:0		16.53	17.86
18:1n-9		15.57	15.77
20:4n-6		7.24	6.93
20:5n-3		6.15	6.37
16:1n-7		6.31	5.72
Percidae	85.70		
16:0		19.08	20.08
18:1n-9		18.14	17.62
22:6n-3		14.45	14.66
16:1n-7		11.78	11.5
20:4n-6		5.17	5.41
20:5n-3		4.94	5.24

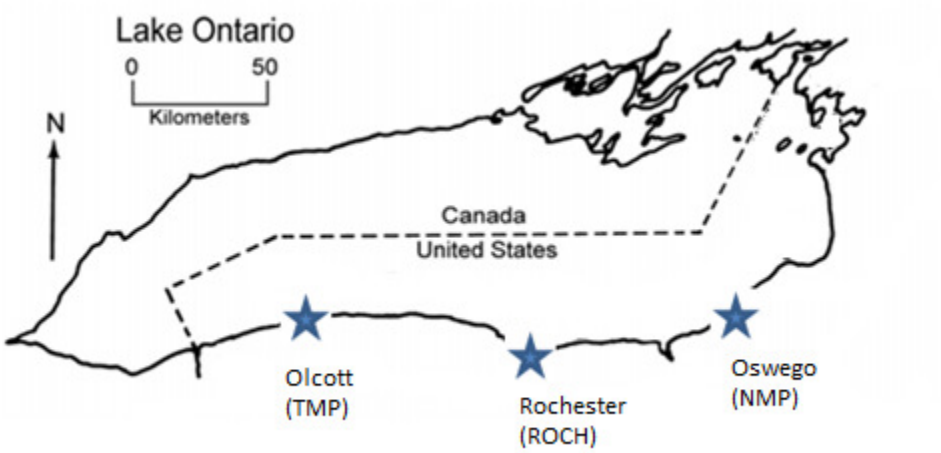
Table 15. Predator fish from Lake Ontario: SIMPER routine for dissimilarity among family groups. Fatty acids (% detected) that contribute to ≥ 70% of cumulative dissimilarity are reported.

Group comparison	Average dissimilarity (%)	Average abundance (%)		Contribution (%)
Esocidae, Percidae	18.25	Esocidae	Percidae	
16:1n-7		6.31	11.78	16.17
22:6n-3		18.84	14.45	16.08
18:1n-9		15.57	18.14	14.21
16:0		16.53	19.08	11.18
20:4n-6		7.24	5.17	6.61
18:2n-6		4.23	2.54	5.12
18:0		4.36	2.85	4.60
Salmonidae, Percidae	21.31	Salmonidae	Percidae	
18:1n-9		24.48	18.14	18.86
22:6n-3		8.34	14.45	14.52
16:1n-7		7.27	11.78	12.15
16:0		14.99	19.08	11.86
18:3n-3		3.84	1.82	4.95
18:2n-6		4.16	2.54	4.41
20:4n-6		3.58	5.17	3.83
Salmonidae, Esocidae	21.45	Salmonidae	Esocidae	
22:6n-3		8.34	18.84	24.64
18:1n-9		24.48	15.57	20.87
20:4n-6		3.58	7.24	8.57
16:1n-7		7.27	6.31	6.58
16:0		14.99	16.53	5.19
18:3n-3		3.84	2.38	3.71
14:0		3.55	2.16	3.50

Table 16. Comparison of prey and predator species FAS using 1-Way ANOSIM pairwise tests. Degree of difference between prey and predator species groups are represented by R-values.

	Alewife	Rainbow smelt	Round goby
Lake trout	0.57	0.76	0.84
Brown trout	0.54	0.79	0.85
Chinook salmon	0.38	0.8o	0.98
Coho salmon	0.28	0.62	0.86
Steelhead trout	0.29	0.68	0.89
Northern pike	0.48	0.39	0.90
Chain pickerel	0.58	0.26	0.76
Walleye	0.72	0.82	0.96
Yellow perch	0.67	0.57	0.50

a)



b)

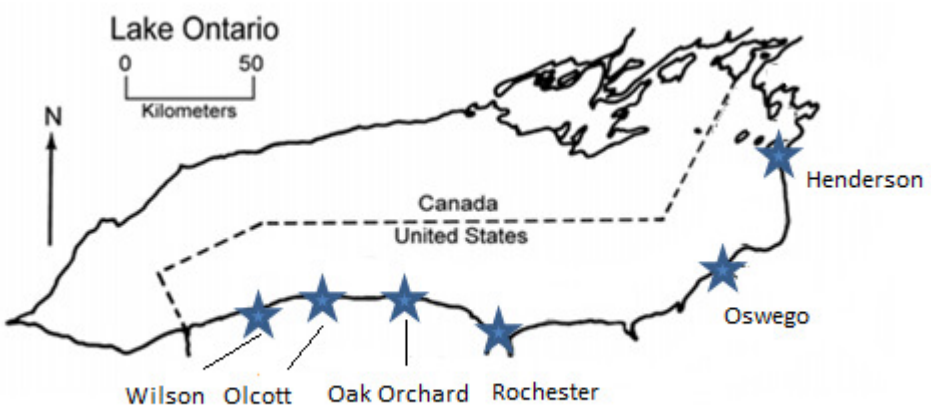


Figure 1. Study area: Lake Ontario. Stars indicate sampling locations for prey species (a) and predator species (b). Locations for prey sampling are Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP). Adapted from USGS (2012).

Figure 2. Preservation of prey fish (alewife).

Figure 3. Section of belly flap removed from predator fish for lipid and fatty acid analysis.

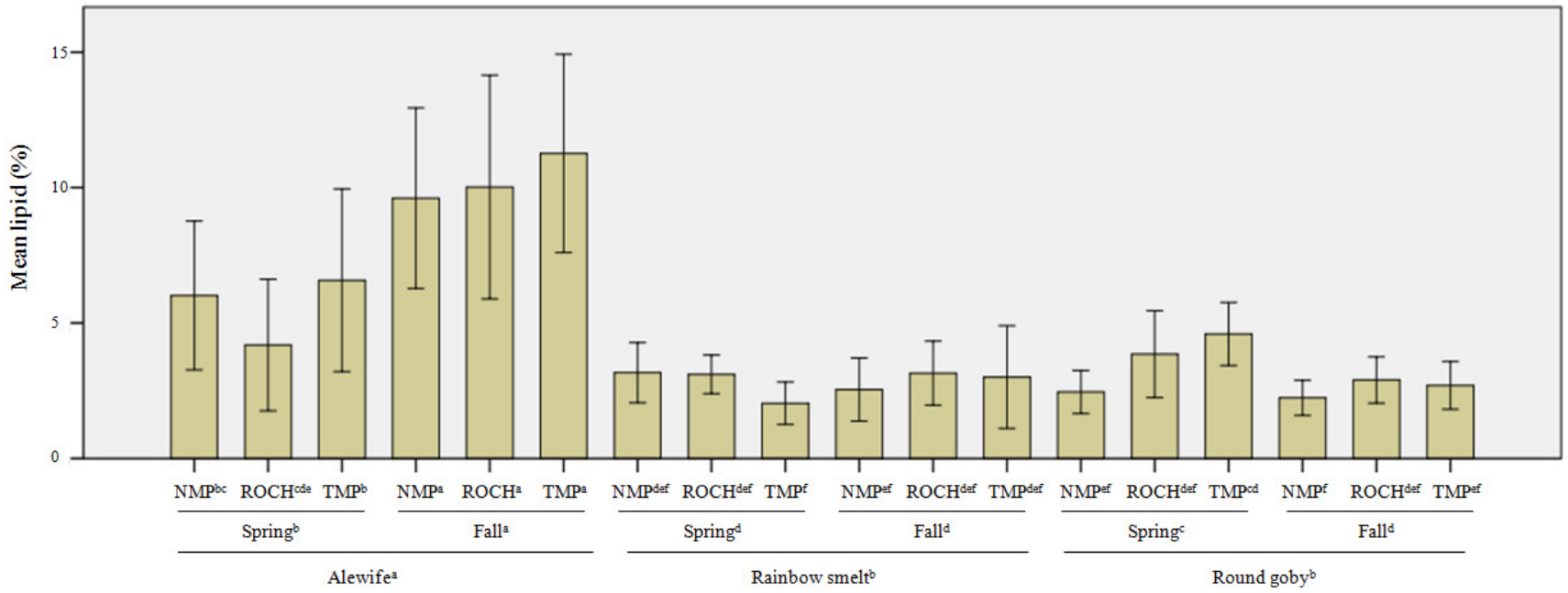


Figure 4. Lake Ontario prey fish mean lipid content separated by location, season, and species, respectively. Locations are Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP). Each separation was analyzed separately using the Kruskal-Wallis test. Means with different superscript letters indicate statistical difference ($p < 0.05$). Error bars indicate standard deviation.

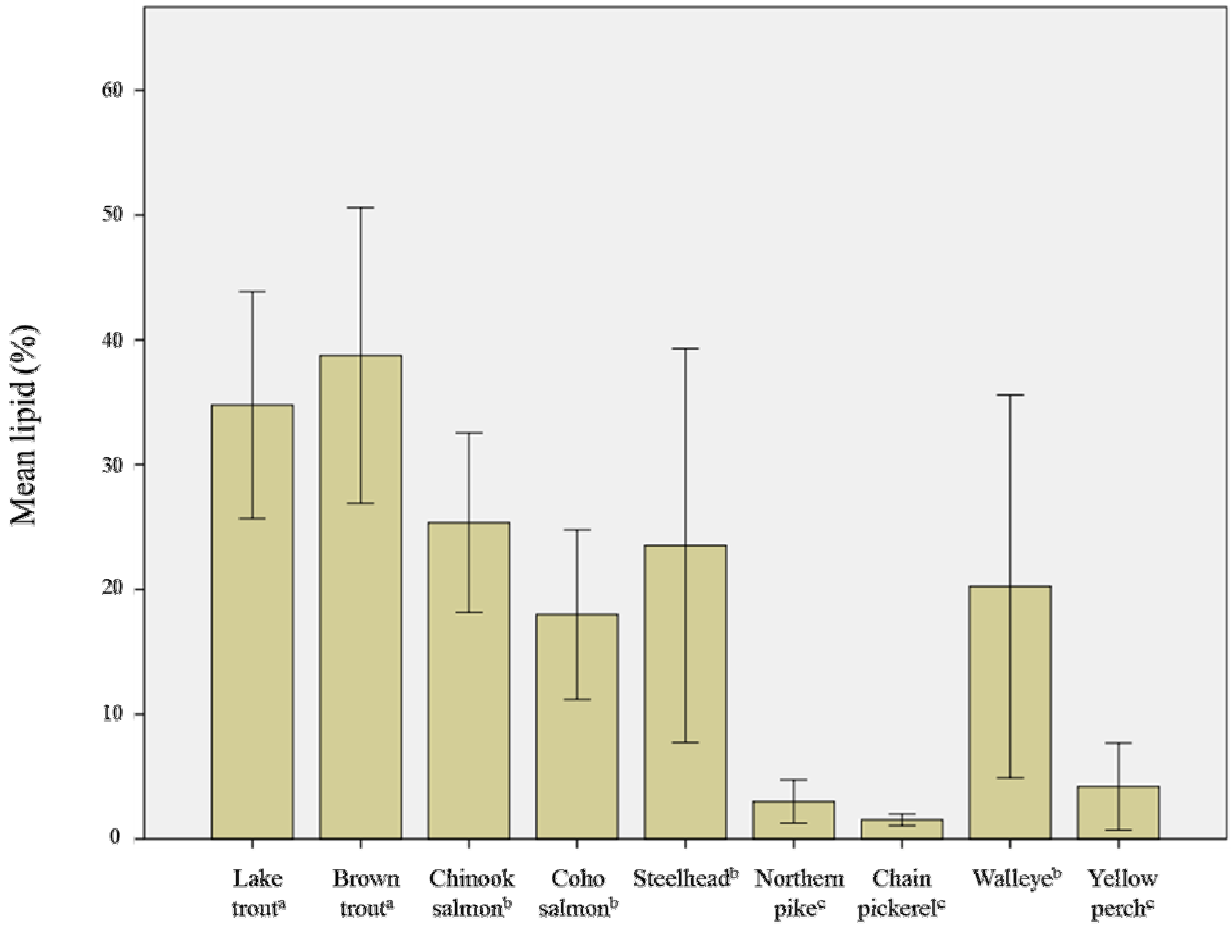


Figure 5. Lake Ontario predator fish mean lipid content separated by species. Differences in lipid content analyzed using Kruskal-Wallis test. Means with different superscript letters indicate statistical difference ($p < 0.05$). Error bars indicate standard deviation.

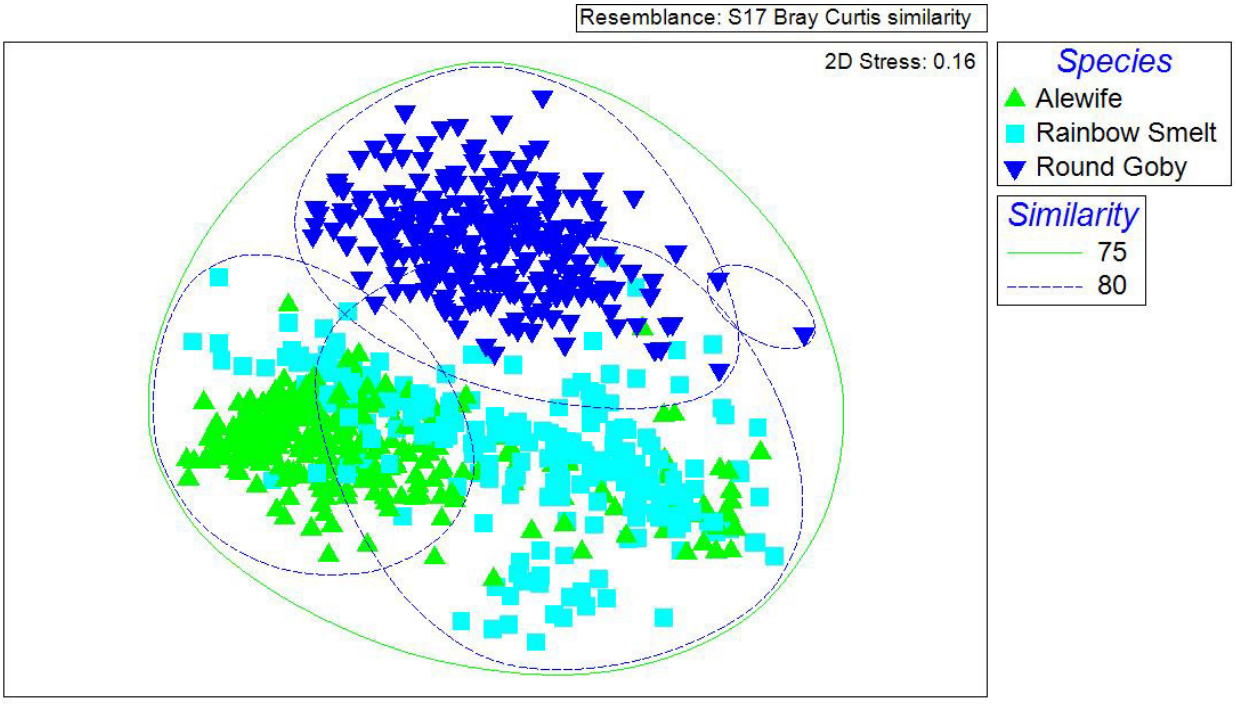


Figure 6. nMDS plot of all prey samples from three different locations during the spring and fall of 2013. Prey fish are separated by species and plotted individually based on similarity of FAS. Contours are a result of cluster analysis and represent 75% and 80% similarity.

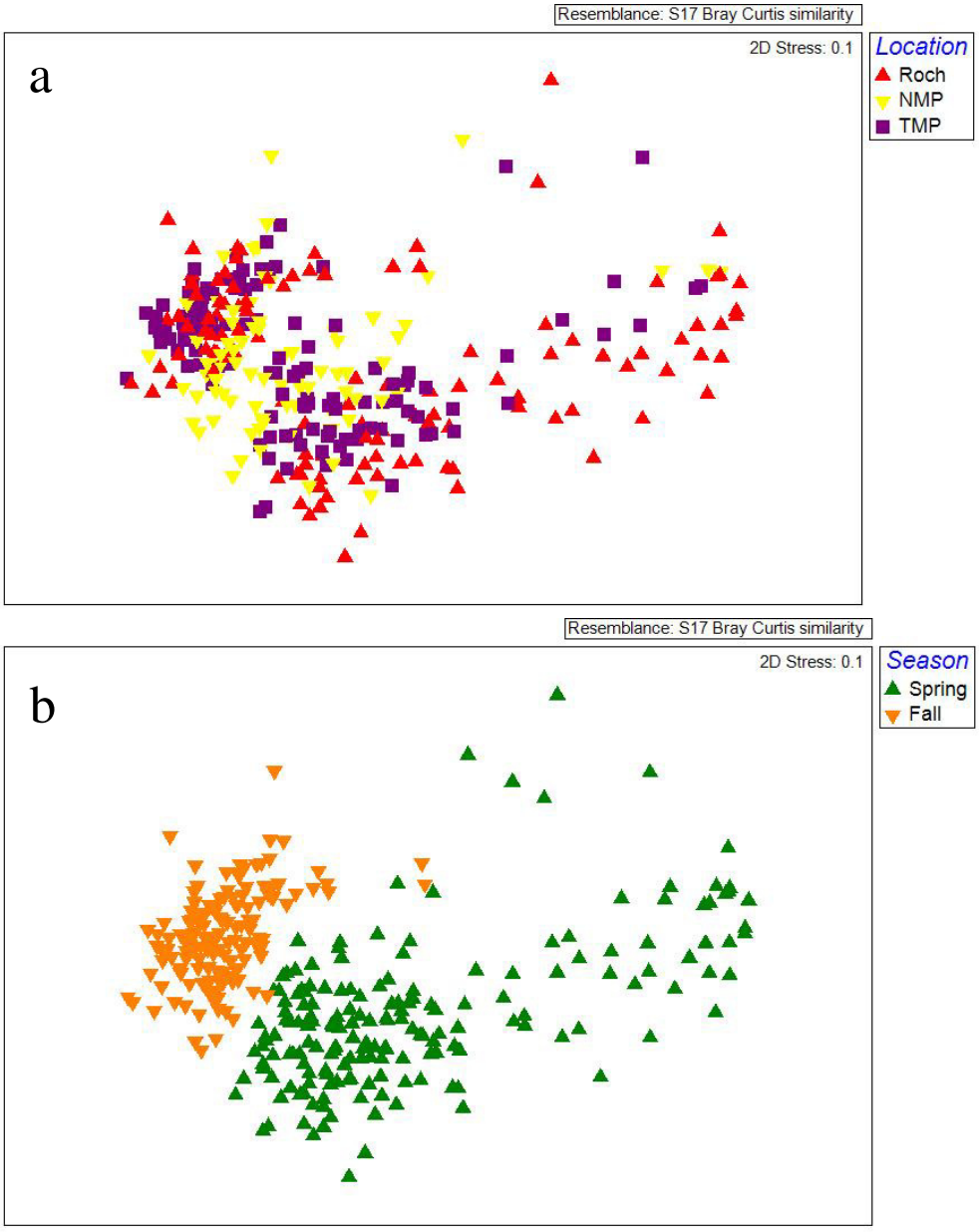


Figure 7. nMDS plot of alewife samples from three different locations during the spring and fall of 2013. Locations are Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP). Samples are plotted individually based on similarity of FAS. Samples are plotted by (a) location and (b) season.

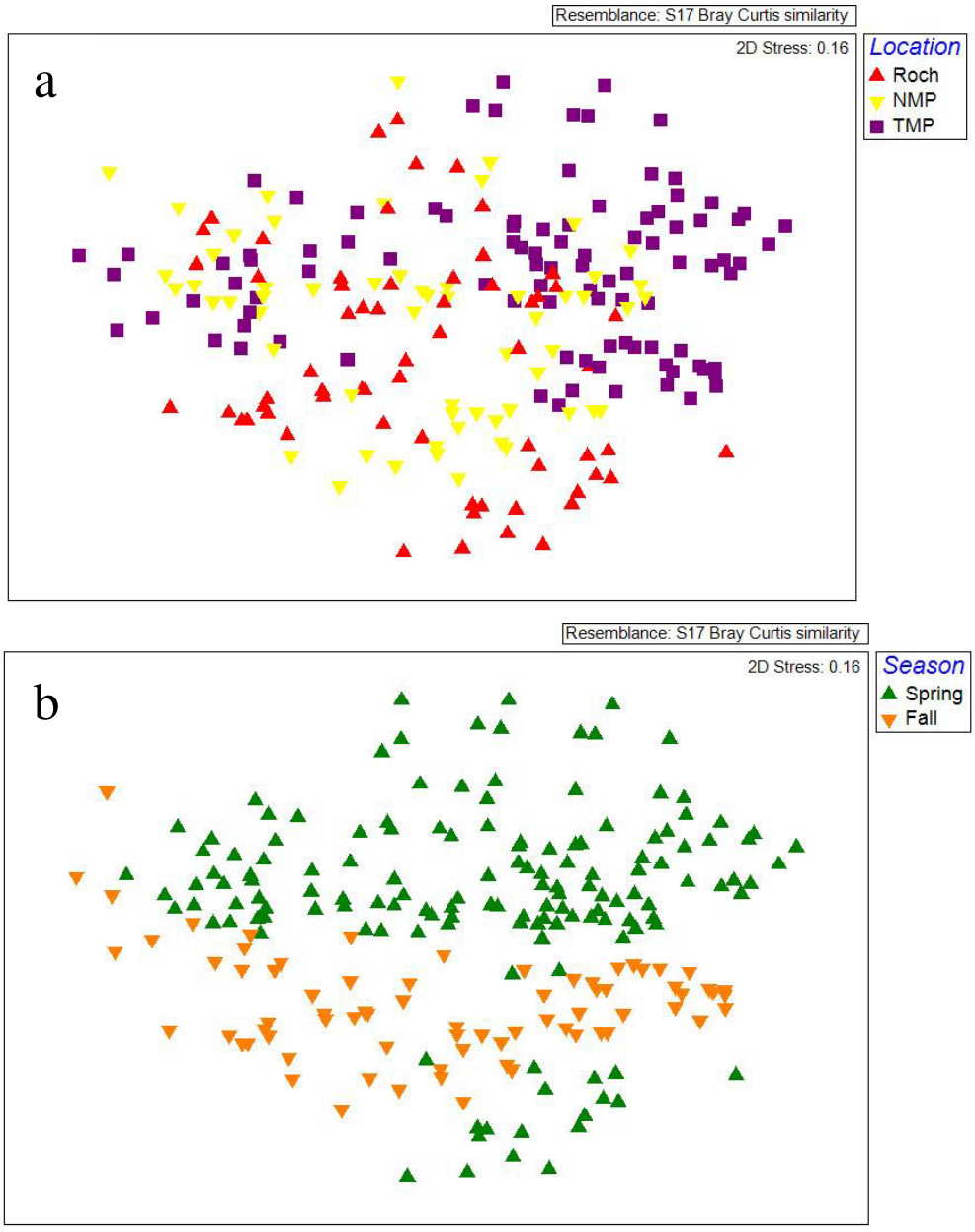


Figure 8. nMDS plot of rainbow smelt samples from three different locations during the spring and fall of 2013. Locations are Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP). Samples are plotted individually based on similarity of FAS. Samples are plotted by (a) location and (b) season.

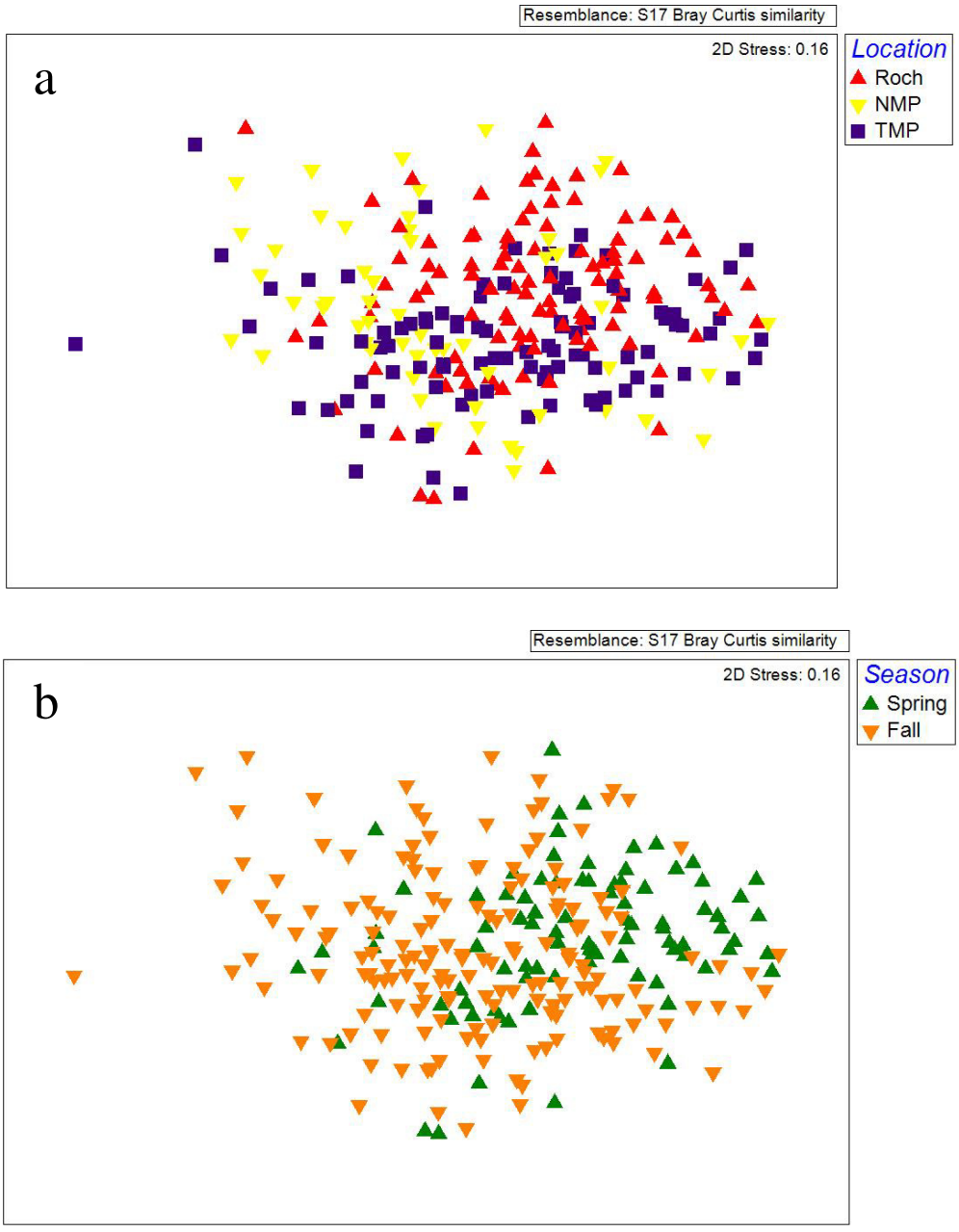


Figure 9. nMDS plot of round goby samples from three different locations during the spring and fall of 2013. Locations are Thirty Mile Point (TMP), Rochester (ROCH), and Nine Mile Point (NMP). Samples are plotted individually based on similarity of FAS. Samples are plotted by (a) location and (b) season.

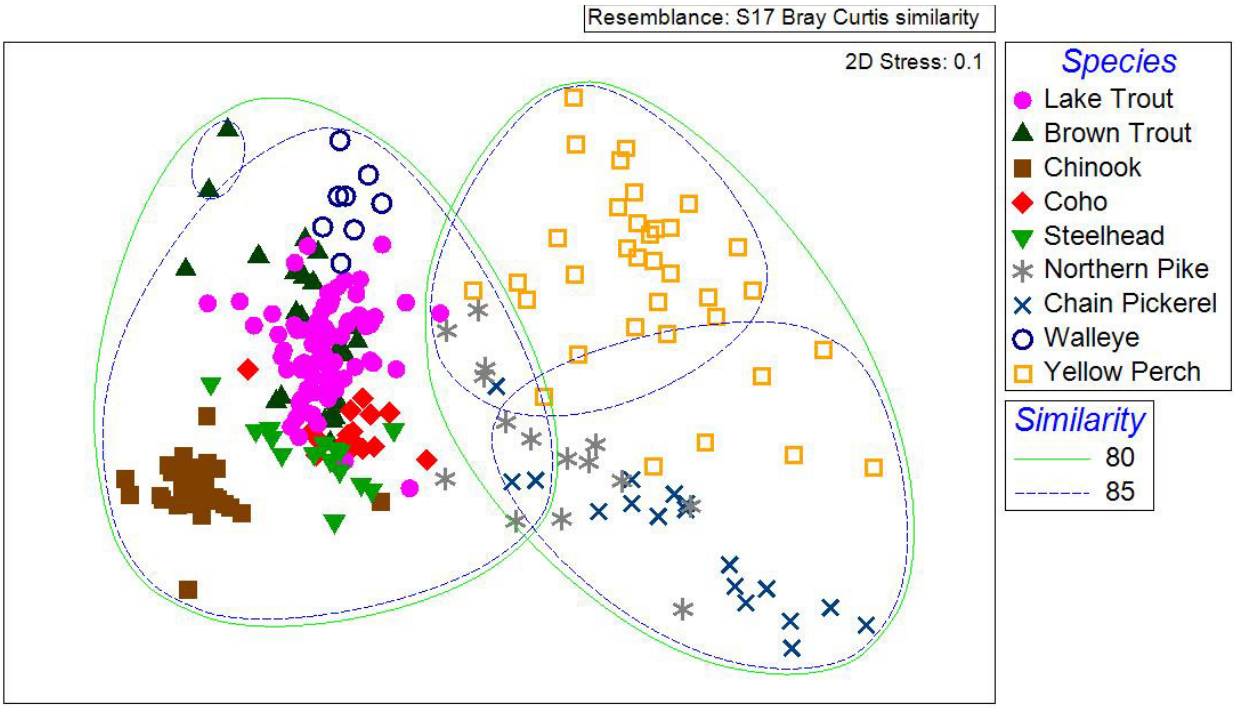


Figure 10. nMDS plot of all predator samples from the south shore of Lake Ontario during the summer and fall of 2013. Predator fish are separated by species and plotted individually based on similarity of FAS. Contours are a result of cluster analysis and represent 80% and 85% similarity.

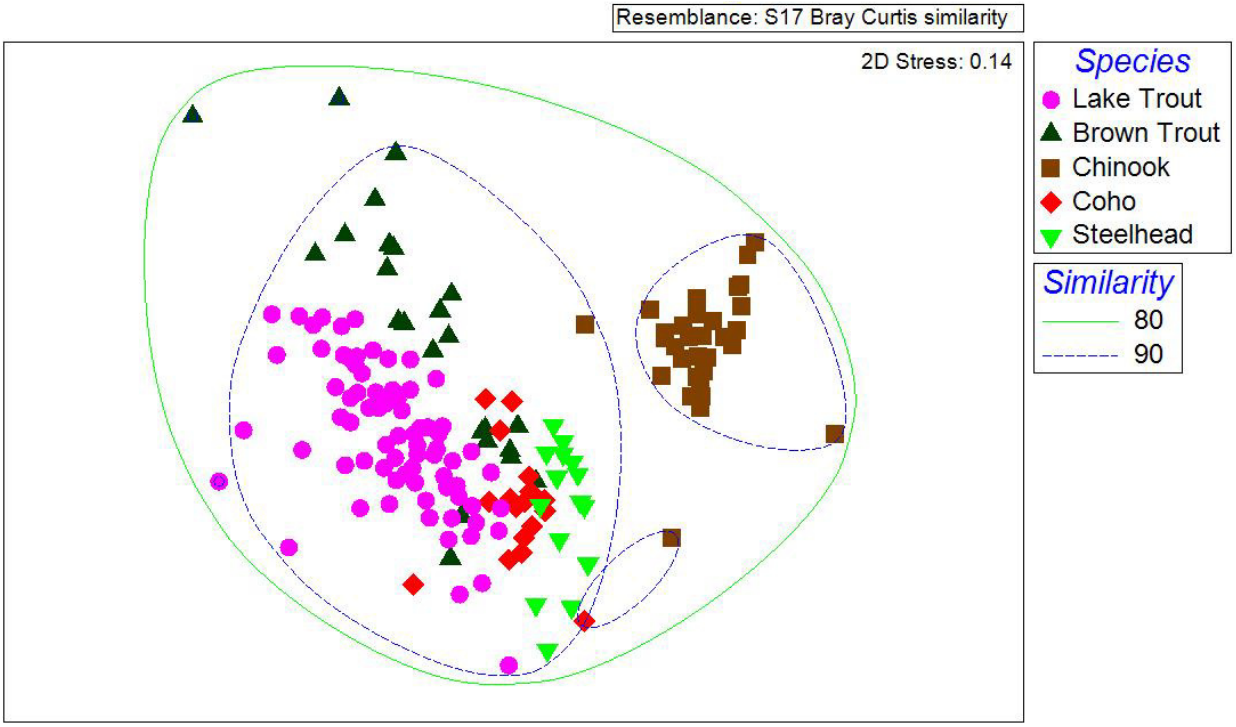


Figure 11. nMDS plot of all salmonid predator samples from the south shore of Lake Ontario during the summer and fall of 2013. Salmonids are separated by species and plotted individually based on similarity of FAS. Contours are a result of cluster analysis and represent 80% and 90% similarity.

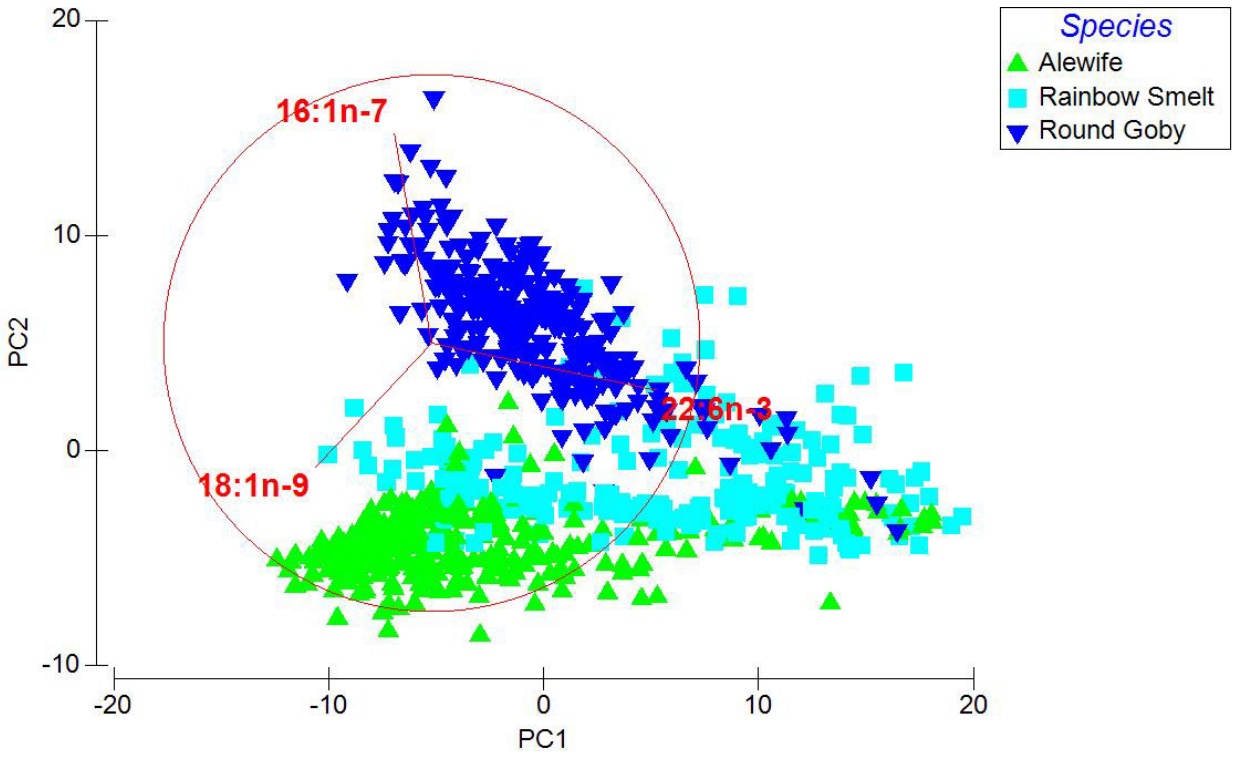


Figure 12. PCA plot of all prey fish by species. Species groups include samples from both seasons and from all locations. PC1 (49.0%) and PC 2 (24.8%) account for 73.8% of the variance in the data. Vectors selected based on correlations above 0.4.

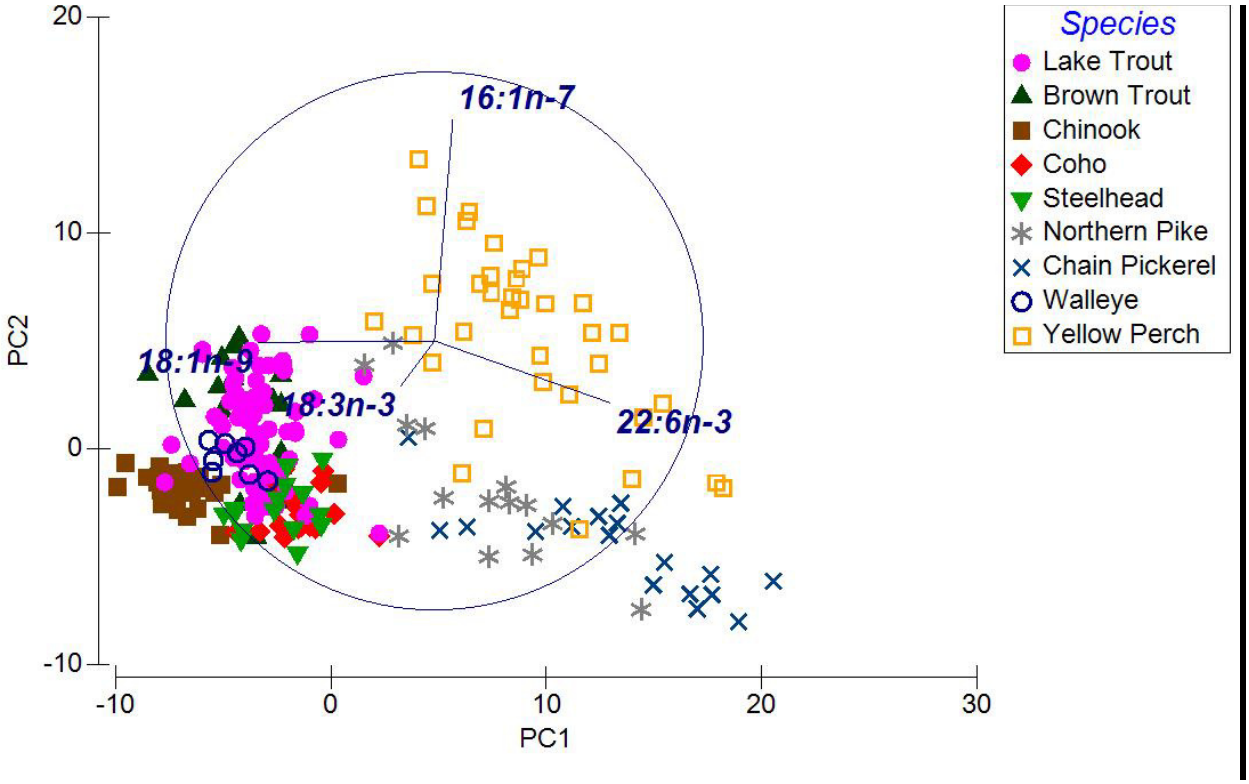


Figure 13. PCA plot of all predator fish by species. PC1 (65.0%) and PC 2 (18.6%) account for 83.6% of the variance in the data. Vectors selected based on correlations above 0.4.